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Examples of Weak, If Not Absent, Form-Function Relations in the Vertebrate Heart

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Abstract: That form and function relates, is the maxim to anatomy and physiology. Yet form-function relations can be difficult to establish. Human subjects with excessive trabeculated myocardium in the left ventricle, for example, are diagnosed with non-compaction cardiomyopathy, but the extent of trabeculations may be without relation to ejection fraction. Rather than rejecting a relation between form and function, we may ask whether the salient function is assessed; is there a relation to electrical propagation, mean arterial blood pressure, propensity to form blood clots, or all? And how should extent of trabeculated muscle be assessed? While reviewing literature on trabeculated muscle, we applied Tinbergen's four types of causation - how does it work, why does it work, how is it made, and why did it evolve - to better parse what is meant by form and function. The paper is structured around cases that highlight advantages and pitfalls of applying Tinbergen's questions. It further uses the evolution of lunglessness in amphibians to argue lung reduction can impact on chamber septation, and it considers the evolution of an arterial outflow in fishes to argue that reductions in energy consumption may drive structural changes with little consequences to function. Concerning trabeculations, we argue they relate to pump function in the embryo in the couple of weeks before the onset of coronary circulation. In fetal and postnatal stages, a spectrum of trabeculated-to-compact myocardium makes no difference to cardiac function and in this period form and function may appear unrelated.

Key words: evolution; development; physiology; structure

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INTRODUCTION

Four weeks into human embryonic development, a single vessel connects the forming pulmonary vasculature to the left atrium [1]. 11 weeks later, pulmonary venous tissue has been incorporated to the left atrium and 4 separate pulmonary veins now open to the left atrium [2]. This ontogenetic acquisition of veno-atrial connections varies between individuals and abnormal connections, for instance 3 or 5, occur in approximately 1 out of 4 people [3]. However, it is inconsequential to a person's health whether there are 3, 4, or 5 pulmonary veins connecting to the left atrium [3], suggesting there is no relation between the normal variation in number of pulmonary veins, i.e. the *form*, and its *function*. Streaming of blood in the left atrium will of course be impacted on by the number of veins giving blood to the cavity, but is this not an unimportant functional relation if there are no consequences to whole organ and body performance?

The example of the number of pulmonary veins illustrates some of the pitfalls of establishing relation between form and function. First, at what level of biological organization should form-function relations be assessed; tissue, chamber, organ, organism, *etc*.? Second, which function to assess? Any structure of the body impacts on (parts of) the body and it is in principle possible to established a consequence to the presence of the structure. But consequence is different from adaptation in evolutionary biology. Adaptation is a trait that has been selected for by natural selection and thus relates directly or indirectly to the reproductive success of the organism [4]. Third, are we seeking proximal answers, such as *how* are left atrial blood streams affected by the number of pulmonary veins, or ultimate answers, such as *why* is the number of pulmonary veins variable?

When attempting to establish causality, Nobel laureate Niko Tinbergen suggested the application of 4 types of questions which are derivatives of the 4 categories of causes of Aristotle (given in parenthesis):

- *Mechanism* (material cause), how does it work?

  Example: The heart pumps blood.
- *Function* (finale cause), why does it work?

  Example: to drive perfusion of blood in the tissues?
- *Ontogeny* (formal cause), how is it made?

  Example: cardiogenic mesoderm surrounds a blood-filled cavity.
- *Phylogeny* (efficient cause), why did it evolve?

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> Example: the propulsion of blood through the tissues compensates for the increased diffusion distance between tissue and environment associated with greater body sizes.

August Krogh, also a Nobel laureate, proposed that for "a large number of problems there will be some animal of choice or a few such animals on which it can be most conveniently studied" [5]. For instance, concerning the biology around blood pressure, would one not want to study giraffes, the animal with the highest known systemic blood pressures [6]? In comparing different animal species, we may encounter evolutionary differences in mechanisms and functions, that, if understood correctly, can make us understand the *efficient cause*, why something became successful. As always, it is important that a functional advantage is not assumed beforehand. For example, Gould and Lewontin [4] emphasize that the question of "what did the *Tyrannosaurus rex* use its tiny front limbs for?" will likely receive an unsatisfactory and unfalsifiable answer. In contrast, we can reasonably answer "how did the *Tyrannosaurus rex* get its tiny front limbs" because the fossil record shows a conspicuous reduction of the front limbs concomitant with increments in the size of the hind limbs and head [4]. Viewed such, the reduction in the front limbs allows the prioritization of (energy to) the hind limbs and head. Similarly, we are not inclined to ponder the use of the tiny limbs of ancestral snakes or the pair of a claws next to the cloaca of pythons [7] because extant snakes abundantly show that life can be successful without limbs. It is rarely considered for the heart, however, whether there are vestigial features without function [8].

Confusion and poor reasoning, then, may arise from asking the wrong questions and 'why' questions can do us a disfavor by implying purpose. Or, as it is stated in the quote attributed to Ernst Wilhelm van Brücke "teleology is the mistress that the biologist cannot live without, but is too ashamed to be seen with in public". Below, we will focus on trabeculated ventricular muscle because it exhibits more than one function – which is the salient one? - it shows ontogenetic changes – at what stages is it important? – and it shows phylogenetic changes – why is there a reduction of trabeculated muscle in the independent evolution of endothermy in mammals and birds?

CASES

One form and multiple functions – which mechanism (*material cause*)?

The curious case of the crocodilian heart

The crocodilian heart has a full ventricular septum which distinguishes it from the hearts of all other ectothermic vertebrates (fishes, amphibians, and reptiles) (Figure 1A-B) [9, 10, 11]. The ventricular septum has a membranous part and a larger myocardial part which, like all cardiac muscle, propagates the electrical impulse and contracts upon electrical activation [11, 12]. Besides these two functions, the ventricular septum has at least 3 additional functional consequences on organ level. First, the blood pressure of the right ventricle can be substantially lower than the blood pressure of the left ventricle (Figure 1C) [13, 14, 15]. This allows for low blood pressures in the lung circulation, which in turn allows for a thinner blood-gas barrier at the respiratory epithelium [16]. Second, left-to-right shunting, the re-entry of pulmonary venous blood to the pulmonary circulation, which occurs in non-crocodylian reptiles, is anatomically impossible (Figure 1D) [11, 17]. The absence of shunting improves the efficacy of oxygen transport [18, 19]. Third, electrical activation spreads from the ventricular septum rather than from the base to the apex as in non-crocodylians (Figure 1E) [12, 20, 21].

Consequences of the ventricular septum can be established as above, but it is surprisingly difficult to ascertain its functional advantage besides the basic properties of myocardium (electrical propagation and contraction): The specialized manner of ventricular electrical activation does not shorten ventricular activation time as it does in mammals and birds [12]; oxygen consumption-dependent behaviors are not limited by the level of shunting in crocodylians and reptiles [19, 22, 23]; the rates of oxygen consumption in crocodylians are not higher than in lizards despite the thin blood-gas barrier [24, 25]. On the level of the organism, therefore, an advantage to the ventricular septum is not evident. It has then been proposed that the specializations of the crocodilian heart, including the ventricular septum, may have been selected for much earlier in evolution at a time, presumably, when crocodylians would have had much more active behaviors [26]. Although this conjecture is difficult to test, it does emphasize the possibility that the conditions are extinct in which the character provided an advantage to reproductive success.

The case of the crocodylian ventricular septum shows any one form may have multiple functional consequences. Ventricular septation in pythons also leads to pronounced pressure differences and reduction of shunts [27, 28]. In both cases, however, the functional advantage (*final cause*) on the level of the organism is not evident [29] and this calls into the question which form-function relation is the salient one.

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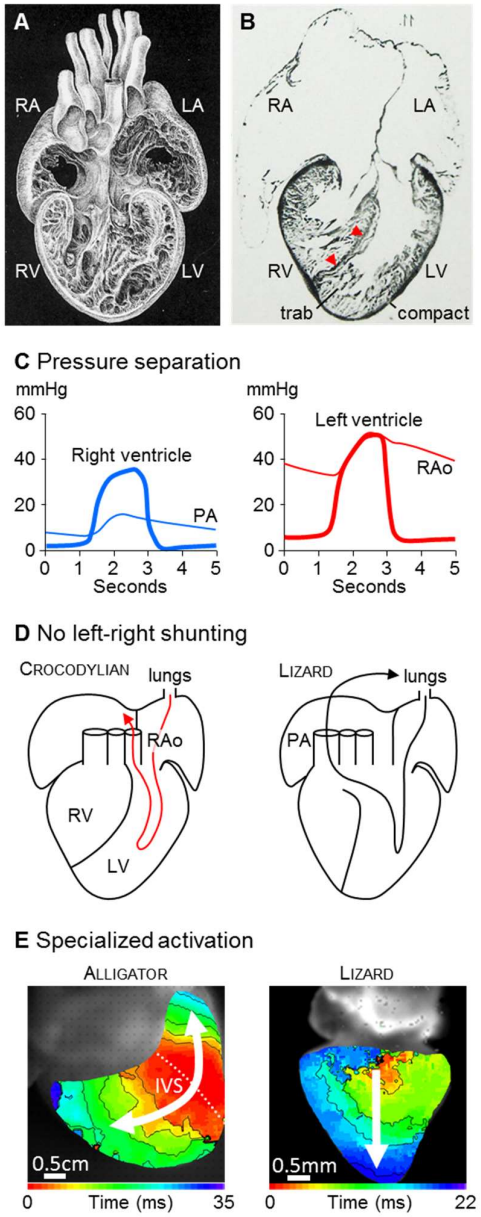

Figure 1. Consequences of the crocodilian ventricular septum on organ level. A. Both left (LV) and right (RV) ventricles of crocodylians are extensively trabeculated. Adapted from [30]. B. By histology, the ventricular wall can be seen to consist of a thin outer shell of compact myocardium and an extensive layer of trabeculated (trab) myocardium. The ventricular septum consists of a thin layer of tightly organized myocardium (red arrowheads). Adapted from [9]. C. Left and right ventricular blood pressures, notice the pulmonary artery (PA) has much lower pressures than the aorta (RAo). Redrawn from [14]. D. In crocodylians, blood returning from the lungs is always ejected through the right aorta (RAo) and left-right shunts are therefore anatomically impossible. In contrast, in non-crocodylian reptiles (e.g. lizard) there is no full ventricular septum and pulmonary venous blood can be shunted back to the lung circulation through the pulmonary artery. E. Optical mapping of electrical activation (depolarization). The pattern of ventricular activation is specialized in the crocodylian ventricle compared to the setting of non-crocodylian reptiles (e.g. lizard) as it spreads laterally from the ventricular septum (IVS) rather than from the base to the apex. Adapted from [12, 21].

Ontogeny (*formal cause*): when is form and function related?

We can postulate that for any structure, there is one or several stages in ontogeny when the form-function relation is the strongest {Richardson, 1999 #20238}. The term 'immature' implies this. In embryonic chicken, electrical propagation develops prior to cardiac contraction [31] and heart formation and pumping commences days before circulation of plasma/blood is necessary for development, as shown in embryos with ligated outflow tract [32]. Cessation of cardiac pumping by genetic perturbations in developing zebrafish and fruit flies have similarly shown that many early features of embryogenesis are not reliant of cardiac pumping [33, 34]. At least in embryogenesis, then, structures may develop before they provide a functional advantage to the organism.

We can also envision the inverse scenario, where structures provide a functional advantage in early stages of ontogeny but they are inconsequential to the adult animal. The extent of trabeculated myocardium of the ventricles of mammals may be such a case. In the embryo, the ventricle is without coronary circulation and to maintain homeostasis any myocardium must be in close proximity to the blood of the ventricular lumen. Trabeculated myocardium is then a solution to growth without coronary circulation, because it is bathed in the blood returning to the heart (Figure 2) [35]. In the embryo, as shown in mice, trabeculated myocardium is richer in mitochondria than the compact wall, suggesting it contributes the most to ventricular work [36]. Unsurprisingly, inhibition of trabecular growth, and therefore ventricular growth, causes hypoplastic ventricles that associate with gestational retardation and lethality [37]. However, in slightly older stages of development, coronary circulation is established, the trabeculated myocardium will have much reduced proliferation and subsequent growth of the ventricle is almost exclusively by the compact wall (Figure 2) [38, 39, 40]. Some trabeculated myocardium persists in the adult heart, but it is proportionally much reduced [41]. If cardiac functional measures are assessed during gestation, such as mean arterial pressure, their development is tied up to the size of the heart, but not to the extent of trabeculated myocardium once coronary circulation is established (Figure 2). The ontogeny of trabeculated myocardium in endotherms suggests, firstly, that trabeculated myocardium is tremendously important in an early and brief period only, and secondly, that it persists, once formed. In the adult heart, trabeculations has a limited ability to remodel Gati [42] and likely contributes to ventricular function on par with the compact wall [43].

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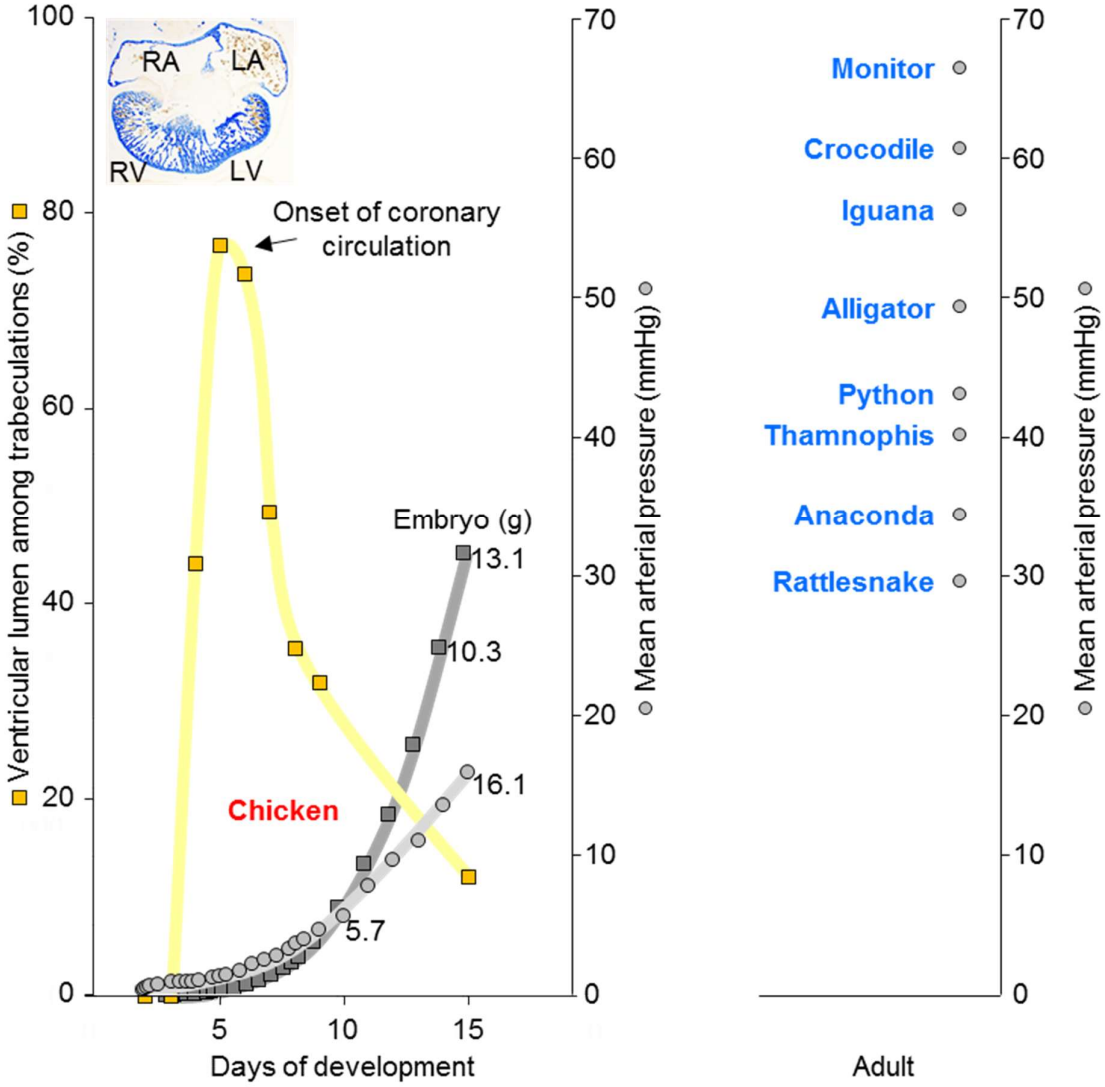

Figure 2. Highly trabeculated ventricles are capable of generating very substantial blood pressures. The left panel shows the *in ovo* development in chicken of body mass (dark grey), blood pressure (grey), and the change in ventricular wall architecture (yellow) from heart tube (2-3 days), to highly trabeculated (6-7 days), and finally to a compact wall (15 days). The insert shows the highly trabeculated left (LV) and right ventricle (RV) at 6 days of development (and the almost a-trabecular left (LA) and right atrium (RA)). From approximately 6 days onwards, when blood pressure is about 3mmHg, trabeculations and blood pressure become inversely related. The right-hand panel, however, shows that ectotherms have much greater blood pressures than developing chicken despite having highly trabeculated ventricles. Assuming cardiac mass is a fixed proportion of embryo weight, which it is in human [44], blood pressure will have a tight relation to cardiac mass (R^2=0.96, blood pressure and embryo weight). In the left panel, values for body mass and blood pressure are from [45] and values for ventricular wall architecture are from [41]. In the right panel, blood pressures of python are from [46] and the remaining values from Jensen [47]).

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Form unrelated to function: no *final cause*?

Trabeculation of the human left ventricle

The human left ventricle is composed of a compact wall with a network of trabecular myocardium on its luminal side (Figure 3). Most prominent of the trabecular myocardium are the papillary muscles which anchor the atrioventricular [48]. The non-papillary trabecular network can be very meagre or extensive, and may make up between near-zero % to some 25% of the left ventricular mass (Figure 3) [49]. Ventricular wall architecture can be measured as the ratio of trabecular-to-compact wall and analysis of thousands of cohort participants have shown the ratio has a log-normal distribution in the population [43]. Surprisingly, the ratio is not related to functional measures like ejection fraction and blood pressure [50, 51], or so poorly it is deemed clinically irrelevant [43]. Even in cases of excessive trabeculation, which can be diagnosed as left ventricular non-compaction cardiomyopathy [52, 53], there is no, or only very poor, relation between the extent of trabecular myocardium and function [49, 51]. Further, adverse outcomes like sudden cardiac death also appear unrelated to the extent of trabecular myocardium, but instead to ventricular dilation and fibrosis [43, 50, 54]. It therefore appears that there is no functional relation to the trabecular-to-compact wall architecture on the level of organ and individual in adult human [55]. This conjecture is supported by the meta-analytical finding that systemic blood pressure is similar across mammal phylogeny and body size [56], despite a substantial variation in the extent of trabeculations [57].

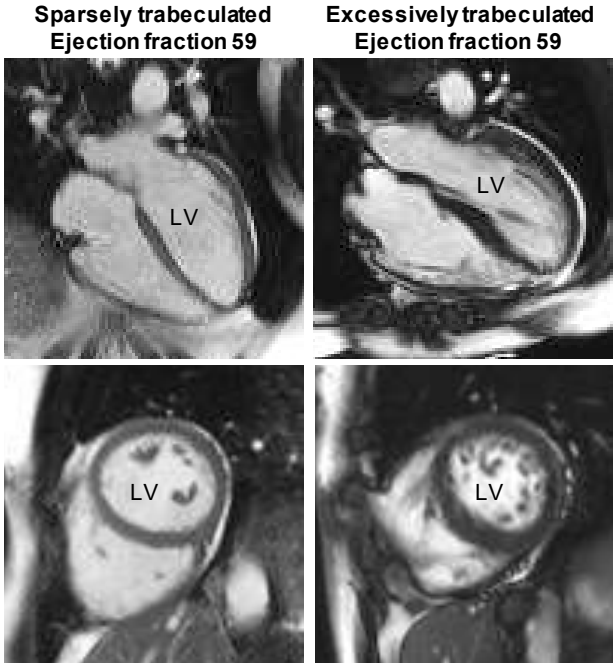

Figure 3. **The architecture of the human left ventricle (LV) appears to have no impact on function**. Normal ejection fractions (stroke volume/end-diastolic volume) occur in 'Sparsely trabeculated' and 'Excessively

trabeculated' ventricles alike. Top row shows so-called 4-chamber views, the bottom row shows transverse views of the two ventricles. Note the much more numerous and extensive trabeculations in the images on the right. Images from published data [41].

Relation of form and function in evolution (*efficient cause*)?

Ventricular trabeculations in vertebrates

The inverse relation of trabeculated wall architecture and blood pressure between 6 and 15 days of development in chicken shown in Figure 2, suggests that coronary circulation favors compact wall growth and causes a decrement in the proportion of trabeculated myocardium. This could be the case in the ontogeny of mammals and birds. Coronary vasculature is found in most ectotherms and can be found within the trabeculated myocardium of the extensively trabeculated ventricles [58, 59, 60]. This comparative analysis suggests that coronary vascularization is a necessary condition for the prioritization of the compact architecture over the trabeculated architecture, but not a sufficient condition. In fact, the adaptive value of compact architecture over the trabeculated architecture is not clear, i.e. the functional advantage (*final cause*) and explanation for its evolution (*efficient cause*).

As trabeculated as a human left ventricle with excessive trabeculations can be (Figure 3), it is much less spongy than the wall architecture of ectotherms (Figure 4) [41]. In ectotherms, the individual trabeculations are typically less than a twentieth of a millimeter wide and the space between trabeculations roughly compares to the distance between grains in fine sand, some 50μm [41]. Conversely, in endotherms, the trabeculated muscle can be orders of magnitude greater, pebble-like rather than sand-like [41](Boukens et al PBMB Under review). Most of the blood in the ventricles of ectotherms is between trabeculations, and, conversely, in endotherms most of the blood is in the central lumen which is without trabeculations [41]. There are at least two primary functions to trabeculated myocardium; propagation of the electrical impulse and contraction [21, 61]. The extensively trabeculated ventricles of some species of ectotherms generate blood pressures approaching those of mammals (Figure 2) [62, 63] and even in the tubular hearts of earthworms blood pressures can exceed 70 mmHg [64]. It is not obvious therefore that the evolution of compact walls directly facilitated the generation of high ventricular blood pressures. Instead, the formation of compact walls yields a trabeculations-depleted ventricular cavity. Such a cavity (which holds a few 'pebbles') will offer less impedance to blood flow than the 'sand-filled' ventricle of ectotherms. We have therefore proposed that the architectural change from trabeculated to compact wall has allowed for a faster filling and emptying of the ventricles and thus it has facilitated the high heart rates

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that characterizes endotherms (Figure 4) [65]. Higher heart rates allow for greater cardiac outputs and therefore higher blood pressure, thus making the compact wall architecture linked to blood pressure *via* heart rates. Also, the faster chamber activation of ectotherms is not explained by higher body temperatures and conduction systems only, suggesting a role for compact walls (Figure 4) (Boukens et al PBMB Under review).

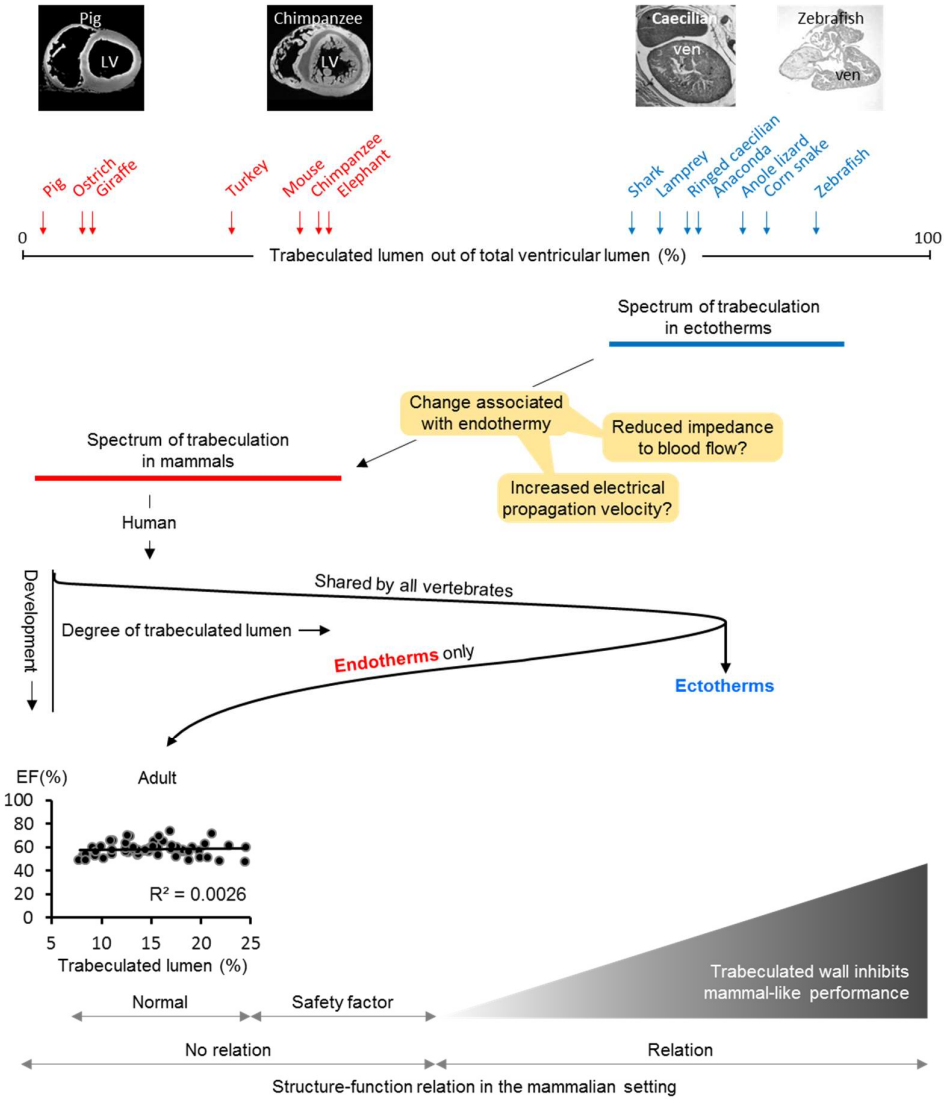

Figure 4. Evolutionary transition from spongy to compact. In the evolution from ectothermy to endothermy, the cardiac chambers became a lot less trabeculated and the chamber walls became compact. This change (orange boxes) may have impacted on the heart by reducing impedance to flow and accelerated chamber activation times, both of which would facilitate a short cardiac cycle and thus the high heart rates that characterizes the endotherms. In development, all vertebrates form highly trabeculated chambers, but only in endotherms this design is reversed by a temporary slow-down in proliferation of the trabeculated muscle while proliferation is maintained in the compact wall [38, 39, 40]. In adult (here human) there is variation in the extent of trabeculated muscle, but this extent does not associate with functional measures. Nonetheless, extremely excessive trabeculated ventricles,

ectotherm-like, appear incompatible with life in endotherms [66]. This suggest that the extent of trabeculated myocardium in endotherms will have a normal range, a safety factor range where excessive extent of trabeculated myocardium is not pathological, and an ectotherm-like range which will be detrimental to pump-function, possibly because of impairment of filling and emptying. Partly based on and adapted from [41].

Can a reduction in energy expenditure have a structural consequence?

The outflow tract of the fish heart

In the evolution of teleost fish there was a change to the arterial pole, whereby the myocardial outflow tract, the conus arteriosus, disappeared and a pear-shaped arterial outlet, the bulbus arteriosus, took its place (Figure 5) [67]. It is debated whether the bulbus of teleost fish should be considered a modification of an older structure or an evolutionary novelty, but the bulbus is evidently much more developed in teleosts than in non-teleosts [68, 69]. A recent study shows in embryonic teleost fish that the myocardial outflow tract undergoes reprogramming to an arterial phenotype mediated by *elnb* [70]. Despite the advances in understanding the phylogenetic appearance, the ontogenetic change, and the mechanism behind it, the functional advantage of an arterial bulbus over a myocardial conus remains elusive. Both the bulbus and conus work as a pressure and flow reservoir that ensures blood flow in the diastolic interval between ventricular ejections [71, 72].

A key difference may be that sarcomeric contraction is the basis of conus function, whereas it is recoil of elastic elements that is the basis of bulbus function [71, 72]. Given the conus comprises between 10 to 30% of the cardiac mass [73] and the metabolic rate of heart muscle [74] is an order of magnitude greater than of arterial wall [75] (values from mammals), having the myocardial conus may be energetically more demanding than having the arterial bulbus (Figure 5). If the bulbus is stiff, ventricular ejection could become expensive, but it is highly compliant and can accommodate a large fraction of cardiac output [76]. To make a theoretical calculation of the energy saved by having a bulbus, we can assume that the disappeared conus was relatively small (10% of cardiac mass), that the metabolic rate of the bulbus was a tenth of the conus, and that the bulbus is relatively noncompliant and more expensive to fill than a conus. The change from conus to bulbus may then constitute a 5% reduction in stroke work, which is the energy spend by the ventricle on ejection in one cardiac cycle. Stroke work in ectotherms is approximately 8 mJ per kg body mass, heart rate of a 3kg sockeye salmon is approximately 40 beats per minute [77, 78], and the saved energy in a course of a year then amounts to approximately 25.000J, or multiple catches of zooplankton, a mainstay of the food of sockeye salmon [79]. These considerations allow for the conjecture

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that the adaptive value of the bulbus is a low energy demand. If so, the form and function of the bulbus are then secondary consequences.

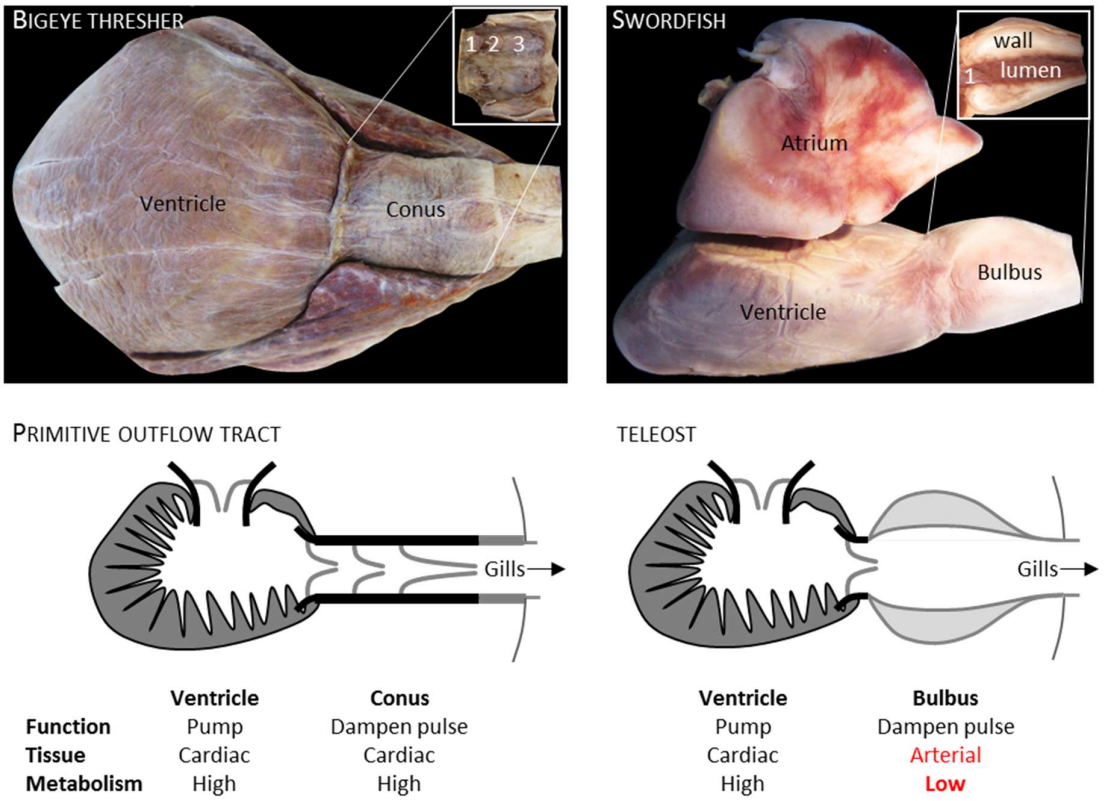

Figure 5. Arterial pole of fishes. The heart (158g) of a 304cm long Bigeye thresher (*Alopias superciliosus*), a shark, had a myocardial outflow tract (Conus, ventral view) that comprised 8.9% of the cardiac mass. In the insert, the conus has been isolated, cut longitudinally, and folded out, exposing its 3 rows of valve leaflets (1-3, 1st to 3rd row of valve leaflets). The heart of an approximately 50kg Swordfish (*Xiphias gladius*), a teleost fish, had an arterial outflow tract (Bulbus, view from the right). In the insert, the interior is exposed of the left half of the bulbus showing its thick arterial wall (1, only 1 row of valve leaflets). The categorization of metabolism as being high or low is based on values for mammals [74, 75].

Lungs, then heart

Can loss of the lungs impact on cardiac septation?

Lunglessness has evolved among amphibians in some aquatic caecilians and salamanders, presumably to reduce buoyancy in fast-flowing environments where oxygen is abundant [80, 81]. Besides having no lungs, these lungless amphibians also exhibit reduced septation in the atria and the outflow tract [80, 82]. We can then ask, whether the reduction in cardiac septation is an adaptation to lunglessness, or whether the reduction in lungs is a direct cause of reduced septation. In all lunged vertebrates, the pulmonary vein develops in the dorsal mesocardium, a

bridge of mesenchyme between the developing atria and the pharyngeal mesoderm in which the lungs develop (Jensen et al *In press* Anat Rec). The dorsal mesocardium projects into the atrial lumen as the dorsal mesenchymal protrusion, which is necessary for the closure of the primary foramen between the left and right atrial cavity [83, 84]. Accordingly, a poorly developed dorsal mesenchymal protrusion leads to atrial septal defects [85]. Further, the pharyngeal mesoderm also contributes cells to the arterial pole of the heart [86]. These observations allow for the conjecture that the reduced cardiac septation of lungless salamanders is a direct consequence of the reduced development of the pharyngeal mesoderm [82], rather than an adaptation related to intracardiac flow patterns.

SYNTHESIS

We have attempted to apply the four causes of Aristotle that Tinbergen adapted to biology. Our motivation came from the confusion and conflicting views concerning the role of trabeculated muscle in vertebrate hearts. 'Function' is a concept that often implies purpose, and teleology may exacerbate the confusion. The application of the four causes, we hope, has revealed that function can be interpreted in several ways. In part, this is because function may be assessed under conditions that are not conducive to test a particular form-function relation. While our cases are based mostly in evolutionary biology, we would argue that there is relevance for medicine: Left ventricular non-compaction cardiomyopathy is characterized as a distinct form of cardiomyopathy by the American Heart Association and is diagnosed when trabeculated myocardium is excessive [52, 53]. (Non-compaction cardiomyopathy has also been reported for a domestic cat [87]). Yet in patients and healthy subjects, left ventricular function does not correlate with the extent of trabeculated myocardium and the diagnostic criteria have very poor sensitivity [50, 51, 54, 88]. These observations suggest that form and function are not related in human when we consider trabeculated myocardium. As argued above, however, when we consider all life stages, a credible argument can be made that function is tightly related to trabeculated muscle in the embryonic heart before coronary circulation is established. Therefore, the extent of trabeculated muscle *is* related to function, but only in certain life stages. If this conjecture is true, it may be futile to use and develop morphometrics to identify true LVNC cases from the general population. Functional assessment remains the crucial readout.

Using the ventricular septum of the crocodylians as a case, we argued that any one feature may have more than one function. One of the functions, is the recently described specialized manner of electrical propagation. The crocodylian septum then exemplifies a case where we may firstly

ask whether there could be salient functions that have not been characterized? Secondly, which of the known functions should be assessed in order to identify the adaptive value of a structure, or should all functions be assessed? In this context, it is interesting to note that considerable effort has been spend on showing the adaptive value of right-to-left shunting in crocodylians, without a clear-cut adaptive value being shown [19].

In most instances, we can rightly assume a structural change to the heart relates to a functional change. However, as argued for the case of lunglessness in amphibians, it is entirely conceivable that a primary adaptation, lunglessness, induces a reduction of cardiac septation (in the atria and the outflow tract) because of developmental changes to the mesoderm that gives rise to both lungs and cells of the septae. That is, if cardiac changes are seen in isolation, we may miss the primary adaptation. It also follows, that it may be futile to assess the functional advantage of the reduction of the septae, because the advantage may not be there anymore. Similar concerns for establishing form-function relations can be expressed when trying to understand the significance of the myocardial to arterial identity change of the outflow tract of fishes. Possibly, the primary adaptation relates to energy consumption, with form-function relations being secondary.

In conclusion, we propose a negative derivative of the Krogh principle, namely that for a form-function relation, there will be a large number of animals of choice and life stages on which it cannot be studied.

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