Extreme blood boosting capacity of an Antarctic fish represents an adaptation to life in a sub-zero environment

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Summary statement

Extreme splenic blood boosting strategy provides bald notothens with an extraordinary facultative aerobic scope that enables an active lifestyle in sub-zero marine environments.

Abstract

Blood doping, the practice of boosting the oxygen carrying capacity of blood, is an illegal strategy used by human athletes to enhance aerobic capacity and athletic performance. Interestingly, the practice of boosting blood oxygen carrying capacity is also naturally prevalent in the animal kingdom via the splenic release of stored erythrocytes. Here we demonstrate that an Antarctic notothenioid fish, the bald notothen (*Pagothenia borchgrevinki*), is a master of this practice. Due to the sub-zero environment these fish inhabit, they sequester a large proportion of erythrocytes in the spleen during times of inactivity to reduce the energetic and physiological costs associated with continuously pumping highly viscous blood around the body. However, in response to metabolically demanding situations (*i.e.* exercise and feeding), these fish contract the spleen to eject stored erythrocytes into circulation, which boosts blood oxygen carrying capacity by up to 207% (*c.f.* exercise-induced increases of ~40-60% in a range of other vertebrates and ~5-25% in blood-doping athletes). By evaluating cardiorespiratory differences between splenectomized (unable to release erythrocytes from the spleen) and sham-operated individuals, we demonstrate the metabolic benefits (*i.e.* aerobic scope increased 103%) and the cardiovascular trade-offs *(i.e.* ventral aortic blood pressure and cardiac workload increased 12% and 30%, respectively) associated with the splenic blood boosting strategy. In conclusion, this strategy provides bald notothens with an extraordinary facultative aerobic scope that enables an active lifestyle in the extreme Antarctic marine environment, while minimizing the energetic and physiological costs of transporting highly viscous blood during times of reduced energetic demand.

Introduction

It is well documented that performance enhancing strategies such as blood doping, the practice of boosting one's blood oxygen carrying capacity, is used by human athletes seeking a competitive advantage (Momaya *et al*., 2015; Tokish *et al*., 2004). The resulting improvements in oxygen delivery affords these athletes substantial increases in their aerobic capacity, which translates into an enhanced athletic performance (Momaya *et al*., 2015; Tokish *et al*., 2004). Increasing blood oxygen carrying capacity can be achieved naturally via high-altitude training or artificially via the administration of illicit substances (*i.e.* erythropoietin, which stimulates an increased production of erythrocytes) and autologous blood transfusions (*i.e.* reinfusing the donor's stored blood back into their bloodstream) (Momaya *et al*., 2015; Tokish *et al*., 2004; WADA, https://www.wada-ama.org). In most professional sports, the use of erythropoietin and autologous blood transfusions are prohibited since they provide an unfair competitive advantage (*i.e.* ~5-25% increase in blood oxygen carrying capacity) and pose a significant risk to health (*i.e.* hypertension, myocardial infarction, pulmonary embolism and an increased risk of thrombosis) (Momaya *et al*., 2015; Tokish *et al*., 2004). Interestingly, the practice of boosting the oxygen carrying capacity of blood can also occur naturally via the contraction of the spleen, which results in the ejection of stored erythrocytes into the bloodstream. While humans have a relatively small splenic reservoir and limited use of this strategy, it is much more developed in other members of the animal kingdom. For example, it has been demonstrated that a number of fish species (Axelsson, 2005; Wells and Weber, 1990; Yamamoto *et al.*, 1980), marine mammals (Hurford *et al.*, 1996; Zapol, 1987), and terrestrial mammals (Hannon *et al.*, 1985; Turner and Hodgetts, 1959; Perrson *et al.*, 1973a,b) are able to boost their blood oxygen carrying capacity by ~40-60% in response to metabolically demanding situations. In fact, the spleen of Weddell seals (*Leptonychotes weddellii*) has been referred to as a natural 'scuba tank', as the release of oxygenated red blood cells tightly regulates the arterial oxygen content during diving (Hurford *et al.*, 1996; Zapol, 1987). However, the most remarkable capacity for splenic blood boosting in vertebrates can be observed in an Antarctic notothenioid fish species, the bald notothen (*Pagothenia borchgrevinki*), which have been reported to more than double the oxygen carrying capacity of their blood during exercise (Davison *et al.*, 1988; Franklin *et al.*, 1993).

The opening of the Drake Passage (~40 million years ago), followed by the Tasmanian Gateway (~25 million years ago), allowed water to flow around the entirety of the Antarctic continent and ultimately led to the formation of one of the most thermally extreme and challenging environments that marine teleosts currently inhabit (Beers and Jayasundara, 2015; Clarke and Johnston, 1996; Eastman, 2005). The combination of stable water temperatures approaching the freezing point of sea water (-1.87°C), the presence of sea ice, and the seasonality of primary production poses substantial ecological and physiological challenges for Antarctic notothenioid fishes, such as the bald notothen (Beers and Jayasundara, 2015; Clarke and Johnston, 1996; DeVries and Eastman, 1981; Eastman, 2005). Since these fish are subjected to temperatures well below the equilibrium freezing point of their tissue fluids (-0.8°C), a necessity for survival was the evolution of antifreeze molecules that are secreted into the bloodstream (DeVries, 1988). However, the blood viscosity of these fish is substantially elevated due to the combination of a high concentration of antifreeze molecules, the extremely low temperatures and circulating erythrocytes, which cumulatively increase the workload on the heart (Axelsson, 2005; Farrell; 1991; Graham and Fletcher, 1985; Hemmingsen, 1991). The energetic cost and cardiovascular consequences (*i.e.* chronic hypertension, increased risk of thrombosis, increased risk of myocardial and cerebral infarction, increased cardiac wear and tear, and chronic ischaemia) of transporting highly viscous blood through the cardiovascular system may constitute important driving forces for the numerous cardiovascular cold adaptations present in Antarctic fishes (Axelsson, 2005; Beers and Jayasundara, 2015; Farrell; 1991; Hemmingsen, 1991; Lowe, 1992; Sidell and Driedzic, 2012; Willis and Patterson, 2013).

In order to reduce blood viscosity and cardiac workload, it has been proposed that an early cardiovascular cold adaptation in Antarctic fishes was the coupling of a low vascular resistance with substantial reductions in circulating erythrocytes (Axelsson, 2005; Buckley *et al.*, 2014; Hemmingsen, 1991). In fact, a unique evolutionary development of the notothenioids is the complete loss of functional erythrocytes in adult members of the family Channichthyidae (icefish), while in red-blooded notothenioids, the numbers of circulating erythrocyte cells at rest is approximately half of the number found in temperate teleost species (Axelsson, 2005; Graham and Fletcher, 1985; Hemmingsen, 1991). While the complete loss or decrease of erythrocytes in notothenioids is associated with a substantial reduction in blood viscosity, it also

coincides with similar reductions in blood oxygen carrying capacity, which has inevitably led to the development of a range of other cardiovascular modifications (Axelsson, 2005; Axelsson *et al.*, 1992; Hemmingsen, 1991; Joyce *et al.*, 2018, 2019). In the icefish group, these adjustments consist of larger blood volumes, enlarged hearts and significantly reduced vascular resistances, whereas in the red-blooded notothenioids, the level of circulatory adjustments is believed to differ depending on the ecology of the varying species (Axelsson, 2005; Axelsson *et al.*, 1992; Buckley *et al.*, 2014; Joyce *et al.*, 2018, 2019). It has been proposed that the next step in the evolutionary sequence for nototheniod fishes was an increase in their capacity for systemic oxygen delivery (*i.e.* the product of cardiac output and blood oxygen content) (Axelsson, 2005; Axelsson *et al.*, 1992; Buckley *et al.*, 2014). For example, the more active, cryopelagic red-blooded notothenioid (*i.e.* the bald notothen) tends to have a higher cardiac output and relative ventricular/atrial masses than the relatively sedentary, benthic species (*i.e.* the Emerald rockcod, *Trematomus bernacchii*), which are able to perform daily activities with a reduced oxygen carrying capacity due to the high oxygen solubility of seawater/plasma and their low metabolic demand (Axelsson *et al.*, 1992). In addition, we argue that the splenic blood boosting strategy of bald notothens represents a cardiovascular cold adaptation for increasing systemic oxygen delivery, as it allows them to match their oxygen carrying capacity with their energetic demand (Axelsson, 2005; Davison *et al.*, 1988; Franklin *et al.*, 1993). For example, when in a resting and unfed state, these fish should be able to reduce blood viscosity, and thus cardiac workload, by sequestering a large proportion of their erythrocytes in the spleen (Davison *et al.*, 1988; Franklin *et al.*, 1993). However, during instances of elevated metabolic demand (*e.g.* exercise and acute stress), these fish are extremely adept at rapidly increasing arterial oxygen carrying capacity and venous oxygen reserve by releasing erythrocytes from the spleen (Davison *et al.*, 1988; Franklin *et al.*, 1993). This highly flexible strategy of being able to utilize a reserve of oxygen carrying capacity only when it is most beneficial, should provide interspecific advantages with regards to competition for ecological resources and/or when escaping from predators. However, the metabolic and cardiovascular pros and cons of splenic blood boosting in the bald notothen has not yet been determined, and thus the potential selective advantages of this strategy remain speculative.

The overall aim of the present study was to quantify the magnitude, as well as the cardiorespiratory implications, of splenic blood boosting in bald notothens in response to metabolically demanding situations. Specifically, we hypothesized that bald notothens would substantially increase their blood oxygen carrying capacity via splenic blood boosting to match the metabolic demands associated with enforced exercise and/or ingestion of a meal. Furthermore, the increase in blood oxygen carrying capacity via splenic blood boosting in bald notothens was hypothesized to coincide with metabolic benefits (due to an increased capacity for systemic oxygen delivery) and cardiovascular tradeoffs (due to the physiological effects of an increased blood viscosity on cardiovascular performance). Therefore, we initially measured changes in the haematocrit of uninstrumented individuals following enforced exercise and/or ingestion of a meal. Then we determined the cardiorespiratory implications of an elevated blood oxygen carrying capacity following enforced exercise by evaluating the differences between splenectomized individuals (*i.e.* unable to release erythrocytes from the spleen into the circulation to boost oxygen carrying capacity) and sham-operated individuals. By exploring the eco-physiological costs and benefits of the splenic blood boosting strategy, we aimed to provide further insights on how the bald notothens were able to radiate into, as well as dominate, the seasonally high productive and relatively underutilized cryopelagic zone of the waters surrounding the Antarctic continent (Beers and Jayasundara, 2015; Clarke and Johnston, 1996; Eastman, 2005).

Material and methods

Experimental animals

Bald notothens (*Pagothenia borchgrevinki*, Boulenger, 1902) were caught using line and hook through a hole in the sea ice at Evans Wall, Ross Island, McMurdo Sound, Antarctica (n=73, mean±s.d body mass=87±26 g). Following capture, the fish were transported back to the Crary Lab at McMurdo Station, Antarctica (Fig. 1). Fish were randomly assigned into one of two identical 2000 L tanks containing fresh, aerated seawater at a temperature of -1.6°C and held at a natural photoperiod of 24 h daylight. Fish in one tank were fed (diet=fish flesh, *ad libitum* every second day), whereas fish in the other tank remained unfed. All fish were allowed >1 week to recover prior to experimentation. Animal care and all experimental procedures were performed in accordance with national regulations and was covered by an ethical permit (264-2011) issued by the regional ethical committee on animal research in Gothenburg, Sweden.

Sample sizes for the treatment groups described in the following sections were based on our previous experiences with similar types of comparative physiological experiments, as well as previously published data on bald notothens (Davison *et al.*, 1988; Franklin *et al.*, 1993).

Experimental protocols

Determining the extent of splenic blood boosting during exercise and feeding

To investigate the changes in blood oxygen carrying capacity of the bald notothen in response to exercise and feeding, we determined the haemotocrit (%, an indicator for blood oxygen carrying capacity), absolute spleen mass (g), and relative spleen mass (% body mass) of 40 uninstrumented fish (Fig. 2A-D). The fish were randomly divided into four groups, which consisted of *i*) 'unfed resting fish' (*i.e.* fasted for 7 days and then sampled in a resting state, n=9, mean±s.d body mass =55±17 g), *ii*) 'unfed exercised fish' (*i.e.* fasted for 7 days and then sampled directly following enforced exercise as described in detail below, n=9, mean±s.d body mass=89±13 g), *iii*) 'fed resting fish' (*i.e.* fed and then sampled 24 h later in a resting state, n=13, mean±s.d body mass=87±29 g), and *iv*) 'fed exercised fish' (*i.e.* fed and then sampled 24 h later directly following enforced exercise as described in detail below, n=9, mean±s.d body mass=59±10 g). Fed fish were sampled 24 h after feeding, as a previous study indicated that bald notothens are still in a post-prandial state at this stage (*e.g.* 58±2% of the ingested meal still remained in the stomach of uninstrumented bald nothothens after 24 h, Sandblom *et al.*, 2012).

'Unfed resting fish' and 'fed resting fish' were captured from their respective holding tanks with a net and sampled immediately (Fig. 2A-B). Sampling consisted of fish being killed by a cranial blow, promptly sampled for blood via a caudal puncture with a heparinized syringe and a 20-gauge needle, and then weighed and measured. The sampling procedure took less than 1 minute. In addition, the body cavity of the fish was opened and the spleen carefully dissected out and weighed to calculate relative spleen mass (% body mass). The blood samples were subsequently analysed for haematocrit (%), as the fractional red cell volume upon centrifugation of a subsample of blood in 80 µL microcapillary tubes at 10,000 rcf for 5 min. No food contents remained in the gut of 'unfed, resting fish' indicating that these fish were in a pre-prandial state, whereas the remaining food contents in the gut of 'fed, resting fish' accounted for 6.6±1.4% of individual body weight (mean±s.e.m) indicating that these fish were in a post-prandial state.

'Unfed exercised fish' and 'fed exercised fish' were captured from their respective holding tanks with a net and placed individually into respirometers (Fig. 2C-D). 'Unfed exercised fish' (Fig. 2C) were allowed to recover in the respirometers for 48 h prior to determining whole animal oxygen uptake (M_{O_2}) before and after a period of enforced exercise (see *Respirometry* for details concerning respirometry techniques used to determine M_{O_2}). The period of enforced exercise involved manually chasing the fish inside the respirometer for 10 min using plastic tubing inserted through an opening located at the top of the respirometer (Seth *et al.*, 2013). Fish displayed a strong aversive behavioural response to the plastic tubing, which resulted in the fish frantically swimming back and forth the inside of the respirometers for the majority of the enforced exercise period. All individuals were visibly exhausted after 10 min of enforced exercise, as highlighted by a lack of response to an experimenter tapping the caudal fin of the fish with the plastic tubing (Clark *et al.*, 2013; Seth *et al.*, 2013). M_{O_2} measurements obtained from 'unfed exercised fish' before and after the period of enforced exercise were used to estimate resting and maximum metabolic rates (see *Respirometry* for metabolic rate calculations), which were subsequently used to evaluate whether the instrumented fish (described in the section below) had sufficiently recovered from the stressors associated with capture, handling and surgical instrumentation prior to experimentation. In contrast, 'fed exercised fish' (Fig. 2D) were immediately subjected to the period of enforced exercise upon entry into the respirometers and thus M_{O_2} was only determined after exercise to estimate maximum metabolic rate. This was due to the fact that these fish did not voluntarily feed inside the respirometers and thus no attempt was made to determine resting M_{O_2} in a fed state. Following the period of enforced exercise, the fish were removed from the respirometers and immediately sampled in a similar fashion as described above for the 'unfed resting fish' and 'fed resting fish'. No food contents remained in the gut of 'unfed, resting fish' indicating that these fish were in a pre-prandial state, whereas the remaining food contents in the gut of 'fed, resting fish' accounted for 14.8±1.6% of individual body weight (mean±s.e.m) indicating that these fish were in a post-prandial state.

Quantifying the cardio-respiratory implications of splenic blood boosting

To investigate the cardiorespiratory implications of the splenic blood boosting strategy employed by the bald notothen during metabolically demanding situations, we measured M_{O_2}, cardiac output, heart rate and ventral aortic blood pressure of 'splenectomized' (*i.e.* splenic vessels were ligated to prevent the exercise-induced increase in hematocrit, n=14, mean±s.d body mass=106±19 g, Fig. 2E) and 'sham-operated' (n=19, mean±s.d body mass=101±17 g, Fig. 2F) fish before and after a period of enforced exercise (see *Surgical instrumentation* for details concerning surgical instrumentation and methodology for determining cardiorespiratory parameters). From the measured cardiorespiratory parameters, we estimated metabolic rates (see *Respirometry* below), stroke volume (*i.e.* cardiac output divided by heart rate), total vascular resistance (*i.e.* ventral aortic blood pressure divided by cardiac output), and cardiac power index (*i.e.* an estimate of cardiac workload, which was calculated by multiplying ventral aortic blood pressure with cardiac output).

Fish from both groups were fasted for 7 days before being captured from the holding tank, anaesthetized and instrumented (*i.e.* fish from both groups were surgically instrumented with custom-made Doppler flow probes and catheters, whereas whether fish had their spleens surgically ligated or underwent a sham operation was randomly determined). After surgery, fish were individually placed into respirometers and allowed to recover for 48 h. All fish were visually observed to be in a calm, resting state following the recovery period. Resting levels of the cardiorespiratory parameters were determined by calculating means for each parameter in undisturbed fish for 15 min directly prior to the fish being subjected to enforced exercise. Once resting levels were determined, a 200 µL sample of blood was carefully withdrawn via the implanted catheter and subsequently analysed for haematocrit. Fish were then subjected to a period of enforced exercise, which as described previously involved manually chasing the fish inside the respirometers for 10 min. Maximum cardiorespiratory parameters following enforced exercise were determined by calculating the mean of each parameter during the 5 min period directly after enforced exercise, as this period corresponded with the highest determinations of M_{O_2} (*i.e.* the steepest section of the decline in the partial pressure of oxygen in the water within the respirometers, see *Respirometry* below). Another 200 µL of blood was then withdrawn for haematocrit analysis. At the end of the protocol, fish were removed from the respirometers, killed by a cranial blow,

measured and weighed, and the spleen was dissected out and weighed in order to calculate relative spleen mass.

Surgical instrumentation and respirometry

Surgical instrumentation

Fish were anaesthetized in seawater (-1.6°C) containing 100 mg L^{-1} MS222 (ethyl-3-aminobenzoate methanesulphonic acid, Sigma-Aldrich Inc., St. Louis, Missouri, USA) buffered with 200 mg L^{-1} $NaHCO_3$. Anaesthetized fish were transferred to an operating table covered with soft, water-soaked foam. To maintain anaesthesia, the gills were continuously flushed with aerated seawater containing 50 mg L^{-1} MS222 buffered with 100 mg L^{-1} $NaHCO_3$. at 0ºC.

To determine cardiac output, the ventral aorta was carefully dissected free, ensuring that the pericardium and nearby nerves and blood vessels remained intact (Axelsson *et al.*, 1992). A 20 MHz Doppler flow crystal (Iowa Doppler products, Iowa City, IA, USA) mounted in 1.3-2.0 mm cuffs (depending on the diameter of the artery) was placed around the vessel. The flow probe was secured with silk sutures to the skin near the area of placement and in front of the dorsal fin. Cardiac output was measured with a directional-pulsed Doppler flowmeter (model 545C-4, Iowa Doppler products, USA). The cardiac output signal, as well as the other signals described below, were relayed to a PowerLab 8/30 system (ADInstruments, Castle Hill, Australia) and data were collected on a PC using ADInstruments acquisition software LabChart™ 5 Pro v7.2.5, at a sampling rate of 10 Hz. Heart rate was determined by quantifying the number of blood flow peaks (*i.e.* cardiac systole) per minute in the acquisition software.

To determine ventral aortic pressure, the third afferent branchial artery was cannulated (Axelsson and Fritsche, 1994). A 4-0 silk suture was placed around the gill arch, and then the artery was punctured upstream from the ligation at the base of the gill filaments. A PE-31 catheter (Natsume, Tokyo, Japan) filled with heparinized saline (25 IU ml^{-1}) was advanced into the vessel toward the ventral aorta. The catheter was secured using the ligation suture, an additional suture placed around the gill arch ~5 mm below the point of insertion, and a skin suture. The ventral aortic catheter was connected to a pressure transducer (model DPT-6100; Pvb Medizintechnik, Kirchseeon, Germany) and calibrated against a static water column with the water level in the experimental chamber as the zero reference. The blood pressure signal was amplified using a Senselab 4ChAmp amplifier (Somedic Sales, Hörby, Sweden).

For the splenectomized fish, a 2 cm mid-ventral incision was made into the body cavity to access the spleen, where after a silk suture was placed around the splenic vessels and tied firmly in order to prevent blood flow to and from the spleen (Franklin *et al.*, 1993). Blood loss from the mesenteric vessels and from the body wall where the incision was made was minimal, and after a successful ligation, the incision was closed with interrupted silk sutures. For the sham-operated fish, the same surgical procedure was employed with the exception of tying of the splenic vessels. The total time taken for the surgical procedures (*i.e.* time from when the fish was initially anaesthetized till their placement in the respirometers) was ~15-30 min.

Respirometry

Fish were individually housed in one of four identical custom-made Perspex respirometers (volume=3.1 L) that were submerged in a larger experimental tank with recirculating aerated seawater at (-1.6°C) for 48 h prior to determinations of M_{O_2} to allow sufficient time to recover from preceding stressors. The partial pressure of oxygen in the water within the respirometer was measured continuously using fibre-optic oxygen meters calibrated in accordance with the supplier's manual (Oxy-4 Micro with oxygen Micro-optode PSt1, PreSens Gmbh, Regensburg, Germany). Due to the high demand for the fibre-optic oxygen meters at the Crary Lab in McMurdo Station during the limited research season, resting and maximum metabolic rates of bald notothens in the present study could only be estimated via determinations of M_{O_2} directly before and after a period of enforced exercise, respectively. However, all fish were visually observed to be in a calm, resting state prior to estimating resting metabolic rates.

When determining M_{O_2} before and after enforced exercise, the flush pumps that continuously refreshed the water in the respirometers were shut off for 15 min. During these times, the slope of the decline in the partial pressure of oxygen in the water within the respirometers were used to calculate M_{O_2}. The entire 15 min slope was used to calculate resting M_{O_2} (as the gradient of the slopes did not vary during this measurement period), whereas only the first 5 min of the slope was used to calculate maximum M_{O_2} (as this was the steepest section of the slope). Both resting and maximum M_{O_2} were calculated using the following formula (Clark *et al.* 2013):

$$\text{Eqn 1: } M_{O_2} = [(V_r - V_f) \times \Delta C_{wO2}] / (\Delta t \times M_f),$$

where V_r is the volume of the respirometer, V_f is the volume of the fish (assuming that the overall density of the fish is 1 g per ml of tissue, thus V_f = mass of the fish, M_f), ΔC_{wO2} is the change in the oxygen concentration of the water within the respirometer (C_{wO2} is the product of the partial pressure and capacitance of oxygen in the water, the latter being dependent on salinity and temperature) and Δt is the time during which ΔC_{wO2} is measured. Aerobic scope was calculated by subtracting resting metabolic rate from maximum metabolic rate.

Statistical analyses

Statistical analyses were performed using SPSS Statistics 25 (IBM Corp., Armonk, NY, USA). All data used were assessed to ensure that they did not violate the assumptions of the specific models outlined below. F-, t- and *P*-values obtained from the statistical analyses are reported throughout the text and all *P*-values <0.05 were considered statistically significant. Unless otherwise specified, all data are presented as means±s.e.m. All data supporting the paper is readily available in the supplementary information (S1-5).

For the analyses regarding the haematocrit of uninstrumented fish, we used a two-way ANOVA. The model used 'feeding state' (*i.e.* unfed *vs.* fed) and 'exercise state' (*i.e.* resting *vs.* exercised) as fixed factors, as well as the interaction between these factors. If an interaction was detected from either a significant interaction term between fixed factors and/or visual inspection of the means plot, we carried out an analysis of the simple main effects. If no interaction between fixed factors was detected, then the main effects for the fixed factors produced by the model were reported. For the analyses regarding the spleen mass of uninstrumented fish, we used a two-way ANCOVA. The model used 'feeding state' (*i.e.* unfed *vs.* fed), 'exercise state' (*i.e.* resting *vs.* exercised) and the interaction between these factors as fixed factors, while using body mass as a covariate since it was linearly related to spleen mass. Linear regressions were used to assess the relationship between haematocrit and relative spleen mass in uninstrumented fish.

In order to statistically analyse changes in the metabolic rates of uninstrumented, unfed fish before and after a period of enforced exercise, whilst comparing these rates with those of sham-operated, unfed fish (for control purposes), we used a two-way mixed ANOVA. The model used 'instrumentation state' (*i.e.* uninstrumented *vs.* sham-operated) as a between-subjects factor, 'exercise state' as a

within-subjects factor, and the interaction between these factors. To statistically analyse the differences in maximum metabolic rate between uninstrumented unfed and fed fish, we used a one-way ANCOVA (*i.e.* unfed, exercised *vs.* fed, exercised fish), which used 'feeding state' as a fixed factor and body mass as a covariate (due to the significant differences in body mass between these two groups).

For the analyses regarding the cardiorespiratory variables of instrumented, unfed fish, we used two-way mixed ANOVAs. The model used 'spleen functionality' (*i.e.* sham-operated *vs.* splenectomized) as a between-subjects factor, 'exercise state' as a within-subjects factor, and the interaction between these factors. In order to meet the assumptions of the model, we applied a natural logarithmic transformation on cardiac output and cardiac power index data. For the analyses regarding the scope of the absolute increase in the cardiorespiratory variables of instrumented and unfed fish in response to enforced exercise, we used independent t-tests with 'spleen functionality' as the between-subjects factor. Linear regressions were used to assess the relationship between haematocrit and blood pressure, as well as haematocrit and aerobic scope in instrumented fish.

Results

Determining the extent of splenic blood boosting during exercise and feeding

A statistically significant interaction ($F_{1,36}$=15.457, P<0.001) was detected between feeding state (*i.e.* unfed *vs.* fed fish) and exercise state (*i.e.* resting *vs.* exercised) on the haematocrit of uninstrumented bald notothens. Analysis of simple main effects revealed that both exercise and feeding had a significant effect on the haematocrit of bald notothens (Fig. 3A). In a resting and unfed state, the haematocrit of uninstrumented fish was 8.6±1.6%. Haematocrit was ~3.1-fold higher in unfed fish subjected to a period of enforced exercise (*i.e.* 'unfed resting fish' *vs.* 'unfed exercised fish', $F_{1,36}$=75.187, P<0.001, Fig. 3A) or ~2.4-fold higher in fish that were fed but still in a resting state (*i.e.* 'unfed resting fish' *vs.* 'fed resting fish', $F_{1,36}$=41.801, P<0.001, Fig. 3A). In addition, haematocrit of fed fish increased further following a period of enforced exercise (*i.e.* 'fed resting fish' *vs.* 'fed exercised fish', $F_{1,36}$=13.099, P=0.001, Fig. 3A) to reach a level that did not significantly differ to that observed in exercised, unfed fish (*i.e.* 'unfed exercised fish' *vs.* 'fed exercised fish', $F_{1,36}$=0.367, P=0.549, Fig. 3A).

The interaction between feeding state and exercise state on absolute spleen mass of uninstrumented bald notothens when adjusted for body mass was not significant ($F_{1,35}$=1.375, P=0.249). The main effects of the model reveal that absolute spleen masses of bald notothens were significantly lower in fish subjected to a period of enforced exercise when compared to fish in a resting state (0.22±0.02 g *vs.* 0.37±0.02, respectively, for a 74 g fish, $F_{1,35}$=24.836, P<0.001). This is also reflected in the statistically significant and strong negative correlations between relative spleen mass and haematocrit for both unfed and fed fish (unfed: $F_{1,16}$=19.950, P<0.001; fed: $F_{1,20}$=14.187, P=0.001, Fig. 3B-C). No significant differences in absolute spleen masses were observed between unfed and fed fish (0.31±0.02 g *vs.* 0.28±0.02, respectively, for a 74 g fish, $F_{1,35}$=1.399, P=0.245).

To evaluate whether the instrumented fish (see next section) had sufficiently recovered from the stressors associated with capture, handling and surgical instrumentation prior to experimentation, we compared haematocrit and metabolic rates of unfed and uninstrumented fish with those from sham-operated fish. A statistically significant interaction ($F_{1,26}$=11.362, P=0.002) was detected between instrumentation state (*i.e.* uninstrumented *vs.* sham-operated) and exercise state (*i.e.* resting *vs.* exercised) on the haematocrit of bald notothens. Analysis of simple main effects revealed that resting haematocrit in uninstrumented fish was significantly lower than that observed in sham-operated fish (8.6±1.6% *vs.* 16.1±1.0%, $F_{1,26}$=17.721, P<0.001), while haematocrit levels following enforced exercise did not significantly differ between the groups (~26-27%, $F_{1,26}$=0.070, P=0.793). With regards to metabolic rates, the interaction between instrumentation state and exercise state was not significant ($F_{1,26}$=1.797, P=0.192). The main effects of the model reveal that although exercise significantly increased metabolic rate in both groups ($F_{1,26}$=179.368, P<0.001), no significant differences were observed in either resting or maximum metabolic rates between uninstrumented and sham-operated fish ($F_{1,26}$=0.322, P=0.575). In addition, when adjusted for body mass, no significant differences were observed in the maximum metabolic rate of uninstrumented bald notothens with regards to feeding state (*i.e.* unfed *vs.* fed, $F_{1,15}$=1.842, P=0.195).

Quantifying the cardio-respiratory implications of splenic blood boosting

Statistically significant interactions were detected between spleen functionality (*i.e.* sham-operated *vs.* splenectomized) and exercise state on the haematocrit and metabolic rate of bald notothens (Table 1). Analysis of simple main effects revealed no significant differences between the groups in the resting levels of these parameters (haematocrit: $F_{1,31}$=2.856, *P*=0.101, metabolic rate: $F_{1,31}$=0.091, *P*=0.765, Table 1). As expected, haematocrit of sham-operated fish was substantially higher than that of splenectomized fish following a period of enforced exercise (25.9±1.1% *vs.* 15.3±2.0%, $F_{1,31}$=24.901, *P*<0.001, Table 1), which coincided with a significantly higher maximum metabolic rate (164±7 mg O_2 kg^{-1} h^{-1} *vs.* 119±10 mg O_2 kg^{-1} h^{-1}, $F_{1,31}$=24.901, *P*<0.001, Table 1). Consequently, sham-operated fish had a significantly higher aerobic scope than splenectomized fish (t_{31}=-4.388, *P*<0.001, Fig 4). In addition, a statistically significant and strong positive correlation was observed between haematocrit and aerobic scope of instrumented bald notothens ($F_{1,31}$=38.517, *P*<0.001, Fig. 5A).

The interactions between spleen functionality and exercise state were not significant with regards to cardiac output, heart rate, stroke volume and total vascular resistance of instrumented bald notothens (Table 1). The main effects of the models revealed no significant differences between the groups with regards to these cardiovascular parameters (Table 1). As expected, exercise significantly increased cardiac output, heart rate, and stroke volume whilst decreasing the total vascular resistance in bald notothens (Table 1, Fig. 4).

Interactions between spleen functionality and exercise state were detected in the ventral aortic blood pressure and cardiac power index of bald notothens (Table 1). Analysis of simple main effects revealed no significant differences in the resting levels of these cardiovascular parameters (ventral aortic blood pressure: $F_{1,31}$=0.012, *P*=0.915, cardiac power index: $F_{1,27}$=0.006, *P*=0.940, Table 1). Yet, both ventral aortic blood pressure and cardiac power index were significantly elevated in the sham-operated fish when compared to splenectomized fish following exercise ($F_{1,31}$=4.379, *P*=0.045 and $F_{1,27}$=4.712, *P*=0.039, respectively, Table 1, Fig. 4). In addition, a statistically significant and strong positive correlation was observed between haematocrit and ventral aortic blood pressure in instrumented bald notothens ($F_{1,31}$=31.012, *P*<0.001, Fig. 5B).

Discussion

Extent of splenic blood boosting during exercise and feeding

Here we reveal the unprecedented extent to which the bald notothen can elevate their blood oxygen carrying capacity via the splenic blood boosting strategy when faced with metabolically demanding situations.

In a resting and unfed state, the haematocrit of uninstrumented fish (~9%) was lower than previously reported resting levels for this species (~15%) (Davison *et al.*, 1988; Franklin *et al.*, 1993). This is most likely due to the fact that fish in the present study were given a longer period of time to recover from capture and handling prior to experimentation (*i.e.* >1 week *vs.* 48 h in the other studies) (Davison *et al.*, 1988; Franklin *et al.*, 1993; Wells *et al.*, 1984). When subjected to a period of enforced exercise, the haematocrit of these fish substantially increased from ~9% to ~27%. Thus, in response to enforced exercise, the bald notothen are able to increase their blood oxygen carrying capacity by ~207%, which is higher than previously reported exercise-induced increases of 110-136% for the bald notothen (Davison *et al.*, 1988; Franklin *et al.*, 1993). The exercise-induced elevation in the blood oxygen carrying capacity of bald notothens is also substantially higher than the relative increases reported in other notothenioid species in response to exercise, heat stress and hypoxia (*i.e.* ~-5-76%, Davison, 2001; Davison and Franklin, 1994; Davison *et al.*, 1995; Egginton *et al.*, 1991; Forster *et al.*, 1998), as well as the exercise-induced increases typically reported for other vertebrates (*i.e.* ~40-60%, Hannon *et al.*, 1985; Hurford *et al.*, 1996; Perrson *et al.*, 1973a,b; Turner and Hodgetts, 1959; Wells and Weber, 1990; Yamamoto *et al.*, 1980; Zapol, 1987).

We also demonstrate that the bald notothen utilizes the splenic blood boosting strategy to meet the cardiorespiratory demands associated with the ingestion and processing of a meal (Sandblom *et al.*, 2012; Secor, 2009), as fed fish in a resting state had a higher haematocrit than unfed fish (*i.e.* ~20% *vs.* ~9%). Thus, to match the moderate and prolonged periods of energetic demand associated with processing a meal (Sandblom *et al.*, 2012; Secor, 2009), bald notothens are able to increase blood oxygen carrying capacity by ~131% to reach an intermediate level. Interestingly, in response to enforced exercise, fed fish were still able to further increase their haematocrit to reach a level that was not significantly different to that of unfed, exercised fish (*i.e.* ~26-28%). This indicates that bald notothens in a postprandial state (*i.e.* following food ingestion)

still retain enough scope to further increase blood oxygen carrying capacity for other metabolically demanding situations (*e.g.* when escaping predators) (Axelsson *et al.*, 1992; Buckley *et al.*, 2014; Franklin *et al.*, 1993, Gallaugher *et al.*, 1995).

The rapid exercise-induced increase in the haematocrit of bald notothens in the present study was clearly due to the substantial recruitment of erythrocytes from the spleen (Nilsson *et al.*, 1996), as spleen masses were 41% lower in fish subjected to a period of enforced exercise when compared to fish in a resting state (demonstrating that the spleen has contracted and ejected erythrocytes out into the circulation). Although the substantial splenic release of erythrocytes is mainly responsible for the elevated haematocrit in the bald notothen following exercise, previous studies have shown that factors such as significant decreases in mean cell haemoglobin concentration (an indicator for cell swelling) and slight increases in plasma chloride concentrations (an indicator for haemoconcentration as water moves out of the plasma) can also play minor roles (Franklin *et al.*, 1993; Wells *et al.*, 1984). Interestingly, although feeding clearly induced an increase in the haematocrit of bald nothothens, absolute spleen masses in fed fish were not significantly lower than unfed fish (*i.e.* ~0.28 g *vs.* 0.31 g, respectively, for a 74 g fish). Although the underlying reason for this finding currently remains unknown and further research is warranted, it could be speculated that erythrocytes were ejected into the circulation in response to feeding, after which the spleen may have refilled with plasma during the 24 h between feeding and sampling.

Prior to determining the cardiorespiratory pros and cons of splenic blood boosting in the bald notothen, we compared the haematocrit and metabolic rates of uninstrumented fish with sham-operated fish. It was observed that resting haematocrit was significantly lower in uninstrumented fish when compared with sham-operated fish, which was most likely due to the stress-induced release of erythrocytes that commonly occurs during anaesthesia and surgery (Wells *et al.*, 1984). However, even though significant differences in resting haematocrit were observed, no significant differences in resting or maximum metabolic rates were observed between the two groups, which indicates that surgical instrumentation did not have a detrimental impact on the aerobic scope of these fish (Clark *et al.*, 2013). Yet, it should be noted that resting metabolic rates (~71 mg O_2 kg^{-1} h^{-1} for a 104 g fish) reported in the present study are higher than previously reported values for similarly sized bald notothens (~35-50 mg O_2 kg^{-1} h^{-1}, Forster *et al.*, 1987; Robinson and Davison, 2008). This is most likely related to the fact that the resting metabolic rate for each individual in the present study

could only be determined from a single M_{O_2} measurement taken directly before the period of enforced exercise (due to our limited access to fibre-optic oxygen meters at the Crary Lab in McMurdo Station during the busy research season) instead of over a longer period of time as recommended in best practice guides (Clark *et al.*, 2013). Despite the abovementioned differences in resting metabolic rates, the maximum metabolic rates induced by our enforced exercise protocol (~164 mg O_2 kg^{-1} h^{-1} for a 104 g fish), as well as absolute aerobic scope (~92 mg O_2 kg^{-1} h^{-1} for a 104 g fish) fell within the range of previously reported values for similarly sized bald notothens exercised in a swim tunnel (~125-195 mg O_2 kg^{-1} h^{-1} and ~91-146 mg O_2 kg^{-1} h^{-1}, respectively, Forster *et al.*, 1987; Robinson and Davison, 2008).

Cardiorespiratory pros and cons of splenic blood boosting

The present study is the first to demonstrate the substantially enhanced aerobic scope that splenic blood boosting provides for bald nothothens, as well as the significant cardiovascular trade-offs associated with the strategy.

As expected, the haematocrit in sham-operated fish was substantially higher than that of splenectomized fish following a period of enforced exercise (~26% *vs.* ~15%). Consequently, the blood oxygen carrying capacity of sham-operated fish was ~69% higher than splenectomised fish, which coincided with the former being able to increase their metabolic rate to a far greater extent than the latter. Since systemic oxygen delivery is the product of cardiac output and blood oxygen content, it seems that the average difference in aerobic scope between sham-operated and splenectomized fish (~103%) is largely due to the substantial increase in blood oxygen content (via the splenic release of erythrocytes), as no significant differences were observed in cardiac output (Axelsson, 2005; Farrell, 1991). Indeed, in the present study, haematocrit levels of bald notothens following a period of enforced exercise explained 55% of the variation in aerobic scope.

In humans, the performance enhancing benefits of blood doping are well documented, as increases in blood oxygen carrying capacity of up to 25% can increase maximum oxygen uptake by up to 13% and significantly improve performance times on treadmill tests, cross-county skiing, long-distance running and cycling (Momaya *et al.*, 2015; Tokish *et al.*, 2004). In fish, by experimentally altering the blood oxygen carrying capacity of rainbow trout (*Oncorhynchus mykiss*), it was demonstrated that

swimming performance and maximum oxygen uptake was lowest in aneamic individuals (*i.e.* haematocrit of 12-17%) and highest in polycythaemic individuals (*i.e.* haematocrit of 47-48%) (Gallaugher *et al.*, 1995). Based on the collective findings from the present study and Franklin *et al.* (1993), the substantially enhanced aerobic scope of bald notothens following splenic blood boosting clearly provides performance enhancing benefits during metabolically demanding activities, as splenectomised individuals were unable to complete an exercise regime and fatigued twice as fast as sham-operated individuals due to their reduced capacity for systemic oxygen delivery.

However, the abovementioned ecophysiological benefits of the splenic blood boosting strategy in the bald notothen are also associated with some cardiovascular trade-offs, which may underlie why these animals only utilize this strategy during instances of elevated metabolic demand. On average, ventral aortic blood pressure of sham-operated fish was ~12% higher than splenectomized fish in response to enforced exercise. Since no significant differences in cardiac output or total vascular resistance were observed between the two groups following enforced exercise, it seems that the increase in ventral aortic blood pressure is largely due to the increased blood viscosity associated with the substantial elevation in circulating erythrocytes (Axelsson, 2005; Farrell, 1991; Graham and Fletcher, 1985). Indeed, in the present study, haematocrit levels of bald notothens following a period of enforced exercise explained 50% of the variation in ventral aortic blood pressure. Consequently, cardiac power index (an estimate of cardiac workload) following enforced exercise was ~30% higher in sham-operated fish. Since cardiac metabolism is directly related to cardiac workload, our findings indicate that there is a considerable energetic cost associated with transporting the highly viscous blood through the cardiovascular system following the splenic release of erythrocytes (Axelsson, 2005; Farrell, 1991; Sidell and Driedzic, 2012). Finally, although not thoroughly investigated in fish, the adverse effects of pumping increasingly viscous blood around the body in humans are well documented (Momaya *et al*., 2015; Tokish *et al*., 2004), and thus it may, at least in part, explain why other Antarctic fish species do not utilize this strategy to the same extent as bald notothens.

Conclusions

The present study is the first to demonstrate the substantial metabolic benefits, as well as the cardiovascular trade-offs, associated with the splenic blood boosting strategy of bald notothens inhabiting the thermally extreme Antarctic marine environment. The advantage of this remarkable strategy is that these fish are able to utilize a reserve of oxygen carrying capacity only when it is most beneficial, which undoubtedly improves a range of aerobic performance traits during metabolically demanding situations whilst minimizing the energetic and physiological costs associated with pumping highly viscous blood around the body during times of reduced oxygen demand. This in turn should incur a selective advantage for this species and may underlie their success in the predator rich, yet seasonally productive and relatively underutilized cryopelagic zone of Antarctica.

Competing interests

The authors declare they have no competing interests.

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Data availability

All data supporting the paper is readily available in the supplementary information (see S1-5), and for any additional correspondence or requests for materials email albin.grans@slu.se.

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Figures

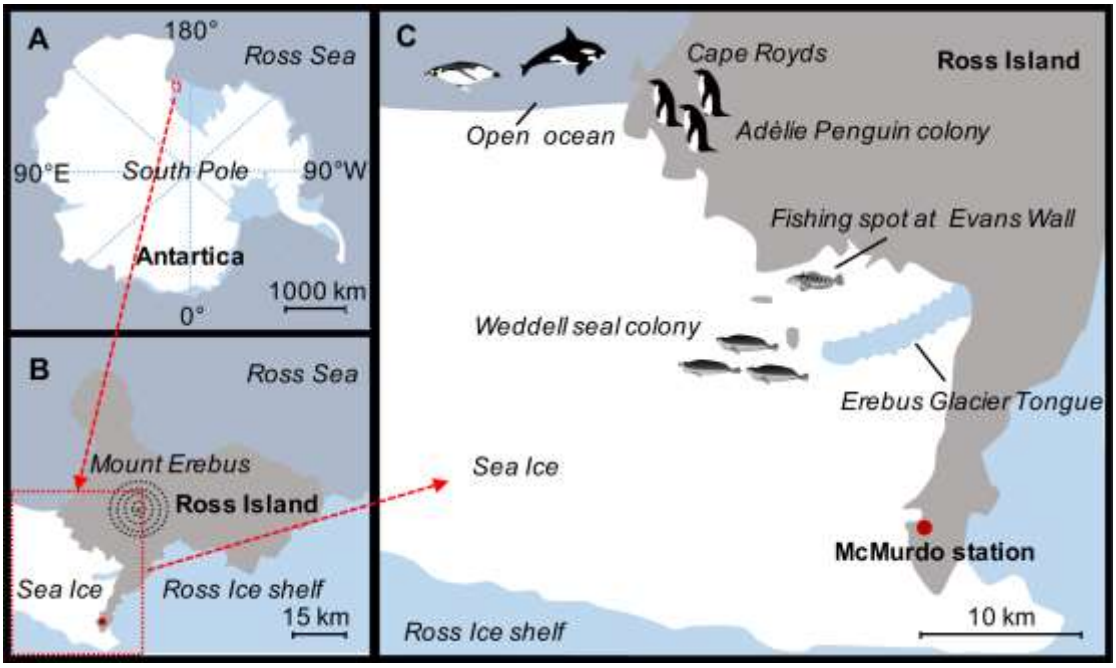

Figure 1. The Antarctic oceans constitute some of the most thermally extreme and challenging environments that marine teleosts have adapted to. Where the Ross Sea (A) connects with the Ross Ice Shelf (B) via the McMurdo Sound (C) lies McMurdo Station (77°51′S, 166°40′E). Bald nothothens used for the experiments were caught from the sea ice ~20 km from the station near Evans Wall.

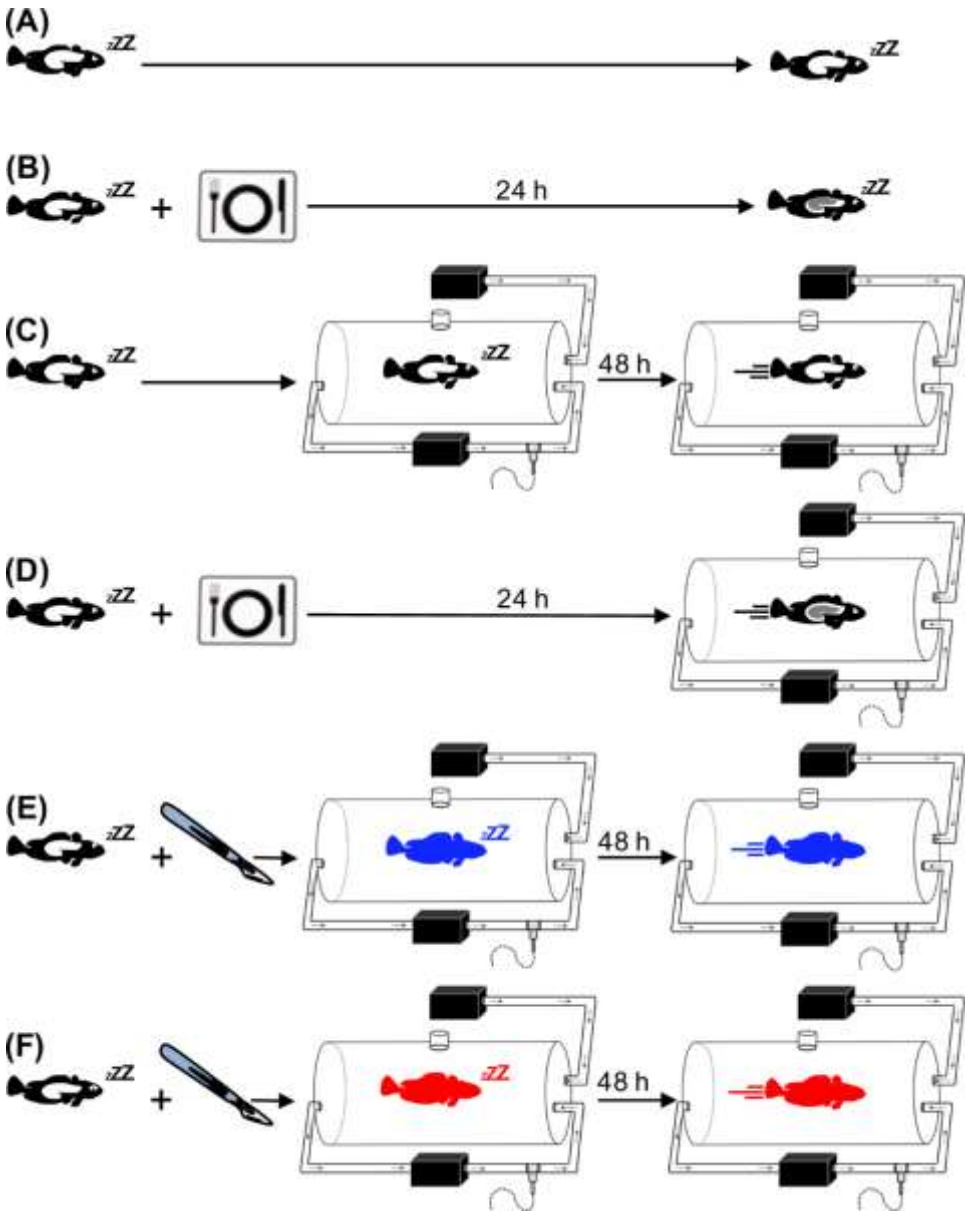

Figure 2. Overview of the varying experimental protocols performed on uninstrumented and instrumented bald notothens. (A-D) Haematocrit and relative spleen mass was determined in uninstrumented bald notothens after being (A) fasted for 7 days and then directly captured from the holding tank ('unfed resting fish', n=9), (B) directly captured from the holding tank 24 h after the ingestion of a meal ('fed resting fish', n=13), (C) fasted for 7 days, captured and placed in a respirometer, and then left undisturbed for 48 h prior to determining resting and maximum $\dot{M}O_2$ before and after a period of enforced exercise, respectively ('unfed exercised fish', n=9), and (D) captured from the holding tank 24 h after the ingestion of a meal, placed in a respirometer, and immediately subjected to a period of enforced exercise to determine

maximum M_{O_2} ('fed exercised fish', n=9). (E-F) Fish were captured from the holding tank and surgically instrumented with a blood flow probe and catheter in order to determine cardiac output, heart rate, stroke volume, ventral aortic blood pressure, cardiac power index, as well as to withdraw blood samples without disturbing the fish. In addition, fish were either (E) splenectomised to prevent splenic blood boosting in response to enforced exercise (n=14) or (F) sham-operated (n=19). Fish from both treatments (E-F) were then placed in respirometers and left undisturbed for 48 h to recover prior to determining resting and maximum levels of the cardiorespiratory variables before and after a period of enforced exercise, respectively.

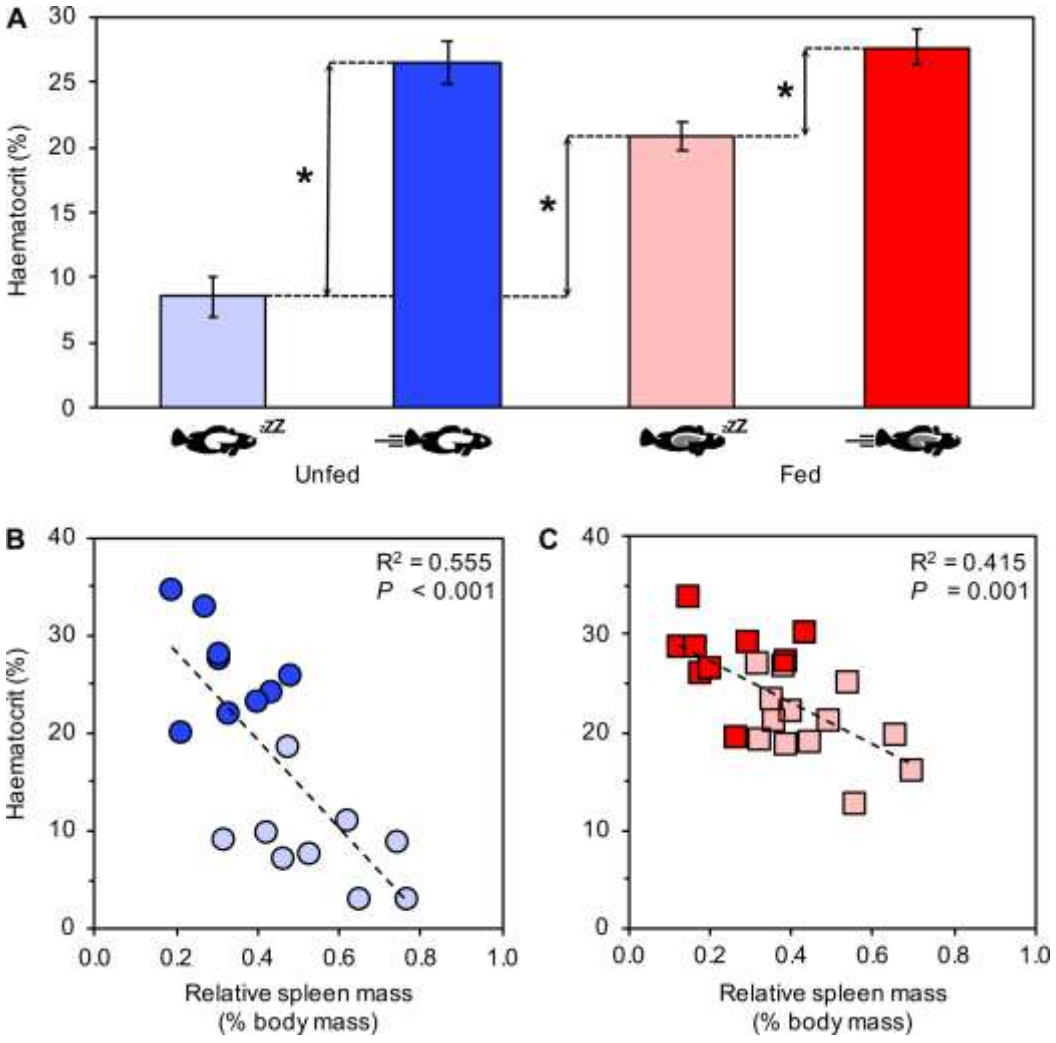

Figure 3. Blood oxygen carrying capacity of the bald notothen (*P. borchgrevinki*) is substantially elevated due to splenic release of erythrocytes in response to enforced exercise and ingestion of a meal. (A) Haematocrit levels of 'unfed, resting fish' (light blue bar, n=9), 'unfed, exercised fish' (dark blue bar, n=9), 'fed, resting fish' (light red bar, n=13), and 'fed, exercised fish' (dark red bar, n=9). Data in (A) are presented as means±s.e.m. and were analysed using a two-way ANOVA. Due to the significant interaction between the fixed factors ($F_{1,38}$=11.785, P=0.001), an analysis of the simple main effects was performed with * indicating significant differences between the respective groups (P<0.05). (B-C) Simple linear regressions displaying the significant relationships between relative spleen mass and haematocrit in (B) unfed (blue circles, light blue = resting, dark blue = exercised) and (C) fed fish (red squares, light red = resting, dark red = exercised). Raw data used to compile Fig. 3 can be found in the supplementary information (Dataset S1).

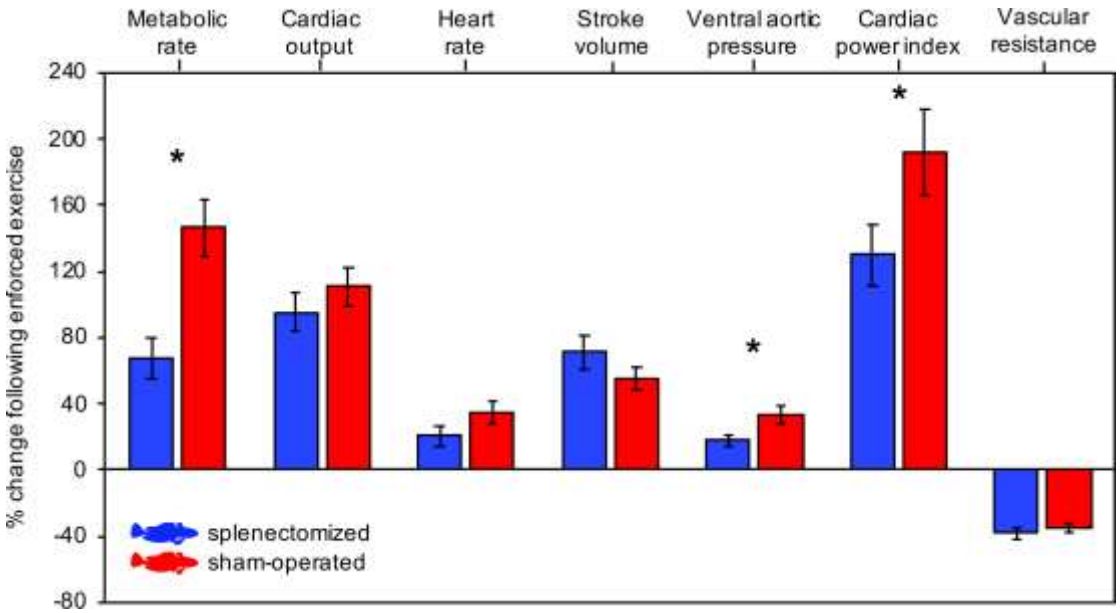

Figure 4. The rapid splenic release of erythrocytes by the bald notothen (*P. borchgrevinki*) in response to enforced exercise allows for a substantially greater increase in metabolic rate at the cost of an increased blood pressure and cardiac workload. Relative changes in metabolic and cardiovascular parameters in splenectomised (blue bars, n=14) and sham-operated individuals (red bars, n=19) following a period of enforced exercise. For illustrative purposes, the figure displays the relative changes that occur in each parameter, however, the statistical analyses were performed on the absolute changes in each parameter (*i.e.* absolute scope = absolute value after enforced exercise – absolute value prior to enforced exercise). Statistical differences between treatments were analysed using independent samples t-tests for each dependent variable and significant differences (P<0.05) between groups are indicated with * (*i.e.* metabolic rate: t_{31}=-4.388, p<0.001, ventral aortic blood pressure: t_{31}=-2.239, p=0.032, and cardiac power index: $t_{25.634}$=-2.926, p=0.007). Data are presented as means±s.e.m. Raw data used to compile Fig. 4 can be found in the supplementary information (Dataset S2-S5).

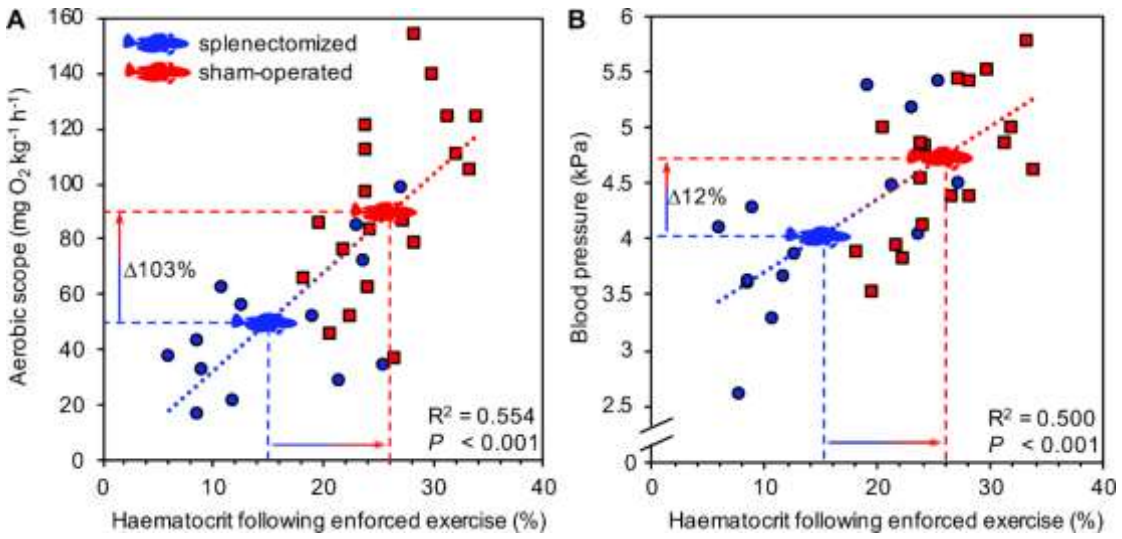

Figure 5. The aerobic benefit and cardiovascular compromise of the substantial increase in blood oxygen carrying capacity of the bald notothen (*P. borchgrevinki*) in response to exercise. Simple linear regressions displaying the significant relationships between haematocrit levels following a period of enforced exercise and (A) aerobic scope and (B) blood pressure in instrumented individuals (blue circles = splenectomised, n=14, red squares = sham operated, n=19). The blue and red fish represent the mean levels for both the x and y variables in each panel. Raw data used to compile Fig. 5 can be found in the supplementary information (Dataset S2-S3).

Table

Table 1. Cardiorespiratory implications of splenic blood boosting in the bald notothen (*P. borchgrevinki*) following a period of enforced exercise. Haematocrit (%), metabolic rate (mg O_2 kg^{-1} h^{-1}), cardiac output (arbitrary units/a.u.), heart rate (beats min^{-1}), stroke volume (a.u.), ventral aortic blood pressure (kPa), vascular resistance (a.u.) and cardiac power index (a.u.) of sham-operated and splenectomized bald notothens before and after a period of enforced exercise.

Variable	Treatment	Resting		Enforced exercise	Statistical summary
Haematocrit	Sham-operated (n=19)	16.1±1.0	*	25.9±1.1 †	**I: F1,31=26.838, *P*<0.001**
	Splenectomized (n=14)	13.1±1.6	*	15.3±2.0	
Metabolic rate	Sham-operated (n=19)	71±4	*	164±7 †	**I: F1,31=19.257, *P*<0.001**
	Splenectomized (n=14)	73±7	*	119±10	
Cardiac output	Sham-operated (n=16)	0.6±0.0	*	1.2±0.1	I: F1,27=0.871, *P*=0.359
					WS: F1,27=278.692, *P*<0.001
	Splenectomized (n=13)	0.6±0.1	*	1.1±0.1	BS: F1,27=0.002, *P*=0.965
Heart rate	Sham-operated (n=18)	19.5±0.9	*	25.3±0.5	I: F1,31=3.113, *P*=0.088
					WS: F1,31=69.225, *P*<0.001
	Splenectomized (n=15)	21.2±0.8	*	24.9±0.4	BS: F1,31=0.545, *P*=0.466
Stroke volume	Sham-operated (n=18)	0.03±0.00	*	0.05±0.00	I: F1,27=0.219, *P*=0.644
					WS: F1,27=195.184, *P*<0.001
	Splenectomized (n=15)	0.03±0.00	*	0.05±0.00	BS: F1,27=0.535, *P*=0.471
Ventral aortic pressure	Sham-operated (n=16)	3.6±0.1	*	4.7±0.1 †	**I: F1,31=5.012, *P*=0.032**
	Splenectomized (n=13)	3.5±0.2	*	4.2±0.2	
Vascular resistance	Sham-operated (n=16)	6.6±0.6	*	4.2±0.4	I: F1,27=0.067, *P*=0.798
					WS: F1,27=73.547, *P*<0.001
	Splenectomized (n=13)	6.2±0.7	*	3.6±0.3	BS: F1,27=0.549, *P*=0.465
Cardiac power index	Sham-operated (n=16)	2.1±0.2	*	5.7±0.5 †	**I: F1,27=3.273, *P*=0.082**
	Splenectomized (n=13)	2.1±0.3	*	4.4±0.3	

Cardiorespiratory data were analysed using two-way mixed ANOVAs. If an interaction (I) was detected (via significant interaction term or by visual inspection of means plot), then analyses for the simple main effects were performed with * indicating a significant within-subjects difference (*i.e.* resting *vs.* enforced exercise), while † indicates a significant between-subjects difference (*i.e.* sham-operated *vs.* splenectomized). F- and *P*-statistics for simple main effects are stated in the main text. If no significant interaction was detected, then the main effects of the model were reported (WS=within-subjects effect, *; BS=between-subjects effect, †). Raw data used to compile Table 1 can be found in the supplementary information (Dataset S2-S5).

Dataset S1: Body mass, haematocrit (Hct), absolute spleen mass, relative spleen mass (RSM) and whole animal oxygen uptake (MO_2) of uninstrumented unfed and fed bald notothens during rest and following enforced exercise.

Treatment	ID	Mass	Hct	Spleen mass	RSM	Routine MO_2	Max MO_2	Scope MO_2
		(g)	(%)	(g)	(% body mass)	(mg O_2 kg^{-1} h^{-1})	(mg O_2 kg^{-1} h^{-1})	(mg O_2 kg^{-1} h^{-1})
Unfed-Rest	1	59.3	9.0	0.19	0.32			
Unfed-Rest	2	47.1	8.7	0.35	0.74			
Unfed-Rest	3	84.4	2.9	0.65	0.77			
Unfed-Rest	4	45	9.8	0.19	0.42			
Unfed-Rest	5	76	7.5	0.4	0.53			
Unfed-Rest	6	32	11.0	0.2	0.63			
Unfed-Rest	7	54.4	18.6	0.26	0.48			
Unfed-Rest	8	55.3	3.1	0.36	0.65			
Unfed-Rest	9	41	7.0	0.19	0.46			
Unfed-Exercise	10	97	24.1	0.42	0.43	36.30	226.72	190.42
Unfed-Exercise	11	106.2	25.9	0.51	0.48	44.51	204.50	159.99
Unfed-Exercise	12	97	20.0	0.21	0.22	54.11	167.80	113.69
Unfed-Exercise	13	85	27.6	0.26	0.31	54.14	171.83	117.69
Unfed-Exercise	14	67	21.9	0.22	0.33	73.08	199.23	126.14
Unfed-Exercise	15	97.1	28.1	0.3	0.31	54.05	97.83	43.79
Unfed-Exercise	16	92.3	23.1	0.37	0.40	67.04	113.17	46.13
Unfed-Exercise	17	73	32.8	0.2	0.27	136.65	224.69	88.04
Unfed-Exercise	18	89	34.5	0.17	0.19	68.10	204.30	136.20
Fed-Rest	19	56.5	16.1	0.39	0.70			
Fed-Rest	20	115.4	26.7	0.44	0.38			
Fed-Rest	21	73.8	21.0	0.36	0.49			
Fed-Rest	22	109.3	22.0	0.44	0.40			
Fed-Rest	23	49.3	19.2	0.16	0.32			
Fed-Rest	24	88.1	27.0	0.28	0.32			
Fed-Rest	25	120.34	19.6	0.79	0.66			
Fed-Rest	26	56.4	23.2	0.20	0.35			
Fed-Rest	27	49.83	18.7	0.19	0.39			
Fed-Rest	28	65.8	12.5	0.37	0.56			
Fed-Rest	29	113.6	25.0	0.62	0.54			
Fed-Rest	30	111.7	21.0	0.40	0.36			
Fed-Rest	31	120.76	19.0	0.54	0.44			
Fed-Exercise	34	60.2	30.1	0.26	0.43		273.58	
Fed-Exercise	35	49.6	28.7	0.06	0.12		229.81	
Fed-Exercise	36	60.7	19.4	0.16	0.26		226.99	
Fed-Exercise	37	74.2	25.9	0.13	0.18		262.46	
Fed-Exercise	38	60.6	28.6	0.1	0.17		258.43	
Fed-Exercise	39	61.3	33.8	0.09	0.15		244.46	
Fed-Exercise	40	59	27.1	0.23	0.39		224.54	
Fed-Exercise	41	64.5	29.2	0.19	0.29		196.70	
Fed-Exercise	42	39.9	26.4	0.08	0.20		256.04	

Dataset S2: Body mass, haematocrit (Hct), absolute spleen mass, relative spleen mass (RSM) and whole animal oxygen uptake (MO_2) of instrumented unfed bald notothens during rest and following enforced exercise.

Treatment (1=Splct, 2=Sham)	ID	Body mass (g)	Hct Control (%)	Hct Stress (%)	Spleen mass (g)	RSM (% body mass)	Routine MO_2 (mg O_2 kg^{-1} h^{-1})	Max MO_2 (mg O_2 kg^{-1} h^{-1})	Scope MO_2 (mg O_2 kg^{-1} h^{-1})	Scope MO_2 (%)
1	43	110	7.69	8.59	0.27	0.25	82.39	99.23	16.84	20.44
1	44	89	22.22	25.42	0.21	0.24	63.61	98.04	34.42	54.12
1	45	154	23.73	27.12	0.34	0.22	62.25	161.34	99.09	159.18
1	46	132	11.28	11.71	1.30	0.98	113.95	135.85	21.89	19.21
1	47	106	16.39	23.62	0.46	0.43	68.12	140.61	72.49	106.42
1	48	122	10.77	12.60	0.72	0.59	65.36	121.53	56.18	85.95
1	49	111.4	8.73	8.96	0.45	0.40	88.44	121.68	33.24	37.58
1	50	99.1	17.39	19.08	0.20	0.20	135.99	188.25	52.25	38.42
1	51	98.6	5.38	5.97	0.54	0.55	49.16	86.87	37.71	76.71
1	52	97	15.87	21.37	0.21	0.22	50.00	78.76	28.77	57.53
1	53	98	6.06	10.77	0.44	0.45	68.45	130.79	62.35	91.09
1	54	96	9.16	7.69	0.40	0.42	42.23	40.84	-1.38	-3.28
1	55	86.6	10.77	8.53	0.21	0.24	71.58	114.69	43.10	60.22
1	56	89.3	17.91	23.08	0.54	0.60	58.92	143.94	85.02	144.30
2	57	123.8	14.93	24.04	0.99	0.80	93.10	155.87	62.77	67.43
2	58	113.1	15.09	27.27	0.25	0.22	66.03	152.51	86.48	130.97
2	59	98.3	14.66	33.33	0.67	0.68	58.77	164.16	105.38	179.31
2	60	107.4	15.38	26.53	0.21	0.20	80.76	117.75	36.99	45.80
2	61	110	9.30	20.55	0.16	0.14	96.83	142.53	45.71	47.20
2	62	99	12.90	18.18	0.28	0.28	98.58	164.30	65.72	66.67
2	63	105	21.31	23.81	0.33	0.31	48.59	145.78	97.18	200.00
2	64	136.5	22.14	28.23	0.42	0.31	82.17	160.97	78.80	95.91
2	65	124	15.87	23.81	0.65	0.52	47.27	168.36	121.09	256.18
2	66	107	17.65	22.39	0.47	0.44	86.02	138.01	51.99	60.43
2	67	119	14.93	32.01	0.38	0.32	55.99	166.85	110.86	198.02
2	68	97	15.08	19.53	0.61	0.63	48.63	134.92	86.30	177.46
2	69	87	7.69	21.74	0.24	0.28	54.39	131.00	76.61	140.85
2	70	89	13.04	31.25	0.37	0.42	59.12	183.35	124.23	210.13
2	71	87.3	16.67	24.29	0.31	0.36	72.52	156.49	83.97	115.79
2	72	89	13.24	28.26	0.16	0.19	74.09	228.25	154.16	208.08
2	73	77.4	19.40	23.91	0.15	0.19	85.51	197.80	112.29	131.31
2	74	74	22.64	33.91	0.32	0.43	87.73	212.54	124.81	142.27
2	75	84	23.08	29.77	0.14	0.17	45.27	185.05	139.78	308.77

Dataset S3: Cardiac output (CO) and ventral aortic blood pressure (VAP) of instrumented unfed bald notothens during rest and following enforced exercise.

Treatment (1=Splct, 2=Sham)	ID	Routine CO (a.u.)	Max CO (a.u.)	Scope CO (a.u.)	Scope CO (%)	Routine VAP (kPa)	Max VAP (kPa)	Scope VAP (kPa)	Scope VAP (%)
1	43	1.26	1.37	0.10	8.03	3.54	3.61	0.07	2.11
1	44					5.11	5.42	0.31	6.13
1	45	0.56	1.06	0.49	87.74	4.19	4.50	0.31	7.37
1	46	0.70	1.27	0.57	80.89	3.37	3.67	0.30	8.90
1	47	0.27	0.73	0.46	167.97	3.16	4.06	0.90	28.50
1	48	0.63	1.12	0.49	76.99	3.33	3.87	0.54	16.31
1	49	0.39	0.92	0.53	137.70	3.17	4.29	1.12	35.26
1	50					3.77	5.39	1.62	42.90
1	51	0.70	1.39	0.69	98.03	3.56	4.11	0.55	15.49
1	52	0.68	1.23	0.55	80.47	3.24	4.48	1.24	38.12
1	53	0.57	1.31	0.74	131.10	3.23	3.29	0.06	1.86
1	54	0.79	1.37	0.57	72.16	2.48	2.62	0.14	5.72
1	55	0.45	0.87	0.42	94.62	3.52	3.64	0.12	3.49
1	56	0.51	1.07	0.55	108.32	3.82	5.18	1.36	35.45
2	57	0.82	1.61	0.80	97.27	3.20	4.13	0.93	29.20
2	58	0.78	1.60	0.82	105.04	3.17	5.45	2.28	71.96
2	59	0.45	1.14	0.70	156.76	3.33	5.78	2.45	73.59
2	60	0.54	1.13	0.59	108.67	3.01	4.38	1.37	45.70
2	61	0.63	1.11	0.48	76.66	3.95	5.00	1.04	26.32
2	62					3.76	3.89	0.13	3.41
2	63	0.55	1.05	0.50	91.12	3.76	4.76	1.00	26.73
2	64	0.59	1.52	0.93	156.95	3.12	5.42	2.30	73.93
2	65					3.46	4.54	1.08	31.26
2	66	0.46	0.99	0.52	113.42	3.57	3.83	0.26	7.33
2	67	1.21	2.00	0.79	65.08	3.67	5.01	1.34	36.56
2	68	0.67	1.43	0.76	114.18	2.59	3.52	0.94	36.14
2	69	0.53	0.94	0.41	76.97	3.34	3.95	0.61	18.28
2	70	0.36	1.26	0.90	248.74	2.98	4.86	1.88	63.13
2	71	0.65	1.38	0.73	111.51	3.50	4.84	1.35	38.56
2	72	0.57	1.19	0.62	108.08	3.83	4.39	0.56	14.76
2	73	0.34	0.87	0.53	156.33	4.22	4.87	0.65	15.53
2	74	0.50	0.79	0.29	58.20	4.43	4.62	0.19	4.28
2	75	0.45	0.65	0.20	43.68	4.68	5.52	0.83	17.75

Dataset S4: Heart rate (HR) and stroke volume (SV) of instrumented unfed bald notothens during rest and following enforced exercise.

Treatment (1=Splct, 2=Sham)	ID	Routine HR (beats min^{-1})	Max HR (beats min^{-1})	Scope HR (beats min^{-1})	Scope HR (%)	Routine SV (a.u)	Max SV (a.u)	Scope SV (a.u)	Scope SV (%)
1	43	23.12	21.87	-1.25	-5.40	0.05	0.06	0.01	14.19
1	44	17.26	21.88	4.62	26.79				
1	45	21.32	26.63	5.31	24.91	0.03	0.04	0.01	50.30
1	46	23.20	25.52	2.32	10.01	0.03	0.05	0.02	64.42
1	47	24.12	25.96	1.84	7.62	0.01	0.03	0.02	149.00
1	48	20.73	26.16	5.43	26.21	0.03	0.04	0.01	40.23
1	49	21.04	27.64	6.60	31.39	0.02	0.03	0.01	80.91
1	50	12.74	24.16	11.42	89.60				
1	51	21.20	25.98	4.78	22.55	0.03	0.05	0.02	61.59
1	52	23.25	24.28	1.03	4.43	0.03	0.05	0.02	72.81
1	53	25.25	25.52	0.27	1.07	0.02	0.05	0.03	128.65
1	54	22.31	24.94	2.63	11.80	0.04	0.05	0.02	53.99
1	55	21.52	23.57	2.05	9.53	0.02	0.04	0.02	77.69
1	56	19.28	24.86	5.58	28.94	0.03	0.04	0.02	61.56
2	57	22.16	30.00	7.84	35.36	0.04	0.05	0.02	45.74
2	58	19.66	25.50	5.84	29.68	0.04	0.06	0.02	58.11
2	59	13.59	23.03	9.44	69.46	0.03	0.05	0.02	51.52
2	60	27.17	27.51	0.35	1.27	0.02	0.04	0.02	106.05
2	61	25.32	28.07	2.75	10.86	0.02	0.04	0.01	59.35
2	62	23.23	24.02	0.79	3.40				
2	63	11.90	20.59	8.69	73.00	0.05	0.05	0.00	10.47
2	64	19.07	24.02	4.95	25.95	0.03	0.06	0.03	104.01
2	65	20.83	25.81	4.98	23.92				
2	66	21.37	27.08	5.71	26.70	0.02	0.04	0.01	68.44
2	67	19.56	25.37	5.81	29.73	0.06	0.08	0.02	27.25
2	68	20.41	24.53	4.12	20.20	0.03	0.06	0.03	78.18
2	69	20.08	25.43	5.35	26.66	0.03	0.04	0.01	39.73
2	70	11.74	25.81	14.08	119.95	0.03	0.05	0.02	58.55
2	71	15.10	25.29	10.19	67.50	0.04	0.05	0.01	26.27
2	72	19.56	25.67	6.11	31.24	0.03	0.05	0.02	58.54
2	73	20.37	26.85	6.48	31.79	0.02	0.03	0.02	94.51
2	74	21.10	25.10	4.00	18.96	0.02	0.03	0.01	32.99
2	75	18.32	20.76	2.44	13.33	0.02	0.03	0.01	26.78

Dataset S5: Total vascular resistance (Rtot) and cardiac power index (CPI) of instrumented unfed bald notothens during rest and following enforced exercise.

Treatment (1=Splct, 2=Sham)	ID	Routine Rtot (a.u)	Max Rtot (a.u)	Scope Rtot (a.u)	Scope Rtot (%)	Routine CPI (a.u)	Max CPI (a.u)	Scope CPI (a.u)	Scope CPI (%)
1	43	2.80	2.65	-0.15	-5.48	4.47	4.93	0.46	10.31
1	44								
1	45	7.46	4.27	-3.19	-42.81	2.36	4.75	2.39	101.58
1	46	4.79	2.89	-1.91	-39.80	2.37	4.66	2.30	96.99
1	47	11.65	5.58	-6.06	-52.05	0.86	2.95	2.09	244.34
1	48	5.28	3.47	-1.81	-34.28	2.10	4.32	2.22	105.84
1	49	8.16	4.64	-3.52	-43.10	1.23	3.96	2.73	221.50
1	50								
1	51	5.09	2.97	-2.12	-41.68	2.49	5.71	3.21	128.70
1	52	4.75	3.63	-1.11	-23.47	2.21	5.52	3.31	149.27
1	53	5.70	2.51	-3.19	-55.92	1.83	4.31	2.48	135.40
1	54	3.12	1.92	-1.20	-38.59	1.97	3.59	1.62	82.01
1	55	7.84	4.17	-3.67	-46.83	1.58	3.18	1.60	101.40
1	56	7.47	4.86	-2.61	-34.98	1.96	5.52	3.57	182.17
2	57	3.91	2.56	-1.35	-34.51	2.61	6.66	4.05	154.88
2	58	4.06	3.41	-0.66	-16.13	2.47	8.72	6.25	252.59
2	59	7.48	5.06	-2.42	-32.39	1.48	6.61	5.13	345.71
2	60	5.58	3.89	-1.68	-30.18	1.62	4.93	3.31	204.03
2	61	6.32	4.52	-1.80	-28.50	2.47	5.52	3.05	123.15
2	62								
2	63	6.84	4.53	-2.30	-33.69	2.06	5.00	2.94	142.20
2	64	5.28	3.58	-1.71	-32.31	1.84	8.21	6.37	346.91
2	65								
2	66	7.74	3.89	-3.85	-49.71	1.65	3.78	2.13	129.07
2	67	3.03	2.50	-0.52	-17.28	4.44	10.02	5.57	125.44
2	68	3.87	2.46	-1.41	-36.43	1.73	5.05	3.32	191.59
2	69	6.31	4.22	-2.09	-33.16	1.77	3.71	1.94	109.32
2	70	8.27	3.87	-4.40	-53.22	1.07	6.11	5.04	468.90
2	71	5.35	3.50	-1.84	-34.49	2.29	6.70	4.41	193.07
2	72	6.67	3.68	-2.99	-44.85	2.20	5.24	3.05	138.80
2	73	12.40	5.59	-6.81	-54.93	1.43	4.25	2.81	196.13
2	74	8.89	5.86	-3.03	-34.08	2.21	3.65	1.44	64.98
2	75	10.38	8.51	-1.87	-18.05	2.11	3.57	1.46	69.19

Table S1: Body mass, haematocrit (Hct), absolute spleen mass, relative spleen mass (RSM) and whole animal oxygen uptake (MO_2) of uninstrumented unfed and fed bald notothens during rest and following enforced exercise.

Treatment	ID	Mass	Hct	Spleen mass	RSM	Routine MO_2	Max MO_2	Scope MO_2
		(g)	(%)	(g)	(% body mass)	(mg O_2 kg^{-1} h^{-1})	(mg O_2 kg^{-1} h^{-1})	(mg O_2 kg^{-1} h^{-1})
Unfed-Rest	1	59.3	9.0	0.19	0.32			
Unfed-Rest	2	47.1	8.7	0.35	0.74			
Unfed-Rest	3	84.4	2.9	0.65	0.77			
Unfed-Rest	4	45	9.8	0.19	0.42			
Unfed-Rest	5	76	7.5	0.4	0.53			
Unfed-Rest	6	32	11.0	0.2	0.63			
Unfed-Rest	7	54.4	18.6	0.26	0.48			
Unfed-Rest	8	55.3	3.1	0.36	0.65			
Unfed-Rest	9	41	7.0	0.19	0.46			
Unfed-Exercise	10	97	24.1	0.42	0.43	36.30	226.72	190.42
Unfed-Exercise	11	106.2	25.9	0.51	0.48	44.51	204.50	159.99
Unfed-Exercise	12	97	20.0	0.21	0.22	54.11	167.80	113.69
Unfed-Exercise	13	85	27.6	0.26	0.31	54.14	171.83	117.69
Unfed-Exercise	14	67	21.9	0.22	0.33	73.08	199.23	126.14
Unfed-Exercise	15	97.1	28.1	0.3	0.31	54.05	97.83	43.79
Unfed-Exercise	16	92.3	23.1	0.37	0.40	67.04	113.17	46.13
Unfed-Exercise	17	73	32.8	0.2	0.27	136.65	224.69	88.04
Unfed-Exercise	18	89	34.5	0.17	0.19	68.10	204.30	136.20
Fed-Rest	19	56.5	16.1	0.39	0.70			
Fed-Rest	20	115.4	26.7	0.44	0.38			
Fed-Rest	21	73.8	21.0	0.36	0.49			
Fed-Rest	22	109.3	22.0	0.44	0.40			
Fed-Rest	23	49.3	19.2	0.16	0.32			
Fed-Rest	24	88.1	27.0	0.28	0.32			
Fed-Rest	25	120.34	19.6	0.79	0.66			
Fed-Rest	26	56.4	23.2	0.20	0.35			
Fed-Rest	27	49.83	18.7	0.19	0.39			
Fed-Rest	28	65.8	12.5	0.37	0.56			
Fed-Rest	29	113.6	25.0	0.62	0.54			
Fed-Rest	30	111.7	21.0	0.40	0.36			
Fed-Rest	31	120.76	19.0	0.54	0.44			
Fed-Exercise	34	60.2	30.1	0.26	0.43		273.58	
Fed-Exercise	35	49.6	28.7	0.06	0.12		229.81	
Fed-Exercise	36	60.7	19.4	0.16	0.26		226.99	
Fed-Exercise	37	74.2	25.9	0.13	0.18		262.46	
Fed-Exercise	38	60.6	28.6	0.1	0.17		258.43	
Fed-Exercise	39	61.3	33.8	0.09	0.15		244.46	
Fed-Exercise	40	59	27.1	0.23	0.39		224.54	
Fed-Exercise	41	64.5	29.2	0.19	0.29		196.70	
Fed-Exercise	42	39.9	26.4	0.08	0.20		256.04	

Table S2: Body mass, haematocrit (Hct), absolute spleen mass, relative spleen mass (RSM) and whole animal oxygen uptake (MO_2) of instrumented unfed bald notothens during rest and following enforced exercise.

Treatment (1=Splct, 2=Sham)	ID	Body mass (g)	Hct Control (%)	Hct Stress (%)	Spleen mass (g)	RSM (% body mass)	Routine MO_2 (mg O_2 kg^{-1} h^{-1})	Max MO_2 (mg O_2 kg^{-1} h^{-1})	Scope MO_2 (mg O_2 kg^{-1} h^{-1})	Scope MO_2 (%)
1	43	110	7.69	8.59	0.27	0.25	82.39	99.23	16.84	20.44
1	44	89	22.22	25.42	0.21	0.24	63.61	98.04	34.42	54.12
1	45	154	23.73	27.12	0.34	0.22	62.25	161.34	99.09	159.18
1	46	132	11.28	11.71	1.30	0.98	113.95	135.85	21.89	19.21
1	47	106	16.39	23.62	0.46	0.43	68.12	140.61	72.49	106.42
1	48	122	10.77	12.60	0.72	0.59	65.36	121.53	56.18	85.95
1	49	111.4	8.73	8.96	0.45	0.40	88.44	121.68	33.24	37.58
1	50	99.1	17.39	19.08	0.20	0.20	135.99	188.25	52.25	38.42
1	51	98.6	5.38	5.97	0.54	0.55	49.16	86.87	37.71	76.71
1	52	97	15.87	21.37	0.21	0.22	50.00	78.76	28.77	57.53
1	53	98	6.06	10.77	0.44	0.45	68.45	130.79	62.35	91.09
1	54	96	9.16	7.69	0.40	0.42	42.23	40.84	-1.38	-3.28
1	55	86.6	10.77	8.53	0.21	0.24	71.58	114.69	43.10	60.22
1	56	89.3	17.91	23.08	0.54	0.60	58.92	143.94	85.02	144.30
2	57	123.8	14.93	24.04	0.99	0.80	93.10	155.87	62.77	67.43
2	58	113.1	15.09	27.27	0.25	0.22	66.03	152.51	86.48	130.97
2	59	98.3	14.66	33.33	0.67	0.68	58.77	164.16	105.38	179.31
2	60	107.4	15.38	26.53	0.21	0.20	80.76	117.75	36.99	45.80
2	61	110	9.30	20.55	0.16	0.14	96.83	142.53	45.71	47.20
2	62	99	12.90	18.18	0.28	0.28	98.58	164.30	65.72	66.67
2	63	105	21.31	23.81	0.33	0.31	48.59	145.78	97.18	200.00
2	64	136.5	22.14	28.23	0.42	0.31	82.17	160.97	78.80	95.91
2	65	124	15.87	23.81	0.65	0.52	47.27	168.36	121.09	256.18
2	66	107	17.65	22.39	0.47	0.44	86.02	138.01	51.99	60.43
2	67	119	14.93	32.01	0.38	0.32	55.99	166.85	110.86	198.02
2	68	97	15.08	19.53	0.61	0.63	48.63	134.92	86.30	177.46
2	69	87	7.69	21.74	0.24	0.28	54.39	131.00	76.61	140.85
2	70	89	13.04	31.25	0.37	0.42	59.12	183.35	124.23	210.13
2	71	87.3	16.67	24.29	0.31	0.36	72.52	156.49	83.97	115.79
2	72	89	13.24	28.26	0.16	0.19	74.09	228.25	154.16	208.08
2	73	77.4	19.40	23.91	0.15	0.19	85.51	197.80	112.29	131.31
2	74	74	22.64	33.91	0.32	0.43	87.73	212.54	124.81	142.27
2	75	84	23.08	29.77	0.14	0.17	45.27	185.05	139.78	308.77

Table S3: Cardiac output (CO) and ventral aortic blood pressure (VAP) of instrumented unfed bald notothens during rest and following enforced exercise.

Treatment (1=Splct, 2=Sham)	ID	Routine CO (a.u.)	Max CO (a.u.)	Scope CO (a.u.)	Scope CO (%)	Routine VAP (kPa)	Max VAP (kPa)	Scope VAP (kPa)	Scope VAP (%)
1	43	1.26	1.37	0.10	8.03	3.54	3.61	0.07	2.11
1	44					5.11	5.42	0.31	6.13
1	45	0.56	1.06	0.49	87.74	4.19	4.50	0.31	7.37
1	46	0.70	1.27	0.57	80.89	3.37	3.67	0.30	8.90
1	47	0.27	0.73	0.46	167.97	3.16	4.06	0.90	28.50
1	48	0.63	1.12	0.49	76.99	3.33	3.87	0.54	16.31
1	49	0.39	0.92	0.53	137.70	3.17	4.29	1.12	35.26
1	50					3.77	5.39	1.62	42.90
1	51	0.70	1.39	0.69	98.03	3.56	4.11	0.55	15.49
1	52	0.68	1.23	0.55	80.47	3.24	4.48	1.24	38.12
1	53	0.57	1.31	0.74	131.10	3.23	3.29	0.06	1.86
1	54	0.79	1.37	0.57	72.16	2.48	2.62	0.14	5.72
1	55	0.45	0.87	0.42	94.62	3.52	3.64	0.12	3.49
1	56	0.51	1.07	0.55	108.32	3.82	5.18	1.36	35.45
2	57	0.82	1.61	0.80	97.27	3.20	4.13	0.93	29.20
2	58	0.78	1.60	0.82	105.04	3.17	5.45	2.28	71.96
2	59	0.45	1.14	0.70	156.76	3.33	5.78	2.45	73.59
2	60	0.54	1.13	0.59	108.67	3.01	4.38	1.37	45.70
2	61	0.63	1.11	0.48	76.66	3.95	5.00	1.04	26.32
2	62					3.76	3.89	0.13	3.41
2	63	0.55	1.05	0.50	91.12	3.76	4.76	1.00	26.73
2	64	0.59	1.52	0.93	156.95	3.12	5.42	2.30	73.93
2	65					3.46	4.54	1.08	31.26
2	66	0.46	0.99	0.52	113.42	3.57	3.83	0.26	7.33
2	67	1.21	2.00	0.79	65.08	3.67	5.01	1.34	36.56
2	68	0.67	1.43	0.76	114.18	2.59	3.52	0.94	36.14
2	69	0.53	0.94	0.41	76.97	3.34	3.95	0.61	18.28
2	70	0.36	1.26	0.90	248.74	2.98	4.86	1.88	63.13
2	71	0.65	1.38	0.73	111.51	3.50	4.84	1.35	38.56
2	72	0.57	1.19	0.62	108.08	3.83	4.39	0.56	14.76
2	73	0.34	0.87	0.53	156.33	4.22	4.87	0.65	15.53
2	74	0.50	0.79	0.29	58.20	4.43	4.62	0.19	4.28
2	75	0.45	0.65	0.20	43.68	4.68	5.52	0.83	17.75

Table S4: Heart rate (HR) and stroke volume (SV) of instrumented unfed bald notothens during rest and following enforced exercise.

Treatment (1=Splct, 2=Sham)	ID	Routine HR (beats min^{-1})	Max HR (beats min^{-1})	Scope HR (beats min^{-1})	Scope HR (%)	Routine SV (a.u)	Max SV (a.u)	Scope SV (a.u)	Scope SV (%)
1	43	23.12	21.87	-1.25	-5.40	0.05	0.06	0.01	14.19
1	44	17.26	21.88	4.62	26.79				
1	45	21.32	26.63	5.31	24.91	0.03	0.04	0.01	50.30
1	46	23.20	25.52	2.32	10.01	0.03	0.05	0.02	64.42
1	47	24.12	25.96	1.84	7.62	0.01	0.03	0.02	149.00
1	48	20.73	26.16	5.43	26.21	0.03	0.04	0.01	40.23
1	49	21.04	27.64	6.60	31.39	0.02	0.03	0.01	80.91
1	50	12.74	24.16	11.42	89.60				
1	51	21.20	25.98	4.78	22.55	0.03	0.05	0.02	61.59
1	52	23.25	24.28	1.03	4.43	0.03	0.05	0.02	72.81
1	53	25.25	25.52	0.27	1.07	0.02	0.05	0.03	128.65
1	54	22.31	24.94	2.63	11.80	0.04	0.05	0.02	53.99
1	55	21.52	23.57	2.05	9.53	0.02	0.04	0.02	77.69
1	56	19.28	24.86	5.58	28.94	0.03	0.04	0.02	61.56
2	57	22.16	30.00	7.84	35.36	0.04	0.05	0.02	45.74
2	58	19.66	25.50	5.84	29.68	0.04	0.06	0.02	58.11
2	59	13.59	23.03	9.44	69.46	0.03	0.05	0.02	51.52
2	60	27.17	27.51	0.35	1.27	0.02	0.04	0.02	106.05
2	61	25.32	28.07	2.75	10.86	0.02	0.04	0.01	59.35
2	62	23.23	24.02	0.79	3.40				
2	63	11.90	20.59	8.69	73.00	0.05	0.05	0.00	10.47
2	64	19.07	24.02	4.95	25.95	0.03	0.06	0.03	104.01
2	65	20.83	25.81	4.98	23.92				
2	66	21.37	27.08	5.71	26.70	0.02	0.04	0.01	68.44
2	67	19.56	25.37	5.81	29.73	0.06	0.08	0.02	27.25
2	68	20.41	24.53	4.12	20.20	0.03	0.06	0.03	78.18
2	69	20.08	25.43	5.35	26.66	0.03	0.04	0.01	39.73
2	70	11.74	25.81	14.08	119.95	0.03	0.05	0.02	58.55
2	71	15.10	25.29	10.19	67.50	0.04	0.05	0.01	26.27
2	72	19.56	25.67	6.11	31.24	0.03	0.05	0.02	58.54
2	73	20.37	26.85	6.48	31.79	0.02	0.03	0.02	94.51
2	74	21.10	25.10	4.00	18.96	0.02	0.03	0.01	32.99
2	75	18.32	20.76	2.44	13.33	0.02	0.03	0.01	26.78

Table S5: Total vascular resistance (Rtot) and cardiac power index (CPI) of instrumented unfed bald notothens during rest and following enforced exercise.

Treatment (1=Splct, 2=Sham)	ID	Routine Rtot (a.u)	Max Rtot (a.u)	Scope Rtot (a.u)	Scope Rtot (%)	Routine CPI (a.u)	Max CPI (a.u)	Scope CPI (a.u)	Scope CPI (%)
1	43	2.80	2.65	-0.15	-5.48	4.47	4.93	0.46	10.31
1	44								
1	45	7.46	4.27	-3.19	-42.81	2.36	4.75	2.39	101.58
1	46	4.79	2.89	-1.91	-39.80	2.37	4.66	2.30	96.99
1	47	11.65	5.58	-6.06	-52.05	0.86	2.95	2.09	244.34
1	48	5.28	3.47	-1.81	-34.28	2.10	4.32	2.22	105.84
1	49	8.16	4.64	-3.52	-43.10	1.23	3.96	2.73	221.50
1	50								
1	51	5.09	2.97	-2.12	-41.68	2.49	5.71	3.21	128.70
1	52	4.75	3.63	-1.11	-23.47	2.21	5.52	3.31	149.27
1	53	5.70	2.51	-3.19	-55.92	1.83	4.31	2.48	135.40
1	54	3.12	1.92	-1.20	-38.59	1.97	3.59	1.62	82.01
1	55	7.84	4.17	-3.67	-46.83	1.58	3.18	1.60	101.40
1	56	7.47	4.86	-2.61	-34.98	1.96	5.52	3.57	182.17
2	57	3.91	2.56	-1.35	-34.51	2.61	6.66	4.05	154.88
2	58	4.06	3.41	-0.66	-16.13	2.47	8.72	6.25	252.59
2	59	7.48	5.06	-2.42	-32.39	1.48	6.61	5.13	345.71
2	60	5.58	3.89	-1.68	-30.18	1.62	4.93	3.31	204.03
2	61	6.32	4.52	-1.80	-28.50	2.47	5.52	3.05	123.15
2	62								
2	63	6.84	4.53	-2.30	-33.69	2.06	5.00	2.94	142.20
2	64	5.28	3.58	-1.71	-32.31	1.84	8.21	6.37	346.91
2	65								
2	66	7.74	3.89	-3.85	-49.71	1.65	3.78	2.13	129.07
2	67	3.03	2.50	-0.52	-17.28	4.44	10.02	5.57	125.44
2	68	3.87	2.46	-1.41	-36.43	1.73	5.05	3.32	191.59
2	69	6.31	4.22	-2.09	-33.16	1.77	3.71	1.94	109.32
2	70	8.27	3.87	-4.40	-53.22	1.07	6.11	5.04	468.90
2	71	5.35	3.50	-1.84	-34.49	2.29	6.70	4.41	193.07
2	72	6.67	3.68	-2.99	-44.85	2.20	5.24	3.05	138.80
2	73	12.40	5.59	-6.81	-54.93	1.43	4.25	2.81	196.13
2	74	8.89	5.86	-3.03	-34.08	2.21	3.65	1.44	64.98
2	75	10.38	8.51	-1.87	-18.05	2.11	3.57	1.46	69.19