Cardiovascular Proportionality of Modern Pigs

Are we breaking the allometric scaling laws ?

Gerard Jan van Essen

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Are we breaking the allometric scaling laws ?

Cardiovasculaire proportionaliteit van het moderne varken

Worden de allometrische schalingswetten verbroken?

Proefschrift

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Voor mijn overleden ouders,
*voor **Leen** de tuinman, die mij leerde met mijn handen te werken en voor **Nolda** die mij, van jongs af aan, heeft voorgehouden: "dat ik mijn goede verstand niet voor niets had gekregen".*

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What
happened to my
progeny

CHAPTER 1

General introduction to Cardiovascular Proportionality of Modern Pigs.

Based on G.J. van Essen, D. Merkus, D.J. Duncker.

Cardiovascular proportionality of modern pigs.

In preparation

General introduction to Cardiovascular Proportionality of Modern Pigs.

1.1 Domestication and cardiovascular alterations.

The ancestor of the modern pig is the wild boar (Giuffra *et al.*, 2000). Domestication of wild boar has probably first occurred in the Near East 9000 BC (Epstein and Bichard, 1984). Darwin (1868) recognized two major forms of domestic pigs, a European (Sus scrofa) and an Asian form (Sus indicus). Based on profound phenotypic differences, these two forms are considered distinct species. Giuffra *et al.* (2000) confirmed independent domestication of European and Asian subspecies using genetic analyses.

Domestication in Western Europe started about 4000 BC both by migration of domestic pigs from the Near East to Europe and by domestication of European wild boar by local farmers. Domestic pigs and European wild boar were mated resulting in hybrid offspring followed by introgression with European wild boar (Giuffra *et al.*, 2000; Larson *et al.*, 2007). Introgression has been defined by Baack and Rieseberg (2007) as the stable integration of genetic material from one species into another through repeated back crossing. Back crossing of hybrids with wild boar has according to Larson et al. (2005) resulted in large impact of wild boar on the genetics of the European domestic pigs. They analysed mitochondrial DNA of 686 wild and domestic pig specimens and identified European wild boar as the principal genetic background of modern European domestic pigs. In the late eighteenth and early nineteenth centuries, British pig breeders imported Chinese pigs renowned for having great mothering characteristics and producing large litters, to improve productivity of local English breeds. Bosse *et al.* (2014) found clear evidence for this human-mediated hybridization in the genome of all investigated Large White pigs.

Domestication and targeted pig breeding has resulted in obvious differences in anatomy and physiology in comparison with the wild ancestor. During the last century, pig breeding has been predominately influenced by selection on economic performance parameters like muscle development, growth rate, feed conversion and reproductive capacity resulting in dramatic increases of body weight, muscularity and litter size (Saunders and Mcleod, 1999; Müller *et al.*, 1999). Also growth rates increased spectacularly in recent

centuries. Thus, whereas in the eighteenth century pigs needed two to three years to reach a body weight of 40 kg, only 100 years later pigs could reach a body weight of 150 kg in just one year (Huisman, 1969). Table 1 gives an overview of successive stages of life of modern fattening pigs with corresponding ages, body weights and growth rates and these data show that today modern fattening pigs can reach a slaughter weight of 115 kg in less than half a year.

Life Stage	**Age (days)**	**Body weight (kg)**	**Mean growth rate (gram/day)**
Birth	0	1	250
Weaning	25	7- 8	400 - 450
Start fattening	70	22.5 - 25	850 - 900
Slaughtering	170	115 - 120	

Table 1. Life stage and corresponding ages, body weights and growth rates of modern fattening pigs with data of van der Peet – Schwering *et al.* (2013).

In addition to the remarkable changes in phenotype and performance, notable differences have also occurred in the cardiovascular system of modern pigs, including lower relative heart weight, blood volume and haemoglobin levels (Von Engelhardt, 1966; Huisman, 1969; Schürmann, 1984). These observations have raised concerns regarding cardiovascular function and adaptability to stress of modern pigs (Von Engelhardt, 1966). Indeed, an increased risk of circulatory insufficiency has been proposed to be responsible for, at least part of, the pathogenesis of multifactorial pig diseases, including transport-associated health problems and oedema disease (Niewold *et al.*, 2000).

Modern pigs, like their ancestor, are truly flight animals. They usually react to sudden changes or threats in their environment with an intense stress response and usually a rapid flight reaction. This flight behaviour is clearly demonstrated by startled piglets running together with loud cries into one corner of their pen where they climb on top of each other. Such a flight response causes a strong increase in oxygen demand of skeletal muscles which, in turn, requires major cardiovascular adaptations in order to increase oxygen transport and supply to the exercising muscle groups. The autonomic nervous system drives, in part via

adrenergic stimulation, the required increases in respiratory minute volume (through elevations in both respiratory rate and tidal volume) and cardiac output (through increases in both heart rate and stroke volume). The exercise-associated increase in sympathetic drive also results in a redistribution of blood flow away from the abdominal organs in favour of the active skeletal muscle groups, to enable increases in muscle perfusion and oxygen supply commensurate with the increased metabolic demands. Finally, as ~ 95% of oxygen in the blood is bound to haemoglobin, the exercise-associated increase in haemoglobin, which is due to splenic contraction and expelling of erythrocyte-rich blood into the circulation (Laughlin *et al.*, 2012), further facilitates oxygen delivery to the active muscle. In addition to exercise, other physiological processes also require cardiovascular adaptations. For example, pregnancy in sows induces increases of total blood volume (Anderson *et al.*, 1970) and cardiac output (Reynolds *et al.*, 1985) to accommodate blood flow and oxygen supply to the placental circulation. In fact, any disruption of homeostasis, including an infection with pathogenic agents, will require a cardiovascular adaptation. Hence it is clear that a well-functioning and adequate cardiovascular system is essential for all animals, including pigs, to maintain homeostasis and to stay healthy. From this point of view the concerns of Von Engelhardt (1966) and Niewold et al. (2001) are logical and understandable. However, while lower relative heart weight, blood volume and haemoglobin levels suggest disproportionality in modern domestic pigs, more knowledge and insight is needed regarding the proportionality of the porcine cardiovascular system in order to determine whether the cardiovascular system of pigs, in particular the porcine heart, is proportional to their body size.

Most investigators have normalized the cardiovascular parameter under study to the pig's body weight (often termed 'index') in order to investigate its cardiovascular proportionality (Wachtel, 1963, Von Engelhardt, 1966 and Schürmann, 1984). Taking this approach they compare these indexed data – including relative heart weight (RHW in g/kg) and cardiac index (CI in ml/min/kg) – to the indexed parameters of wild boar (Wachtel, 1963 and Schürmann, 1984) or even other species (Von Engelhardt, 1966). However, this approach is not optimal and does not yield proof of cardiovascular disproportionality in pigs, because – according to the allometric scaling laws – the relations between heart weight and body weight or between cardiac output and body weight are not linear, but proportional to three quarter power of body weight. (West *et al.*, 1997). In the studies described in this thesis we applied the allometric scaling laws as a leading reference to investigate cardiovascular proportionality

of modern domestic pigs and we used the values of allometric exponents for variables of the mammalian cardiovascular system proposed by West *et al.* (1997), because of the striking similarity between the values predicted by their model with empirical observations of others.

1.2 Allometric Scaling Laws

Since animal scientist and veterinarian Max Kleiber (1932) published his seminal paper demonstrating that standard metabolic rates among mammals varied with three-quarters power of body weight similar allometric scaling phenomena have been widely investigated (Agutter and Wheatly, 2004). In wild mammals, many anatomical parameters and physiological processes scale with body mass. Among the many fundamental variables that obey such scaling laws - termed "allometric scaling " by Julian Huxley – are metabolic rate, life span, growth rate, heart rate, DNA nucleotide substitution rate, length of aortas and genomes etc. (West and Brown, 2005). Also the cardiovascular system and its components, including heart weight, cardiac output, stroke volume and blood volume, have all been proposed to scale allometrically with body weight (West *et al.*, 1997; Agutter and Wheatly, 2004; West and Brown, 2005; Dewey et al., 2008). The observed scaling is typically a simple power law: $Y = a \cdot M^{b}$, where Y is some observable for example CO, a is a constant, and M the mass of the organism and the exponent b almost invariably approximates a simple multiple of ¼ (West *et al.*, 1997; Agutter and Wheatly, 2004; West and Brown, 2005). In wild animals, heart weight and cardiac output have been shown to scale with body mass to the power of 0.75, blood volumes scales with body mass to the power of 0.25 and stroke volume scales with the power of 1.00 (West *et al.*, 1997; Yang and Lin, 1997; West and Brown, 2005).

The value of exponent b has been subject to debate (Agutter and Wheatly, 2004; Painter, 2005; Glazier, 2008). For example, several early interspecies studies have suggested that heart weight scales to body mass with an exponent value of 1.0, whereas intraspecies studies have shown that heart weight scales to body mass with a scaling power of 0.75 (West *et al.*, 1997).

The origin of the scaling laws in biology has fascinated and pre-occupied many scientists but a comprehensive scientific theory was lacking until West *et al.*, (1997) published a general model for the origin of allometric scaling laws in biology. The three quarter power scaling laws originate from the fractal like branching networks that distribute energy, metabolites and information in organism (West *et al.*, 1997). The properties of the network with optimal

architecture or geometry are presumed to be the consequences of natural selection. West and Brown (2005) proposed that metabolic rate plays a central role in determining the scale of biological phenomena. Aerobic metabolism in mammals is fuelled by oxygen. The oxygen concentration in blood is invariant, so cardiac output or blood volume flow rate through the cardiovascular system is a proxy for metabolic rate. Consequently, the characteristics of the circulatory network determine and constrain the scaling of metabolic rate. All biological systems, containing numerous subunits need to be serviced in a relatively "democratic" and efficient way to supply metabolic substrates, remove waste products and regulate activity. West and Brown proposed that natural selection solved this problem by evolving hierarchical fractal-like branching networks, which distribute energy and materials between macroscopic reservoirs and microscopic sites. These networks comply with three conditions. First the networks service all local biologically active regions (they are so called space-filling). Second, the networks terminal units like capillaries are invariant within a class or taxon, and third, the required energy for transport is minimized. So the cardiovascular system of animals and particularly the common properties of the vascular network determine the biological scaling phenomena (West and Brown, 2005).

1.3 Blood Volume, Heart Weight, Stroke Volume and Cardiac Output

1.3.1 Blood Volume

New born animals have a higher relative blood volume (blood volume per kg body weight), than adults. In most species, the adult ratio is already reached a few months after birth and remains nearly constant with advancing age (Huisman, 1969). In contrast, in domestic pigs the decrease in relative blood volume continues with increasing body weight (Huisman, 1969). Von Engelhardt (1966) and Yang and Lin (1997) reported a reduction of relative blood volume of nearly 40% at slaughter weight (~115 kg) and the reported values of, respectively, 55-68 ml/kg are rather low in comparison to the values reported for other large domestic animals such as 70-120 ml/kg in horses and 52-81 ml/kg in cattle (Von Engelhardt, 1966).

Yang and Lin (1997) fitted log blood volume (BV) to log body weight (BW) and they found the allometric power law formula, $BV = 0.36 \bullet BW^{0.62}$. The observed exponent of 0.623 is significantly lower than the exponent 1.0 given by West *et al.* (1997), which is a clear indication of the disproportionality of the circulating blood volume in modern domestic pigs.

1.3.2 Heart Weight

Von Engelhardt (1966) observed a decrease of relative heart weight with increasing body weight and concluded that the pig's body grows more rapidly than does its heart. At slaughter weight (~115 kg) pigs reach a relative heart weight of 0.30%, which is relatively low in comparison to values reported for other large domestic animals, including 0.7-1.1% in adult horses and 0.4-0.5% in adult cattle (Von Engelhardt, 1966).

The relative heart weight of domestic pigs at slaughter weight was still 0.45% at the end of the 19th century, but fell during the 20th century to 0.3%, which is significantly less than the 0.64 % reported for wild boar (Huisman, 1969). Thus, the decline in relative heart weight during the last 100 years (from 0.45 % to 0.30 %) almost equals the decline of the previous 9000 years of domestication (from 0.64 % to 0.45 %). This 50% decline in relative heart weight due to domestication and selection on performance features has been supported and confirmed by the findings of Schürmann (1984) and Müller *et al.* (1999).

Several factors are known to affect heart size in domestic pigs. Heath (1988) varied ambient temperature and the level of food intake to study the influence on organ development in piglets. The heart size was generally more affected by rearing temperature than by food level intake, with hearts of cold-reared pigs being greater. Koong *et al.* (1983) used 15 genetically lean and 15 genetically obese pigs with an initial body weight of 27 kg to investigate the effect of three feeding regimes on organ size and heart weight. The first treatment group (HL) of five lean and 5 obese pigs was fed to gain 19 kg body weight during the first 35 days and to lose 5 kg during the second 35 days. The second treatment group (MM) was fed to gain 7 kg during both periods. The third treatment group (LH) was fed to lose 5 kg during period 1 and to gain 19 kg in period. At the end of period 2 the animals were slaughtered and organ weights were measured. No significant difference was detected for heart weight between the three treatments groups but lean pigs had significantly higher heart weight then their obese counterparts. These results were confirmed by studies of Yang and Lin (1997) who investigated heart size in purebred and crossbred pigs and they found a significant increase of heart weight of growing boars of various genetic backgrounds if they exhibited a better food conversion efficiency or less back fat thickness.

An extensive study was carried out by White *et al.* (1994), who investigated growth and body composition of the Chinese Meishan pigs versus Yorkshire pigs. Five barrows and

five gilts of each of the two breeds were slaughtered at birth and at 41, 71, 123 and 171 days of age and five gilts of each breed were slaughtered at 260 days of age. Meishan pigs are extremely prolific and attain sexual maturity at an early age. Conversely, they have fat carcasses, poor food efficiency and low growth rates. The mean heart weight and body weight at birth of female Yorkshire piglets was 13.4 and 1248 gram and of female Meishan piglets 6.4 and 968 gram. The mean heart weight and body weight on 260 days of age of Yorkshire gilts was 385 gram and 150.2 kg and of Meishan gilts 269 gram and 87.5 kg. The ratio between the mean heart weight and the mean body weight at birth was higher for the female Yorkshire piglets, 1.07% versus 0.66% while at an age of 260 days was this ratio slightly higher for the Meishan gilts, 0.31% versus 0.26%. Despite obvious differences in body composition between Yorkshire and Meishan (figure 1.) were these values, 0.31% and 0.26% at 260 days in the same order of magnitude and they also correspond to the values for relative heart weight of pigs at slaughter weight that were previously given by Von Engelhardt (1966) and Huisman (1969)

Figure 1. Pictures of a modern European pig (left) and the Chinese Meishan pig (right) show the huge differences in anatomy and body composition.

Yang and Lin (1997) were the first to fit log heart weight (HW) to log body weight (BW) and found the allometric power-law formula: $HW = 12.2 \bullet BW^{0.73}$ ($r = 0.96$). The observed exponent of 0.73 is almost equal to 0.75, the exponent given by West *et al.* (1997).

1.3.3 Stroke Volume and Cardiac Output

The first cardiac output data of both wild boar and domestic pigs were collected and published by Wachtel (1963), who measured cardiac output in barbiturate-anesthetized pigs and wild boar using the thermo-dilution method. Wachtel (1963) found a clear decrease of cardiac index (cardiac output per kg body weight) in domestic pigs with increasing body weight, but did not find a clear difference of cardiac index between domestic pigs and wild boar.

Holt *et al.* (1968) studied cardiac stroke volumes in relation to body weight in different mammalian species, including rats, horses and pigs. Average values for stroke volume per kilogram body weight, extending over the wide range of mammals studied, were approximately constant, i.e. 0.9±0.3 ml/kg, indicating that stroke volume is linearly related to body weight, i.e. $BW^{1.0}$. Holt *et al.* (1968) also measured cardiac output in all species and proposed that cardiac output is not a function of body surface but of body weight. Thus, cardiac output is the product of heart rate and stroke volume. Heart rate is a function of $BW^{-0.25}$ and stroke volume is a function of $BW^{1.0}$ and the product HR•SV is a function of $BW^{0.75}$ (Holt *et al.*, 1968; West *et al.*, 1997).

1.4 Aim and Synopsis of the Thesis.

There is evidence that the cardiovascular system of modern domestic pigs differs in unfavourable terms from the cardiovascular system of their ancestor, the wild boar. Modern pigs have for example lower relative heart weight, blood volume and haemoglobin levels (Von Engelhardt, 1966; Huisman, 1969). These findings have raised concerns regarding cardiovascular capacity and adaptability to stress (Von Engelhardt, 1966) and concerns about an increased risk of circulatory insufficiency or failure which might even contribute to multifactorial pig diseases like Oedema Disease and transport associated health problems (Niewold *et al.*, 2000). To date a solid scientific foundation for these concerns is lacking.

Due to a possible role of insufficient cardiovascular capacity and performance in the pathogenesis of swine diseases, it is from a veterinary point of view very interesting and important to investigate the cardiovascular system of pigs. To verify this suggested possible intrinsic animal health risk we investigated the cardiovascular proportionality of modern domestic pigs. Our primary target and scientific aim of this thesis is to gain more insight and knowledge about the proportionality, according the allometric scaling laws, of the porcine

cardiovascular system. Consequently, we tested the hypothesis that the cardiovascular system of the pig, in particular its heart size and function, is proportional to its body weight.

Chapter 1 provides a general introduction to cardiovascular proportionality of modern pigs and to allometric scaling, with a particular emphasis on the quarter-power scaling laws. In wild animals, heart weight and cardiac output have been shown to scale with body weight to the power of 0.75 and stroke volume with the power of 1.0 (West *et al.*, 1997; Yang and Lin, 1997). In addition, it provides a literature review of blood volume, heart weight, stroke volume and cardiac output of wild boar and modern pigs.

In **Chapter 2**, we tested the quarter scaling hypothesis for heart weight, cardiac output and stroke volume in growing pigs with body weights ranging from 22 to 75 kg, both at rest and during strenuous exercise.

Since we found little evidence for disproportionality in cardiac output, stroke volume and heart weight in the young animals, we subsequently tested in **Chapter 3** the quarter scaling hypothesis for heart weight, cardiac output and stroke volume in adult pigs up to 300 kg. The results from that study demonstrated that adult pigs suffer from disproportionally low stroke volume and cardiac output.

In view of the methodological limitations in the study described in Chapter 3, and our interest in understanding the mechanisms underlying the lower stroke volume and cardiac output in adult pigs, we conducted a follow-up study in **Chapter 4**. Here we investigated the proportionality in pigs, over a wide range of body weights, of a single (female) sex, with a similar genetic background, undergoing invasive hemodynamic studies in a single laboratory using a uniform anaesthesia regimen. In this study we assessed possible mechanisms underlying the disproportionally low stroke volume, by assessing left ventricular dimensions as well as left ventricular systolic and diastolic function and by performing myocardial tissue analysis.

In the closing chapter of this thesis (**Chapter 5**) we summarize and discuss our findings with an emphasis on the implications of our findings for the intensive pig industry – and society as a whole – and draw parallels between porcine and human cardiovascular pathology. Finally, a number of recommendations for future research are presented.

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CHAPTER 2

Does cardiovascular performance of modern fattening pigs obey allometric scaling laws?

G.J. van Essen, J.C.M. Vernooij, J.A.P. Heesterbeek, D. Anjema, D. Merkus and D.J. Duncker

J Anim Sci. 2009, 87: 1991 – 1997.

Abstract

In view of the remarkable decrease of the relative heart weight and the relative blood volume in growing pigs we investigated whether cardiac output (CO) and stroke volume (SV) of modern growing pigs are proportional to body mass (M), as predicted by allometric scaling laws: CO (or SV) = $a \cdot M^b$, in which b is a multitude of 0.25 (quarter power scaling law). Specifically, we tested the hypothesis that cardiac output CO scales with M to the power of 0.75 ($CO=a \cdot M^{0.75}$) and SV scales with M to the power of 1.00 ($SV=a \cdot M^{1.0}$), and investigated whether these relations persisted during increased cardiac stress. For this purpose, two groups of pigs (group 1 of 57±3 kg in Lelystad, and group 2 of 28±1 kg in Rotterdam) were chronically instrumented with a flow probe to measure CO and SV; instrumented swine were studied at rest and during strenuous exercise (at ~85% of maximum heart rate). Analysis of both groups of pigs (analysed either separately or combined) under resting conditions, demonstrated that the 95% confidence intervals of power-coefficient b for CO encompassed 0.75 and that for SV encompassed 1.0. During exercise, similar results were obtained, except for SV in group 2, in which the 95% confidence limits remained below 1.0, which may have been due to the relatively small range of weights in group 2. These observations indicate that CO and SV of growing pigs with M less than 75 kg are still proportional to M, even during strenuous exercise, and that CO and SV scale with M according the quarter power scaling laws. In conclusion, the concerns about disproportional growth and development of modern growing pigs with body weights until 75 kg were not confirmed by the present study.

2.1 Introduction

Domestication of pigs started approximately 9000 years ago, resulting in marked differences in anatomy and physiology with its ancestor the wild boar (Darwin, 1868; Hemmer, 1990; Jones, 1998; Giuffra *et al.*, 2000). During the last century in particular, pig breeding has been dominated by selection based on meat quantities and other economic aspects, which has yielded remarkable increases in skeletal muscularity as well as litter size (Müller *et al.*, 1999). This economy-driven selection process has inadvertently resulted in alterations of anatomy and physiology of the cardiovascular system of the domesticated pig, including lower relative heart weight, blood volume and haemoglobin levels (Von Engelhardt, 1966; Huisman, 1969; Schürmann, 1984). Wachtel (1963) measured CO of anesthetized pigs and wild boar and he observed that CO of farm pigs levelled off in pigs above 50 kg, resulting in a marked decrease in relative cardiac output per kg of body weight. Von Engelhardt postulated in 1966 that all these cardiovascular alterations could contribute to an unstable circulatory system in swine, but emphasized that our understanding of cardiovascular physiology of domesticated swine was fragmentary and that there was a serious lack of data, especially of cardiac output (CO) data in conscious animals. Similar concern was raised more recently by Niewold *et al.* (2000) that the relatively small heart, blood volume and low haemoglobin level could limit cardiac output and hence cardiovascular adaptability to stress. The consequent increased risk of circulatory insufficiency or failure might even contribute to persistent pig diseases such as Oedema Disease and transport-associated health problems (Niewold *et al.*, 2000).

In wild animals, heart weight and cardiac output have been shown to scale with body mass (M) to the power of 0.75, blood volume scales with body weight to the power of 0.25 and stroke volume (SV) scales with the power of 1.00 (West *et al.*, 1997; West and Brown, 2005). The present study was undertaken to test the hypothesis that cardiac output and stroke volume of modern growing pigs are proportional to its body size as predicted by the quarter scaling laws (H_0: $CO = a \cdot M^{0.75}$; and H_0: $SV = a \cdot M^{1.0}$). For this purpose, we collected and analysed CO data of 60 chronically instrumented awake pigs with body weights ranging from 22 to 75 kg. Since a deviation from the established scaling law would be most likely detected under conditions of increased physical load, animals were studied not only at rest but also during heavy treadmill exercise.

2.2 Materials and Methods

All studies were performed in accordance with the Council of Europe Convention (ETS123)/ Directive (86/609/EEC) for the protection of vertebrate animals used for experimental and other scientific purposes, and with approval of the Animal Care Committees of the Animal Science Group of Wageningen University and Research Centre (ASG of WUR) (group 1, studies 1-3) or Erasmus University Medical Center Rotterdam (group 2, study 4).

2.2.1 Experimental Design

The present report encompasses the results obtained in four studies in chronically instrumented growing conscious pigs performed at Lelystad (group 1, studies 1, 2 and 3) and in Rotterdam (group 2, study 4). The Lelystad studies were performed to investigate the influence of daily exercise training (study 1), husbandry system (study 2) and genetics (study 3) on cardiac performance during strenuous exercise. The Rotterdam data were obtained as part of ongoing studies pertaining to regulation of cardiac performance during strenuous exercise (study 4).

After arrival in Lelystad and at least one week prior to surgery, all pigs were housed with three pigs in a pen. After surgery, pigs were housed individually on 1.8 square meter indoor space at the animal facility of ASG at Lelystad. After arrival in Rotterdam and at least one week prior to surgery, all pigs were housed separately with one pig in a pen at the animal facility of Erasmus University Medical Center. All pigs were given ad libitum access to regular dry pelleted food. Furthermore, all pigs were familiarized to run on a treadmill prior to surgery. Subsequently, pigs were instrumented for measurement of heart rate, stroke volume and cardiac output at rest and during strenuous exercise testing.

2.2.2 Surgical Instrumentation

Group 1: Studies 1-3.

Pigs received ampicillin (15 mg/kg, intramuscularly; AUV, Cuijk, the Netherlands) 24 hours before surgery and were deprived of food for 16 hours before surgery. Surgical instrumentation was carried out under general anaesthesia. Pigs were sedated with ketamine (10 mg/kg, intramuscularly; AUV) combined with midazolam (0.75 mg/kg, intramuscularly; AUV). Anaesthesia was deepened for induction with sufentanil (1 µg/kg, intravenously) and mivacurium (0.4 mg/kg, intravenously; OPG, Staphorst, the Netherlands). Pigs were intubated

and mechanically ventilated (tidal volume 13 ml/kg and 13 breaths/min.) with a mixture of air and O_2 (3:2) and 2.5% sevoflurane. Anaesthesia was maintained with rocuroniumbromide (5 mg/kg/hr, intravenously; OPG) and sufentanil (1 µg/kg/hr, intravenously, OPG). The chest was opened via the fourth left intercostal space. The pericardial space was opened and the ultrasonic cardiac output flow probe 20A (Transonic Systems Inc, Ithaca, New York) was placed around the pulmonary artery. The pericardial space and the thorax were closed with soluble sutures, and the probe wire was tunnelled from the thorax wound under the skin to the dorsal region just between the shoulders. Ampicillin was repeated on day 1 and day 3 and the pigs received the daily injection of the analgesic, flunixine (2 mg/kg, intramuscularly; AUV), during the first two days after surgery.

Group 2: Study 4.

Pigs were sedated with ketamine (30 mg/kg intramuscularly; AUV), anaesthetized with thiopental (10 mg/kg intravenously; AUV), intubated and ventilated with a mixture of O_2 and N_2O (1:2) to which 0.2-1% (vol/vol) isoflurane was added (Stubenitsky *et al*, 1998; Duncker *et al*, 2001; Merkus *et al.*, 2007). Anaesthesia was maintained with midazolam (2 mg/kg followed by 1 mg/kg per hour, intravenously) and fentanyl (10 µg/kg per hour, intravenously). Under sterile conditions, the chest was opened via the fourth left intercostal space and a fluid-filled polyvinylchloride catheter was inserted into the aortic arch for blood pressure measurement (Combitrans® pressure transducers, Braun, Melsungen, Germany). An electromagnetic flow probe (14 to 15 mm, Skalar, Delft, the Netherlands) was positioned around the ascending aorta for measurement of cardiac output. Catheters were tunnelled to the back and animals were allowed to recover, receiving analgesia (0.3 mg buprenorphine intramuscularly; AUV) for 2 days and antibiotic prophylaxis (25 mg/kg amoxicillin and 5 mg/kg gentamycin intravenously; AUV) for 5 days.

2.2.3 Experimental Groups

Group 1 Lelystad, Study 1: Daily exercise training

To investigate the influence of daily exercise training on cardiovascular performance, nine female Dalland pigs from different litters, age 9.2 ± 0.5 weeks with bodyweight 25.7 ± 1.4 kg (9.2 ± 0.5 wk) were obtained from the breeding farm of ASG of Wageningen University and Research Centre at Raalte, the Netherlands. During the second post-surgical week, pigs

underwent treadmill running at ~2 km/h for 20 min on 3 days (Monday, Wednesday, Friday). Two weeks after surgery they were able to run a full exercise test. The training group (n = 5) was subsequently exercised on a treadmill at 0% grade during 4 wk for 5 consecutive days (Monday through Friday) each week. The training protocol consisted of 3 min of warming- up exercise at 1.2 km/h, followed by 4 min at 1.9 km/h, 5 min at 3 km/hand 10 min. at 3.6 km/h, followed by exercise at a maximal speed of 4.2 km/h, until exhaustion. Hemodynamic measurements were obtained once every week (Friday). The control group (n = 4) stayed in their pens for 4 wk and underwent hemodynamic measurements at the beginning and at the end of the 4-wk period. ; they were tested at the beginning and at the end of the four-week period. For this purpose, pigs were placed on the treadmill and flow probes were connected to the flow meter (Transonic Systems T 450, Ithaca, NY, USA)) that was connected to a computer. The mean flow per (l/min) and the treadmill speed were sampled every 20 seconds by the IOX data acquisition and analysis software (EMKA, Paris, France) on the computer. Heart rate was derived from the flow signal by the IOX software. After resting measurements (heart rate 80 to 120 beats/min.) were obtained a staged exercise protocol was performed, consisting of 3 min of warming-up exercise at 1.2 km/h, followed by 4 min at 1.9 km/h, 5 min at 3 km/h and 10 min at 3.6 km/h, followed by exercise at a maximal speed of 4.2 km/hr until exhaustion during 12.3 ± 2.1 min. Pigs were considered to be exhausted when they stopped running and attempted to sit or lie down on the treadmill.

Group 1 Lelystad, Study 2: Zoo-technical influence: Organic Vs Conventional Husbandry

To investigate the influence of environmental and or zoo-technical variables like food, feeding patterns, housing and freedom of movement on cardiovascular performance, 8 female half-sisters, ¾ great Yorkshire and ¼ Dutch Landrace pigs who shared the same father, were obtained from the breeding centre of ASG at Raalte and subjected to exhaustive treadmill exercise. Four pigs had been reared under organic conditions and the other four under conventional conditions. The organic group was continuously fed organic food (CP: 15.7 %, Lys.: 083 %, CF: 4.0 %, ME: 13.47 MJ/kg) composed of ingredients cultivated without fertilizer, the conventional group was fed a regularly food (CP: 16.3%, Lys.: 0.96 %, CF: 4.4 %, ME 13.55 MJ/kg). Another important difference is that conventionally reared piglets were weaned at 28 days versus the organic piglets on 42 days. Finally, the organic pigs had more freedom of movement at the breeding farm. Thus, conventional pigs had just 0.4 square meter per piglet,

while organic piglets had 0.6 square meter inside room and 0.4 square meter outside room. Upon arrival in Lelystad, BW were 36.3±1.7 kg for conventional pigs and 42.6±4.7 kg for organic pigs. Two weeks after surgery pigs ran their final test on the treadmill, identical to the procedures described for Study 1.

Group 1 Lelystad, Study 3: Influences of Genetics

To investigate the influence of genetic background on cardiovascular performance, two purebred strains (Piétrain and Duroc) and one crossbred strain (Dalland) were compared for cardiovascular performance during exhaustive treadmill exercise. The Duroc and Piétrain strains differ in growth phenotype; Duroc pigs and their offspring have been found to grow faster, but also have more backfat. Piétrain-sired pigs are leaner than Duroc-sired pigs (Edwards et al, 2008). Piétrain pigs have 5 to 10% more meat than comparable pigs of other breeds (Houba and te Pas, 2004). The Dalland pig was a synthetic line of Piétrain and Large White (de Vries and Loenen, 2007). After the Dalland breeding group merged into the TOPIGS group, the Dalland sow was renamed as the TOPIGS 40 sow. For this study, six Duroc, six Dalland and six Piétrain gilts, age were all obtained from TOPIGS (Vught, The Netherlands). All three strains consisted of 2 BW weight groups compassing 3 pigs: Duroc 24.7 ± 0.3 kg (8.0 ± 0.2 wk) and (34.8 ± 1.0 kg (11.9 ± 0.3 wk), Dalland 25 ± 0.4 kg (7.8 ± 0.4) and 35.1 ± 0.6 kg (11.9 ± o.4 wk) and Piétrain 25,3 ± 0.3 kg (8.2 ± 0.5 wk) and 35.4 ± 0.5 kg (12.3 ± 0.5 wk). All 3 BW- matched pigs of each strain were kept in one pen until surgery, after which pigs were housed individually. Two weeks after surgery pigs ran three tests (as described under study 1) on Monday, Wednesday and Friday within one week.

Group 2 Rotterdam, Study 4: Cardiovascular performance in young pigs

Twenty-eight Yorkshire X Landrace pigs, 8-12 weeks old, 23±1 kg at the time of surgery of either sex were obtained from a commercial breeding farm and housed separately. After surgery, pigs performed exercise test at 10±1 days (26 pigs) and 24±1 days (16 pigs). Pigs were placed on the treadmill and aortic blood pressure, cardiac output, heart rate and stroke volume were measured at rest and during a consecutive 5-stage exercise protocol with pigs exercising at 1, 2, 3, 4, and 5 km/hr and each stage lasting 2 -3 minutes.

2.2.4 Statistical analysis

The different experiments have different number of observations per animal during rest and exercise at different weights. The cardiac output of the animals was measured at rest and during exercise at each weight. Therefore a randomly selected weight of each animal was taken for the analysis with the corresponding CO observations at rest and during exercise.

The mean heart rate, the mean cardiac output and mean stroke volume were compared in rest versus during maximal exercise using a linear mixed effects model (Pinheiro, 2007) with a normal distribution for the outcome with pig as random effect to model the correlation between the repeated observations within pig. The random effect was assumed to have a normal distribution.

To model the function $Y= a.M^b$ the data were analysed using a linear mixed effects model (Pinheiro, 2007) with a normal distribution for the outcome with pig as random effect to model the correlation between the repeated observations within pig. This formulae was e-log transformed to estimate the constant a and regression coefficient b: log(Y) = log(a) + b*log(M). The random effect was assumed to have a normal distribution. This model was fitted with cardiac output (Y) as the dependent variable and with the body mass (M) as the independent variable. Also an interaction term between the weight and the physical performance level was added to the model to estimate the coefficients a and b for both performance levels separately. The same model was used with stroke volume as dependent variable. The maximum likelihood method was used to estimate the parameter effects. The best model per experiment was selected using the AIC criterion (Pawitan, 2001). The 95% confidence intervals were based on the degrees of freedom calculated by the analysis. The models were fit using the statistical program R, version 2.5.1 (R Development Core Team, 2007). The Lelystad and Rotterdam data were analysed separately and combined.

2.3 Results

Table 1 shows average hemodynamic data at rest and during strenuous exercise of Lelystad pigs of group 1 (studies 1, 2 and 3) and Rotterdam pigs of group 2 (study 4). Strenuous exercise of both groups of pigs resulted in a near doubling of the CO. This was principally due to an increase in heart rate, as SV was either unchanged (group 2) or even slightly decreased (group 1).

Table 1. Hemodynamics at rest and during strenuous exercise

	Group 1 Lelystad (n=32; 57±3 kg)		**Group 2 Rotterdam (n=28; 28±1 kg)**	
	Rest	Exercise	Rest	Exercise
Cardiac output (L/min)	9.1 ± 0.5	16.4 ± 0.6 *	3.6 ± 0.1	8.0 ± 0.2*
Heart rate (bpm)	125 ± 4	249 ± 4 *	120 ± 4	256 ± 1 *
Stroke volume (mL)	73 ± 3	66 ± 2 *	31 ± 1	32 ± 1

All data are presented as means ± SEM; *$P < 0.05$ versus corresponding Rest.

To test the hypothesis that cardiac output and stroke volume of modern growing pigs are proportional to its body size as predicted by the quarter scaling laws (H_0 of CO : b = 0.75; H_0 of SV : b = 1.0)**, we** plotted CO and SV as a function of M for the Lelystad and Rotterdam pigs (Figure 1).

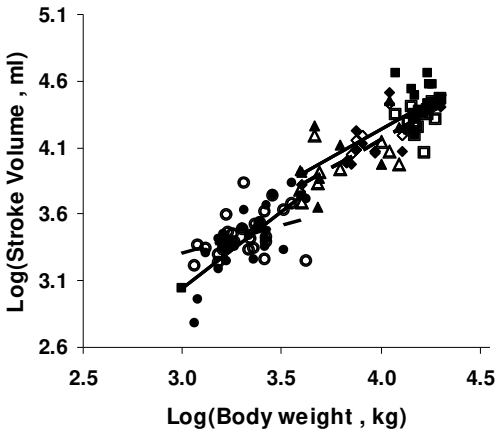

Figure 1 A.

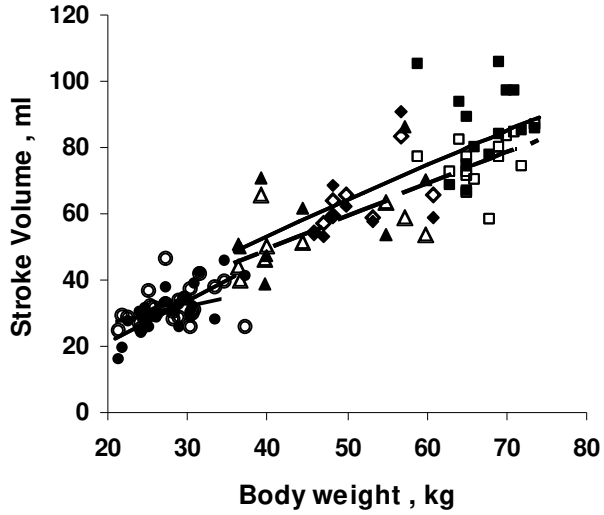

Figure 1 B.

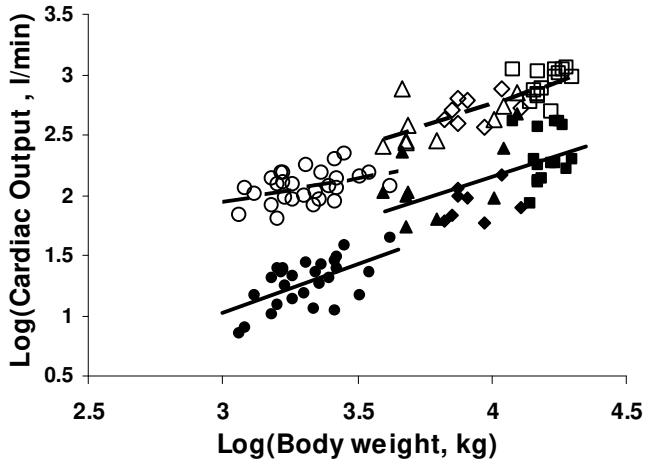

Figure 1 C.

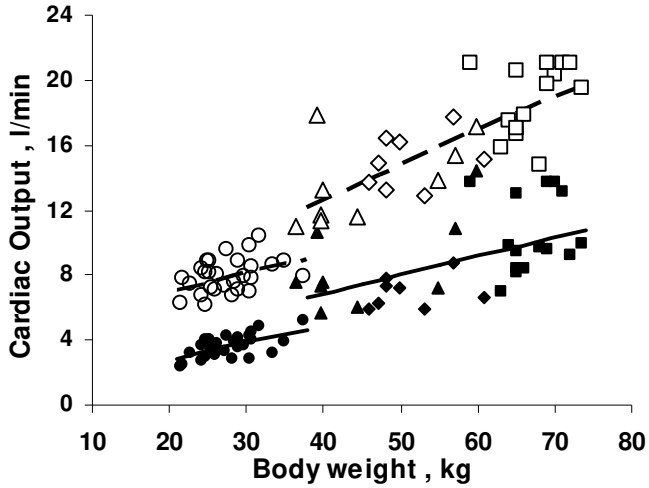

Figure 1 D.

Figure 1: Relations between stroke volume and body weight (panels A and B) and cardiac output and body weight (panels C and D).

Within group 1 there was no effect (*P*>0.05) of exercise training (probably due to the relatively mild intensity and short duration of each training session and overall training period), husbandry or genetic strain on cardiac output and stroke volume. Therefore pigs from Studies 1,2 and 3 were pooled.

Table 2 shows the expected exponent of CO of 0.75 falls within the 95% confidence interval of all the Lelystad and Rotterdam pigs at rest and during exercise. The expected exponent of SV of 1.00 falls also well within the 95% confidence interval of all the Lelystad and Rotterdam pigs at rest and during exercise except the exercising pigs of study 4 (95% CI: 0.02 to 0.85).

Table 2. Confidence intervals of Coefficient b

		Cardiac Output	Stroke Volume
Group 1 Lelystad	Rest	0.41 - 1.04	0.63 - 1.05
	Exercise	0.40 - 1.03	0.63 - 1.05
Group 2 Rotterdam	Rest	0.43 - 1.20	0.74 - 1.50
	Exercise	-0.01 - 0.76	0.02 - 0.85
Total	Rest	1.03 - 1.26	1.05 - 1.25
	Exercise	0.80 - 1.03	0.86 - 1.06

Shown are the 95% confidence intervals for coefficient b for cardiac output ($CO = a\ BW^b$) and stroke volume ($SV = a\ BW^b$). Group 1 Lelystad: n = 32, BW= 57±3 kg; Group 2 Rotterdam: n = 28, BW= 28±1 kg).

2.4 Discussion

Domestication of the pig has resulted in a dramatic increase of muscularity, litter size and growth rate. At the beginning of the 19th century pigs needed 2 to 3 years to reach a body weight of 40 kg (Huisman, 1969). Currently**,** fattening pigs reach their slaughter weight of 110 kg within a half year. The RHW (heart weight normalized to BW) declines during growth. At four weeks the RHW of the piglet is 0.82 % and at 110 kg it is decreased to 0.25 to 0.30%, which is low in comparison with the adult wild boar (0.64 %), horse (0.7 to 1.1%) and man (0.5%) (Von Engelhardt, 1966). The RHW of domesticated pigs at the end of the 19th century was 0.45 % (Huisman, 1969). Thus, the rate of RHW declined from 0.45 to 0.25 % during the last century and is similar to the rate of RHW decline from 0.64 to 0.45% that occurred during the foregoing 9000 years of domestication. Modern growing pigs exhibit a significant decrease of blood volume during growth. The relative blood volume of a 20-kg growing pig was 117 ml/kg decreasing to 68 ml/kg at around 110 kg bodyweight (Yang and Lin, 1997). Wild boar has a substantially higher haemoglobin level; the blood of wild boar is able to carry 25 to 40 % more oxygen than that of modern pigs of the same body weight (Von Engelhardt, 1966). Wachtel (1963) measured CO of anesthetized pigs and wild boar, and observed levelling off of the CO in growing fattening pigs with BW above ~50 kg. Thus, the CO of the

heaviest pigs with body weights ranging from 91-96 kg were almost similar to the CO of the 43-53 kg group. All these observations raise concern regarding a loss of proportionality, scaling and adaptability of the cardiovascular system of growing pigs even at body weights of up to 75 kg, as compared to their weight-matched wild counterparts.

Von Engelhardt (1966) and Niewold et al (2000) reported the relatively small heart, low blood volume and low haemoglobin level of growing pigs and they raised concern about their cardiovascular stability and adaptability. However, information regarding the pig's cardiovascular system in relation to body size is fragmentary and especially cardiac output measurements in conscious growing pigs were lacking to date. Consequently, in the present study we addressed this question by collecting and analysing CO and SV data of 60 conscious growing pigs with body weights ranging from 22 to 75 kg, both at rest and during strenuous exercise. The latter was done, in light of the concern that modern fattening pigs have a poor cardiac adaptability to stress. All pigs showed adaptation of the CO during strenuous exercise with an approximate doubling of CO. In both groups of pigs the increase in CO was mainly due an increase in HR, as SV was unchanged (group 1) or slightly decreased (group 2). An essentially unchanged SV is in accordance with other exercise studies in young farm pigs (Stubenitsky *et al.*, 1998; Merkus *et al.*, 2007) and adult miniature pigs (Hastings *et al.*, 1982; Armstrong *et al.*, 1987).

Many physiological processes scale with animal size in a surprisingly simple fashion. Indeed, the cardiovascular system and its components, like heart weight, cardiac output, heart rate and blood volume scale with quarter power of body mass The observed scaling is typically a simple power law: $Y = a M^{b}$, where Y is some observable for example cardiac output, "a" is a constant, M is the body mass of the animal and the exponent b almost invariably approximates a multitude of 1/4. (West and Brown 2005). We tested the hypothesis that CO and SV of modern growing pigs are proportional to their body size as predicted by the quarter scaling laws (H_0 of CO: b = 0.75; H_0 of SV: b = 1.00). The calculated 95% confidential range (table 2.) of the coefficient b for CO of all groups encompassed 0.75 and the 95% confidential range for SV encompassed 1.00 for all groups except the exercised pig of study 4 (0.02 to 0.85). The different confidence interval of SV of the last group is remarkable and difficult to explain, but may be the result of the relatively small body weight range within the Rotterdam study. This is also suggested by the observation that analysis of pigs from both Lelystad and Rotterdam groups combined yielded 95% confidence limits that stay well above

0.75 (Table 2). Therefore, we submit that our investigation indicates proportionality and scaling of the cardiovascular system of growing pigs with BW until 75 kg.

2.4.1 Methodological Considerations

Although our study of CO and SV in conscious pigs is somewhat limited by BW range 28±1 kg (group 2) and 57±3 kg (group 1)), by different pig breeding, laboratories and slight differences in pig instrumentation, all sixty pigs were modern growing fattening pigs and the cardiac output was by all pigs measured by direct blood flow measurement on the pulmonary artery or ascending aorta. Consequently, any major aberration from the scaling law (i.e. for CO: $b \leq 0.50$ and for SV: $b \leq 0.75$) should have been revealed in the present study. Nevertheless, the results from this study cannot be extrapolated to make any meaningful statements regarding the proportionality of CO and SV with body weight of heavier pigs like (200 to 300 kg). Such future studies in adult heavy modern fattening pigs are clearly warranted to identify important factors in the unexplained pathogenesis of severe multi-factorial animal disease (Niewold *et al.*, 2000). Furthermore, pigs are extremely useful as an animal model to study diseases like obesity, diabetes mellitus and cardiovascular disease (Von Engelhardt, 1966; Verdouw *et al.*, 1998). The supposed disproportional development of the cardiovascular system could be an advantage to study cardiac overload and or cardiovascular consequences of overweight in human beings.

2.4.2 Conclusions

There is growing concern that modern fattening pigs display cardiovascular insufficiency during stressful events like weaning, fighting or transport due to impairments in cardiovascular performance. During stress, blood flow is redistributed away from the intestines toward active muscle groups (Duncker *et al.*, 2001). Circulatory insufficiency will aggravate this redistribution of blood flow away from the intestines, the risk of intestinal ischaemia (Krack *et al.*, 2005). Intestinal ischaemia may subsequently lead to impairment of the gut barrier and result in increased translocation of gut microbes into the bloodstream (Niewold *et al.*, 2000), increasing the likelihood of circulatory disease, impairment of animal welfare, and also a greater risk of food poisoning of pork consumers. Confirmation of this concern may require reconsiderations of adjustments in the current genetic selection process, the way in which pigs are raised or both.

These concerns about disproportional growth and development of modern fattening pigs were, notwithstanding the limitations, not confirmed by the present study in pigs with body weights up to 75 kg. Future studies in adult heavy pigs of over 250 kg body weight are required to assess whether scaling proportionality of cardiac output and stroke volume with body mass are maintained at these extreme body weights.

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CHAPTER 3

Cardiovascular performance of adult breeding sows fails to obey allometric scaling laws.

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Abstract

In view of the remarkable decrease of the relative heart weight (HW) and the relative blood volume in growing pigs we investigated whether HW, cardiac output (CO) and stroke volume (SV) of modern growing pigs are proportional to body weight (BW), as predicted by allometric scaling laws: HW (or CO or SV) = $a \cdot BW^b$, in which b is a multiple of 0.25 (quarter power scaling law). Specifically, we tested the hypothesis that both HW and CO scale with BW to the power of 0.75 (HW or CO = $a \cdot BW^{0.75}$) and SV scales with BW to the power of 1.00 (SV = $a \cdot BW^{1.0}$). For this purpose, two groups of pigs (Group 1, consisting of 157 pigs of 50 ± 1 kg and Group 2, consisting of 45 pigs of 268 ± 18 kg) were instrumented under open-chest anesthetized conditions to measure CO and SV, with a flow probe or thermo-dilution, and for measurement of HW. The 95% confidence intervals of power-coefficient b for HW were 0.74 to 0.80, encompassing the predicted value of 0.75, suggesting that HW increased proportionally with BW according as predicted by the allometric scaling laws. In contrast, the 95% confidence intervals of power-coefficient b for CO and SV as measured with flow probes were 0.40 to 0.56 and 0.39 to 0.61, respectively, while values obtained with the thermo-dilution technique amounted 0.34 to 0.53 and 0.40 to 0.62, respectively. Thus, the 95% confidence limits failed to encompass the predicted values of b for CO and SV of 0.75 and 1.0, respectively. In conclusion, although adult breeding sows display normal heart growth, cardiac performance appears to be disproportionately low for BW. This raises concern regarding the health status of adult breeding sows.

3.1 Introduction

Domestication of pigs started ~9,000 years ago, and has resulted in marked differences in anatomy and physiology between domesticated pigs and their ancestors, the wild boar (Darwin, 1868; Hemmer, 1990; Jones, 1998; Giuffra *et al.*, 2000). During the last century in particular, pig breeding has been dominated by selection based on meat quantity and other economic aspects, yielding remarkable increases in body weight (BW), muscularity and litter size (Müller *et al.*, 1999; Saunders and McLeod, 1999), which has inadvertently resulted in alterations of anatomy and physiology of the cardiovascular system of the domesticated pig, including lower relative heart weight (HW), blood volume and haemoglobin levels (Von Engelhardt, 1966; Huisman, 1969; Schürmann, 1984). This process has raised concerns regarding cardiovascular capacity and adaptability to stress of modern pigs (Von Engelhardt 1966), and the increased risk of circulatory insufficiency might even contribute to Oedema Disease and transport-associated health problems (Niewold *et al.*, 2000).

In wild animals, HW and CO have been shown to scale with BW to the power of 0.75, blood volume scales with BW to the power of 0.25 and stroke volume (SV) scales with the power of 1.00 (West *et al.*, 1997; Yang and Lin, 1997; West and Brown, 2005). Recently we tested the scaling hypothesis for CO and SV in growing domesticated pigs with BW ranging from 22 to 75 kg at rest and during strenuous exercise (van Essen *et al.*, 2009) and found that, in domestic pigs with a BW that is still within the range of their wild ancestors, CO and SV scaled to BW as predicted by the quarter scaling laws (H_0: $CO = a \cdot BW^{0.75}$; and H_0: $SV = a \cdot BW^{1.0}$). Whether CO and SV scale proportionally with BW in pigs with BW of 200 to 350 kg has not been studied to date. This is important, because these heavy pigs appear more prone to health problems (Niewold *et al.*, 2000).

Consequently, in the present study we investigated the proportionality of the cardiovascular system in pigs over a wide range of BW, including adult breeding sows with a BW of 3 to 4 times the average BW of an adult female wild boar. Specifically, we tested the hypothesis that HW and CO scale with BW to the power of 0.75 ($HW = a \cdot BW^{0.75}$ and $CO = a \cdot BW^{0.75}$) and SV scales with BW to the power of 1.00 ($SV = a \cdot BW^{1.0}$).

3.2 Materials and Methods

All studies were performed in accordance with the Council of Europe Convention (ETS123)/ Directive (86/609/EEC) for the protection of vertebrate animals used for experimental and other scientific purposes, and with approval of the Animal Care Committees of the Erasmus University Medical Center Rotterdam (Group 1) and the Animal Science Group of Wageningen University and Research Centre (ASG of WUR) (Group 2).

3.2.1 Experimental Groups

Studies were performed in two groups of pigs. Group 1 consisted of 157 growing Yorkshire x Landrace pigs of either sex (female or neutered male; age 2 to 6 months; weight 23 to 97 kg) which were studied in Rotterdam. Group 2 consisted of 45 sows (TOPIGS; age 11 to 43 months; weight 170 to 338 kg; parity 1 to 11) which were studied in Lelystad. The Rotterdam data were obtained as part of several ongoing cardiovascular studies under general anesthesia (van Kats *et al.*, 2000; van der Velden *et al.*, 2004; Sorop *et al.*, 2008; Duncker *et al.*, 2009; te Lintel Hekkert *et al.*, 2010) and the Lelystad data were obtained from a group of pregnant sows delivering full-term piglets by a caesarean section under general anaesthesia for the porcine-specific pathogen free (SPF) unit.

Pigs of Group 1 arrived one week prior to surgery at Rotterdam, and were denied access to food starting 12 hr before surgery. The pregnant sows of Group 2 arrived from different breeding farms in the early morning on surgery day at the Lelystad operation theatre. They were kept until pre-medication without access to food in a pen in the preparation department of the surgery room.

3.2.2 Surgical Instrumentation and CO Measurements

Group 1.

Pigs were sedated with ketamine (20 mg/kg, i.m.) and midazolam (0.5 mg/kg, i.m.), anesthetized with sodium pentobarbital (15 mg/kg, i.v.) and intubated for ventilation with O_2 and N_2 (1:2 v/v) (van der Velden *et al.*, 2004; Sorop *et al.*, 2008; Duncker *et al.*, 2009; te Lintel Hekkert *et al.*, 2010). A catheter was inserted into the jugular vein and advanced into the superior caval vein for infusion of sodium pentobarbital (10-15 mg/kg·hr^{-1}, i.v.) to maintain anesthesia. In 37 pigs, a Swan-Ganz thermo-dilution catheter (Corodyn^{TD}, Braun, Melsungen, Germany) was inserted into a femoral vein, advanced into the pulmonary artery, and

connected to a CO computer (Edwards Lifesciences, Irvine, California) for measurement of CO (Sorop *et al.*, 2008; Duncker *et al.*, 2009). In 93 other pigs, the chest was opened via the sternum, and an electromagnetic flow probe (Skalar, Delft, The Netherlands) was positioned around the ascending aorta for measurement of CO. After stabilization, CO measurements were obtained with either thermo-dilution (using injections of 10 mL of ice cold saline; measurements were performed in triplicate) or with the Skalar electromagnetic flow probe. In 70 pigs (in 44 of which CO was also measured) the heart was excised, rinsed and weighed.

Group 2.

Sows received azaperone (2 mg/kg, i.m.) as a premedication 15 min before induction of general anesthesia. Ten minutes later, sows were cleaned and prepared for surgery including local infiltration anaesthesia of the left abdominal wall with ca 100 to 150 mL lidocaïne (2 mg/mL, i.m.). After induction with propofol (1 mg/kg, i.v.) sows were intubated and mechanically ventilated (tidal volume 13 mL/kg and 13 breaths/min.) with a mixture of air and O_2 (3:2) and 2.5% sevoflurane. The sows were delivered by a caesarean section via a mid-lateral incision in the left lateral abdominal wall. Uterine- and abdominal incisions were closed with sutures and sows were turned on their back for CO measurements. Anaesthesia was maintained with 2.5% sevoflurane, Midazolam (5 mg/kg·hr^{-1}, i.v.) and sufentanyl (1 µg/kg·hr^{-1}, i.v.). A Swan-Ganz thermo-dilution catheter (131HF7, Edwards Lifesciences) connected to a CO computer (Edwards Lifesciences) was inserted into the right jugular vein and advanced into the pulmonary artery. The chest was opened via a sternotomy, and the pericardial space was opened and a transit-time CO flow probe (20A; Transonic Systems Inc, Ithaca, New York**)** was placed around the ascending aorta. The pericardial space was filled with 0.9% NaCl to carry out reliable CO measurements. A pulse-oximetry sensor was placed on the auricle to measure oxygen saturation and heart rate.

Following stabilization, 10 thermodilution measurements were obtained per sow (10 mL saline solution at room temperature for injection into the Swan-Ganz catheter with 2 min interval time) simultaneously with the CO measurements. At the end of each saline injection the CO value on the flow meter was simultaneously recorded to allow direct comparison of the 2 techniques. At the same moment heart rate was registered using the pulse-oximeter and checked these values with the simultaneously counted heartbeats, visible via the open chest. After completion of these measurements these seven sows, as well as the 37 other

sows that underwent caesarean delivery (but without hemodynamic measurements), were sacrificed. After measurement of the post-partum BW, the heart was removed and the large vessels were cut nearest to the heart base and the atria, after which HW was determined.

3.2.3 Statistical analysis

Inter- (Group 1 versus Group 2) and intra-group (flow probe versus thermo-dilution) comparisons were performed using unpaired and paired t-tests as appropriate. The scaling coefficients of the relations between BW, as an independent variable, and CO, SV and HW as dependent variables were determined using a linear mixed model. Experimental study location (Rotterdam versus Lelystad) was treated as a random effect and assumed to have a normal distribution. To linearize the function $CO = a \cdot BW^b$ the natural logarithm was taken: $\log(CO) = \log(a) + b \cdot \log(BW)$. The same transformation was applied to SV and HW. The AIC criterion was used to select the best model. The assumptions for this model were checked by studying the residuals. The CO and SV were analyzed separately for the measuring technique (flow probe and thermo dilution). The library NLME (Pinheiro et al, 2007) of the statistical package R version 2.7.0 (R Development Core Team, 2008) was used for the analysis. Data are presented as mean ± SE, unless otherwise stated.

3.3 Results

Table 1 shows the characteristics of Groups 1 and 2 for the anatomical and hemodynamical studies. Pigs in Group 2 had considerably higher BW, HW, CO and SV than in Group 1 (all $P < 0.001$), while heart rates were similar in both groups ($P = 0.80$). Moreover, in both groups, CO values as measured with the thermo-dilution technique were significantly higher than those obtained with the flow probe technique in both Group 1 ($P = 0.001$) and Group 2 ($P = 0.03$). These differences can be attributed, at least in part, to the position of the flow probe around the ascending aorta distal to the coronary arteries (which encompass ~5% of CO), but may also be related to slight overestimation of flow by the thermo-dilution technique (see 'Discussion' section below).

Relative values (normalized to BW) of HW, and particularly of CO and SV were consistently lower (all $P < 0.001$) in the adult sows of Group 2, as compared to the younger pigs of Group 1 (Table 1), confirming earlier observations (Von Engelhardt 1966; Huisman, 1969; Schürmann, 1984; Niewold *et al.*, 2000).

Table 1. Anatomical and hemodynamic data

	Group 1 Rotterdam (N = 157)	**Group 2 Lelystad (N = 45)**
Anatomical Study	N = 70	N = 45
BW (kg)	47 ± 3	243 ± 5 *
HW (g)	199 ± 10	717 ± 20 *
HW/BW (g/kg)	4.4 ± 0.1	3.0 ± 0.1 *
Flow Probe Studies	N = 93	N = 7
BW (kg)	49 ± 1	268 ± 18 *
Heart Rate (bpm)	102 ± 2	104 ± 7
CO_{FP} (L/min)	3.6 ± 0.1	8.0 ± 1.1 *
CO_{FP}/BW ($mL \cdot min^{-1} \cdot kg^{-1}$)	76 ± 2	30 ± 4 *
SV_{FP} (mL)	36 ± 1	84 ± 21 *
SV_{FP}/BW (mL/kg)	0.75 ± 0.02	0.32 ± 0.07 *
Thermo-dilution Studies	N = 37	N = 7
BW (kg)	40 ± 2 †	268 ± 18 *
Heart Rate (bpm)	119 ± 2 †	104 ± 7
CO_{TD} (L/min)	4.2 ± 0.2 †	9.9 ± 1.2 *†
CO_{TD}/BW ($mL \cdot min^{-1} \cdot kg^{-1}$)	109 ± 4 †	38 ± 5 *†
SV_{TD} (mL)	36 ± 2	101 ± 20 *†
SV_{TD}/BW (mL/kg)	0.92 ± 0.03 †	0.39 ± 0.08 *†

Data are mean ± SE. BW = body weight; HW = heart weight; CO = cardiac output; SV = stroke volume; FP = flow probe; TD = thermo-dilution. *P<0.05 Group 2 vs corresponding variable in Group 1; †P<0.05 Thermo-dilution Studies vs corresponding value in Flow Probe Studies.

To test the hypothesis that HW, CO and SV of modern growing pigs and adult sows are proportional to BW as predicted by the quarter scaling laws (H_0 of HW : b = 0.75; H_0 of CO : b = 0.75; H_0 of SV : b = 1.0), HW, CO and SV were plotted as a function of BW. Figure 1 shows an

increase of HW that is commensurate with the increase in BW according to the formula HW = a $BW^{0.75}$, indicating that HW follows natural scaling laws.

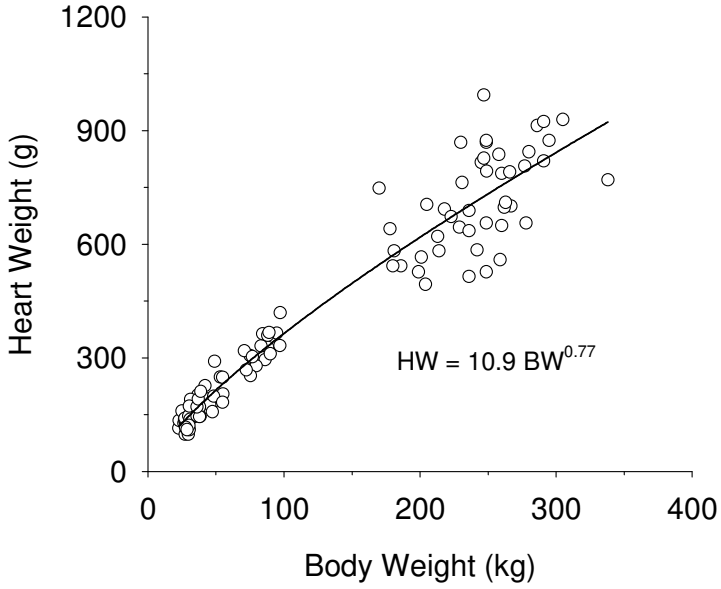

Figure 1. Relation between body weight (BW) and heart weight (HW) in 115 pigs.

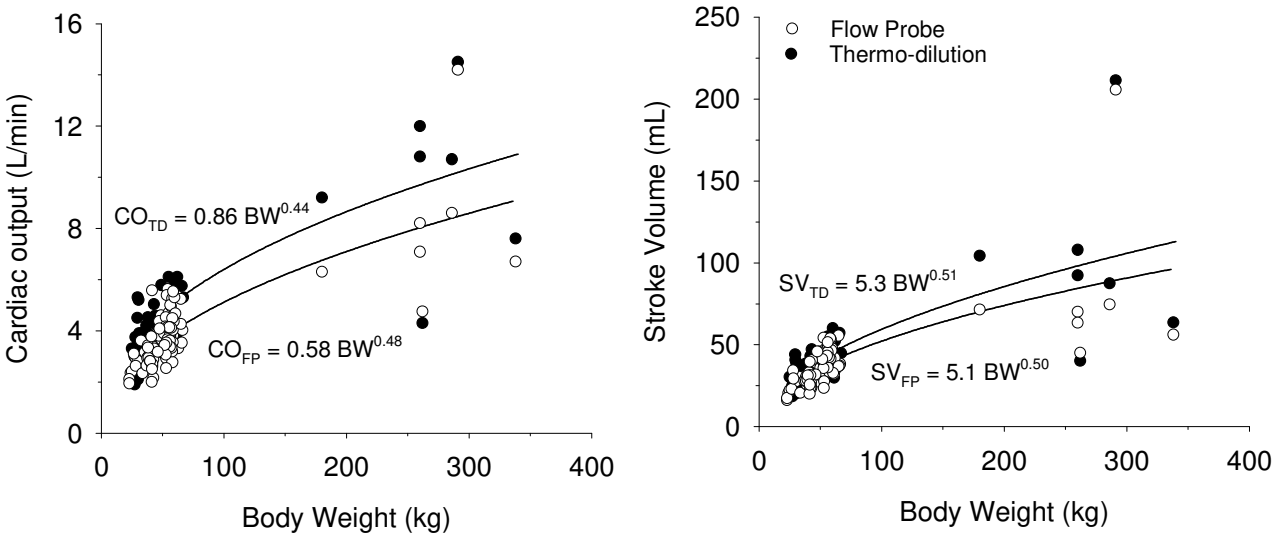

Figure 2. Relations between body weight (BW) and cardiac output (CO) and stroke volume (SV) as measured with a thermo-dilution (TD) catheter in the pulmonary artery (closed circles; n=44) or a flow probe (FP) around the ascending aorta (open circles; n=100).

Table 2 also shows that the expected exponent of HW of 0.75 falls well within the 95% confidence interval. In contrast, Figure 2 shows that CO and SV in the adult sows is lower than expected based on BW, with estimated values of b being close to 0.50 for both CO and SV. Furthermore, the expected exponent of SV of 1.00 and the expected exponent of CO of 0.75 fall well outside the 95% confidence intervals of value b (Table 2), irrespective of the technique that was used to measure CO and SV.

Table 2. Confidence intervals of Coefficient b

	Lower	Mean	Upper
HW	0.74	0.77	0.80
CO_{FP}	0.40	0.48	0.56
CO_{TD}	0.34	0.44	0.53
SV_{FP}	0.39	0.50	0.61
SV_{TD}	0.40	0.51	0.62

Shown are the 95% confidence intervals for and the mean value of exponent b for HW ($HW = a.BW^{b)}$, CO ($CO = a \cdot BW^{b}$) and SV ($SV = a \cdot BW^{b}$). BW = body weight; HW = heart weight; CO = cardiac output; SV = stroke volume; FP = flow probe; TD = thermo-dilution.

3.4 Discussion

Von Engelhardt (1966) and Niewold *et al.* (2000) reported relatively small HW, low blood volume and low haemoglobin level of growing pigs and raised concern about the cardiovascular stability and adaptability of modern fattening pigs. However, information regarding the pig's cardiovascular system in relation to BW is fragmentary and especially CO measurements in growing pigs were lacking to date. Recently we (van Essen *et al.*, 2009) addressed this question by collecting and analysing CO and SV data of 60 growing pigs with BW ranging from 22 to 75 kg, both at rest and during strenuous treadmill exercise. The results of that study showed for the first time that both CO and SV obeyed allometric scaling laws in pigs up to 75 kg, not only under resting conditions, but also during treadmill exercise. However, in that study BW of the pigs under study remained well below the range of BW reached in modern farming with pigs that reach up to 100 to 120 kg for meat production and even up to 250 kg in case of reproduction sows. Consequently, the aim of the present study

was to test the hypothesis that HW, CO and SV of growing pigs and adult pregnant sows are proportional to their body size as predicted by the quarter scaling laws as proposed by West and Brown (2005). Specifically we tested the hypothesis that HW and CO scale with BW raised to the power of 0.75 ($HW = a \cdot BW^{0.75}$ and $CO = a \cdot BW^{0.75}$) and SV scales with BW raised to the power of 1.00 ($SV = a \cdot BW^{1.0}$).

Many physiological processes scale with animal size in a surprisingly simple fashion. The cardiovascular system and its components, like HW, CO, SV and blood volume have been proposed to scale allometrically with BW (West *et al.*, 1997; Agutter and Wheatley, 2004; West and Brown, 2005; Dewey *et al.*, 2008). The observed scaling is typically a simple power law: $Y = a\ BW^{b}$, where Y is an observed variable for example CO, "a" is a constant, and the exponent b almost invariably approximates a multiple of 1/4 (West *et al.*, 1997; Agutter and Wheatley, 2004; West and Brown, 2005), although the value of exponent b is subject to debate (Agutter and Wheatley, 2004; Painter, 2005; Glazier, 2008). For example, several early interspecies studies have suggested that HW scales with BW with an exponent value of 1.0 (see Brown *et al.*, 1997), whereas intraspecies studies have shown that HW scales to BW with a scaling power of 0.75. Thus, in wild pigs (Von Engelhardt, 1966) as well as in cross-bred domestic pigs up to either 110 kg (Yang and Lin, 1997) or 100 kg (present study), HW was found to scale allometrically to BW with a scaling exponent of 0.70 (Von Engelhardt, 1973), 0.75 (Yang and Lin, 1997) and 0.80 with a 95% confidence interval of 0.73 to 0.87 (present study), respectively. The present study showed that the 95% confidence interval of the coefficient b for HW of all pigs encompassed the predicted value of 0.75, demonstrating for the first time that also in pigs well over 100 kg, HW continues to scale to BW raised to the power of 0.75.

In contrast, the 95% confidence intervals of exponent b for CO and SV clearly failed to encompass the predicted values of 0.75 and 1.0, respectively (West and Brown, 2005). The mean estimate of exponent value b for CO was 0.48 for flow probe measurements and 0.44 for thermo-dilution measurements. The calculated mean estimates of exponent b for SV were 0.50 for flow probe data and 0.51 for thermo-dilution data. Taken together, these data indicate that whereas HW scales with BW according to the allometric scaling law: $HW = a.\ BW^{0.75}$, both CO and SV do not scale with BW according to the 0.75 and 1.00 allometric scaling laws, respectively. Thus, cardiac performance of adult pregnant sows is clearly less than expected based on their BW. It should be acknowledged that the exact value of exponent b

with which metabolism (and thus CO) scales to BW (being either 3/4 or 2/3) is subject to debate (West *et al.*, 1997; Agutter and Wheatley, 2004; West and Brown, 2005; Painter, 2005; Glazier, 2008). However, irrespective of whether the exponent b is closer to 3/4 or 2/3, it is important to note that the upper limits of the 95% confidence intervals of exponent b for both CO and SV were even well below the value of 2/3. In contrast, analysis of pigs of Group 1 alone yielded average values of exponent b for CO and SV as determined by flow probe of 0.63 and 0.74, respectively, with 95% confidence intervals of 0.45 to 0.81 and 0.54 to 0.94, respectively, encompassing both the values 2/3 and 3/4. Taken together, these findings clearly indicate that CO and SV were low in the large pigs relative to their BW.

The consequent increased risk of circulatory insufficiency or failure may contribute to persistent pig diseases such as edema disease and transport-associated health problems (Niewold *et al.*, 2000). In the present study, we did not determine regional blood flows to investigate which organs and tissues experienced hypoperfusion. However, visceral organs such as the small intestine, to which blood flow is reduced even during physiological stress such as exercise (Armstrong *et al.*, 1987), are particularly prone to hypoperfusion during exercise in the presence of circulatory insufficiency (Haitsma *et al.*, 2001). In addition, there is evidence that skeletal muscle comprised of principally oxidative fibers displays a reduced vascular flow capacity in animals with circulatory insufficiency and appears to be more sensitive than skeletal muscle comprised principally of fast glycolytic fibers (McAllister *et al.*, 1993). Future studies are required to investigate the consequences for regional organ and tissue perfusion of the relatively low CO in large pigs.

There are several methodological aspects of the present study that need to be taken into account when interpreting our data sets. These include inter-group differences in (*i*) CO measurement techniques, (*ii*) physiology due to pregnancy of sows in Group 2, (*iii*) anesthesia regimen, and (*iv*) potential inter-group differences in body fat percentage. First, studies were performed in two different experimental centers and using different flow probe systems. Thus, in Group 1 CO was assessed using an electromagnetic flow probe whereas in Group 2 transit-time flow probes were used. There is evidence that electromagnetic flow probes may yield slightly (~15%) higher flows than transit-time flow probes (Hartman et al, 1994; Buffington et al, 2004; Flynn et al, 2006), which could have resulted in a lower b value. However, the thermo-dilution technique that was employed in both centers yielded highly similar results in terms of b values as compared to the flow probe data, indicating that flow

probe results were not due to differences in flow probe measurement techniques. Second, studies were performed under different anesthesia regimen in Groups 1 and 2. In Group 1, measurements were performed under pentobarbital anesthesia, whereas in Group 2, pigs were anesthetized with midazolam, sufentanyl and sevoflurane. We have previously shown that in pigs, pentobarbital (van Kats *et al.*, 2000) is significantly more cardiodepressive (in terms of negative inotropic effects) than the combination of midazolam-fentanyl anesthesia (van Woerkens *et al.*, 1992), although CO was only slightly (<10%) lower under pentobarbital than under midazolam-fentanyl anesthesia. Yet, these small differences are unlikely to have had a significant effect on our estimation of b, but if anything will tend to overestimate it, because CO would be expected to be lower in the sows if they had been studied under pentobarbital anesthesia as well. A third confounding factor could be the effects of pregnancy on hemodynamics. Thus, all mature sows in Group 2 had just delivered, as opposed to the adolescent growing pigs in Group 1. Indeed, gestation causes an increase of blood volume, mainly in the last trimester of gestation (Anderson *et al.*, 1970). This physiological volume loading results in elevations of cardiac preload and a 15 to 30% increase in cardiac muscle mass (Mone *et al.*, 1996; Schannwell *et al.*, 2002), that together serve to produce a 10 to 35% increase in CO and SV by the end of pregnancy, which is principally directed towards the uterus and fetuses (Reynolds *et al.*, 1985). In the present study, these cardiovascular adaptations in the pregnant sows may have resulted in overestimations of HW, CO and SV. Hence, a disproportional increase in HW compared to BW, i.e. a low b value, may have been masked by the pregnancy-induced increase in HW in the sows. Conversely, the low b value of CO and SV may actually have been underestimated by pregnancy, indicating that a discrepancy between HW and CO or SV is clearly present. Furthermore, by adding the random effect for "experiment" (Rotterdam versus Lelystad) in the statistical model, differences in experimental circumstances were taken into account for the estimation of effects. Taken together, the differences between the experimental set-ups in Rotterdam and Lelystad may have resulted in masking of a disproportionate increase in HW (i.e. due to the pregnancy status of the adult sows), but cannot explain the disproportionate increase in CO and SV in adult sows.

Finally, it could also be argued that a progressive increase in fat percentage in larger pigs may have contributed to a lower value of exponent b for CO and SV, because metabolic demands and hence perfusion of fat tissue are lower than most other body tissues (Armstrong

et al., 1987). Body and carcass composition during growth of modern pigs appears to increase from ~14% at 20 kg of weight to ~22% at 70 kg (Fowler *et al.*, 1992; Mitchell *et al.*, 2001). Interestingly, the line of sows that was used in our study has an estimated fat percentage in the range of 20 to 24% which is much lower than the 40% fat values reported in earlier studies in pigs of 145 kg (Shields *et al.*, 1983), and likely reflects breeding selection over the past 20 years to further increase meat percentage (up to 60% in the TOPIGS lines). These observations suggest that the low CO in the large sows was not the result of an increase in fat percentage.

In conclusion, we previously reported that the proposed disproportionate development of the cardiovascular system in modern fattening pigs is not apparent in growing pigs with BW up to 75 kg, even when the cardiovascular system is stressed during (sub-)maximal exercise (van Essen et.al., 2009). The present study in young growing and adult pigs demonstrates for the first time that adult sows exhibit levels of CO and SV that are disproportionately low for their BW. Future studies in groups of young growing and (non-pregnant) adult pigs studied in the un-anesthetized state are needed to establish to what extent the low cardiac performance is exaggerated under stress conditions, such as treadmill exercise. Such studies should also include animals in the weights range of 100 to 120 kg in order to assess cardiovascular performance in pigs at slaughter weight.

3.5 References

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CHAPTER 4

Cardiovascular performance of modern pigs does not comply with allometric scaling laws.

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ABSTRACT: In view of increasing concerns regarding cardiovascular capacity and adaptability of modern pigs, we investigated the geometry and function of porcine hearts over a wide range of body weights (25 – 225 Kg). Specifically, we tested the hypothesis that heart weight (HW), left ventricular weight (LVW), cardiac output (CO), stroke volume (SV) and LV end-diastolic volume (LVEDV) scaled with body weight (BW) according to the allometric scaling laws, with HW (or CO) = $a \cdot BW^{0.75}$ and SV (or LVEDV) = $a \cdot BW^{1.00}$. Pigs (n=31) were anesthetized and surgically instrumented for assessment of hemodynamics and LV geometry and function. Following euthanasia, HW and LVW were determined and LV tissue processed for further analysis. The 95% confidence intervals of power-coefficient b for HW and LVW were 0.67 – 0.88 and 0.70 – 0.86, respectively, encompassing the predicted value of 0.75, indicating that both HW and LVW increased proportionally to BW. However, the 95% confidence intervals of power-coefficient b for CO (0.40 – 0.65) and stroke volume (0.52 – 0.83) were below the predicted values of 0.75 and 1.00, respectively, indicating impaired scaling of cardiac performance with BW. The latter did not appear to be the result of impaired LV contractile function, as indices of LV systolic function, including ejection fraction and the maximum rate of rise of LV pressure, were similar across weight ranges. In contrast, the 95% confidence limit of LVEDV (0.57 – 0.99) was below the predicted value of 1.00, suggesting that impaired scaling of SV with BW was due to disproportionally low values of LVEDV at higher BW. Tissue analysis revealed increases in myocardial collagen content and a shift from the compliant N2BA towards the stiff N2B titin isoform in hearts of pigs >150 kg. Together with concentric LV remodeling in response to the elevated systolic arterial pressure in pigs with increasing BW, these changes in collagen and titin may have contributed to impaired scaling of LVEDV and hence reduced SV. The pathological changes in the LV wall of heavy pigs parallels the changes in the heart muscle of obese, deconditioned humans. Thus, the modern heavy pig is a potentially useful animal model to study diastolic dysfunction in humans. In conclusion, our study provides further evidence for the concept that cardiac dimensions and performance do not scale with body weight in modern pigs according to allometric scaling laws. These observations justify the growing concerns regarding health status of modern domestic pigs.

Key words: modern pigs, body mass, cardiovascular system, heart mass, cardiac output, stroke volume, allometric scaling laws

INTRODUCTION

Modern pigs originate from wild boar [1] with domestication starting in the Near East as early as 9000 years B.C. [2]. Particularly, during the last century breeding of pigs has resulted in marked increases in litter size, body weights (BW) and muscularity[3, 4]. This has inadvertently led to alterations of the cardiovascular system, including lower relative heart weight, blood volume and hemoglobin levels[5, 6]. These changes have raised considerable concerns[5, 7] with respect to cardiovascular performance and adaptability of modern pigs to stress.

In mammals, heart weight (HW) mass and cardiac output (CO) have been shown to scale with body weight (BW) to the power of 0.75, and stroke volume (SV) scales with the power of 1.00[8-10]. The observed scaling is typically a simple power law: $Y = a \cdot BW^b$, where Y is an observed variable for example CO, "a" is a constant, BW is body weight of the animal and the exponent b almost invariably approximates a multitude of 0.25.

Previously we tested the scaling hypothesis for CO and SV in modern growing pigs and in adult sows at the end of their gestation, and found that while pigs up to 100 kg scaled proportionally with BW[11], adult sows demonstrated a disproportionally low CO and SV[12]. In our previous studies we included pigs that had a different genetic background, underwent different anesthesia regimen and were studied in different laboratory settings. Moreover, in our previous study we did not further investigate the mechanisms underlying the disproportionally low levels of SV in adult pigs. Consequently, in the present study we investigated the proportionality of the cardiovascular system over a wide range of BW (25 – 225 kg) in pigs of a single (female) sex, with a similar genetic background, undergoing invasive hemodynamic studies in a single laboratory using a uniform anesthesia regimen. Importantly, in the present study we also explored the mechanisms underlying the disproportionally low SV by assessing left ventricular dimensions as well as LV systolic and diastolic function, and by performing comprehensive myocardial tissue analysis.

MATERIALS AND METHODS

All studies were performed in accordance with the Council of Europe Convention (ETS123) and the Directive (2010/63/EU) for the protection of vertebrate animals used for experimental and other scientific purposes, and with approval of the Animal Care Committee of Erasmus University Medical Center Rotterdam.

Animals

Studies were performed in three groups of Yorkshire x Landrace pigs classified according to body mass: group 1 with body mass below 75 kg (n=10), group 2 with body mass between 75 and 150 kg (n=13) and group 3 with body mass of more than 150 kg (n=8). One week prior to surgery, pigs arrived for acclimatization at the central animal housing facility of Erasmus University Medical Center in Rotterdam. Twice a day, pigs were fed compound feed in accordance with their body weight and had free access to drinking water. Twelve hours before surgery, pigs were denied access to food.

Surgical Instrumentation and Measurements

Pigs were sedated with Tiletamine/Zolazepam (5 mg/kg), Xylazine (2.25 mg/kg) and atropine (0.03 mg/kg i.m.), anesthetized with sodium pentobarbital (6 mg/kg i.v.) and intubated for ventilation with O_2 and N_2 (1:3 v/v) A catheter was inserted into the right jugular vein and advanced into the superior vena cava for infusion of sodium pentobarbital (10-15 mg/kg·hr^{-1} i.v.) to maintain anesthesia. A fluid-filled catheter was also inserted into the right carotid artery for the measurement of mean arterial pressure.

A Swan-Ganz catheter (5 corodyn TD F7, Braun, Melsungen, Germany) was inserted into the left jugular vein, via a sheath introducer, and advanced into the pulmonary artery for the measurement of mean pulmonary artery pressure and pulmonary capillary wedge pressure, and for thermodilution-based measurement of CO (Abbott Laboratories, North Chicago, Illinois, USA). A 7F conductance catheter (CD Leycom, Hengelo, The Netherlands) was inserted into the left carotid artery, via a sheath introducer, and advanced into the left ventricle (LV) for measurement of LV volume. The conductance catheter was calibrated using the thermodilution CO measurements and hypertonic saline before LV volume measurements were obtained. Subsequently, the conductance catheter was replaced by a micro-manometer-tipped catheter (SPC-370s, Millar Instruments, Houston, USA) for measurement

of LV pressure (LVP) and its first derivative (LVdP/dt). LVdP/dt at a pressure of 40 mmHg ($LVdP/dt_{P=40}$) was used as an index of afterload-independent systolic cardiac function. Heparin (10.000 I.U., i.v.) was administered before arterial blood gasses and hemodynamics were measured.

The chest was opened via sternotomy and the pericardial space was opened. After stabilization, ultrasound echography recordings were made by placing the ultrasound probe directly onto the epicardium and obtaining short-axis recordings (Aloka SSD 4000; Aloka Company, Tokyo, Japan).

Just before sacrifice left ventricular biopsies were obtained, snap frozen and stored in liquid nitrogen until further analysis. Finally, animals were sacrificed, heart and lungs excised and weighed. After removal of the large vessels, total heart mass was determined. Then, the atria and right ventricle were dissected and LV weight was determined.

Analysis of collagen and titin in cardiac tissue samples

After excision of the heart, LV anterior myocardial tissue samples were cut, fixated in 4% buffered formaldehyde and embedded in paraffin. Slides of 4.5 µm were cut, deparaffinized and stained for histological analyses. Six to ten fields were examined in the endocardial and in the epicardial part of each slide, at 20x magnification. Collagen content was quantified using picrosirius red staining. Using a linear polarization filter, the area occupied by collagen type I, collagen type III fibers, as well as total collagen was measured and expressed as percentage of the myocardial area. All measurements were performed using a microscopy image analysis system (Impak C, Clemex Vision Image analysis system, Clemex Technologies, Quebec, Canada).Titin isoforms were separated on 1% agarose gel and stained with SYPRO Ruby protein stain as described previously[13].

Statistical analysis

Data are presented with pigs being divided into three arbitrarily chosen body weight categories: 25 – 75 kg, 75 – 150 kg, and 150 – 225 kg (Table 1). To assess the statistical significance of differences between variables in the three body weight categories, we used One-Way ANOVA, followed by Student-Newman-Keuls post-hoc testing when appropriate. Using a linear mixed model, the scaling coefficients of the relations between body weight (BW), as an independent variable and cardiac output (CO), stroke volume (SV), heart mass

(HW), left ventricular weight (LVW), left ventricular end-diastolic lumen volume (LVEDV) and left ventricular end-diastolic lumen area (LVEDLA) as dependent variables were determined. To linearize the function $CO = a.BW^b$ the natural logarithm was taken : $\log(CO) = \log(a) + b.\log(M)$. The same transformation was performed for all above mentioned dependent variables.

RESULTS

Table 1 shows the anatomic, hemodynamic, LV function and histology data for the three weight categories. HW and LVW as well as LV dimensions increased significantly with increasing body weights (all $P<0.05$). Cardiac output also increased with increasing body weight, which was the result of an increase in stroke volume (both $P<0.05$), as heart rates were not different significantly between the three weight groups. Mean pulmonary artery pressure and pulmonary capillary wedge pressure did not differ significantly between the three weight categories, but mean arterial pressure and LV systolic pressure were markedly elevated in the highest weight category (both $P<0.05$). LV systolic function, reflected in $LVdP/dt_{P=40}$ and both 2D and 3D ejection fractions, was not different between the weight groups. In contrast, although LV end-diastolic pressures were not different between weight groups, the time constant of relaxation was significantly increased in the highest weight category, indicating slowed LV relaxation. Histological analysis of LV tissue demonstrated an increase in total collagen and collagen type I (thick fibers) in the highest weight category (Table 1), and a shift from the compliant N2BA titin isoform towards the stiff N2B titin isoform evident from a lower N2BA/N2B ratio in the high compared to the low weight pigs (Table 1).

Table 1. Anatomical, hemodynamical, left ventricle functional and histological data

	25-75 kg (n=10)	75-150 kg (n=13)	150-225 kg (n=8)	ANOVA
Anatomical data				
Body Weight (kg)	46 ± 5	106 ± 4 *	182 ± 8 *†	<0.001
Heart Weight (g)	147 ± 10	367 ± 26 *	612 ± 33 *†	<0.001
LV Weight (g)	112 ± 7	233 ± 14 *	381 ± 17 *†	<0.001
LV End Diastolic Lumen Volume (mL)	92 ± 14	161 ± 18 *	288 ± 21 *†	<0.001
LV End Diastolic Lumen Area (mm^2)	1510 ± 90	2600 ± 310 *	2810 ± 160 *	<0.001
Hemodynamics				
Heart rate (bpm)	96 ± 7	85 ± 4	80 ± 8	0.164
Mean Arterial Pressure (mmHg)	84 ± 8	109 ± 4 *	138 ± 12 *†	<0.001
Mean Pulmonary Artery Pressure (mmHg)	22 ± 2	26 ± 2	27 ± 3	0.425
Pulmonary Capillary Wedge Pressure (mmHg)	9 ± 1	12 ± 1	12 ± 1	0.112
Cardiac Output (L/min)	4.3 ± 0.4	6.0 ± 0.4 *	8.5 ± 0.6 *†	<0.001
Stroke Volume (mL/beat)	45 ± 4	75 ± 9	115 ± 16 *†	<0.001
LV function				
LV Systolic Pressure (mmHg)	109 ± 4	122 ± 5	154 ± 14 *†	0.002
LV $dP/dt_{P=40}$ (mmHg/s)	1390 ± 140	1180 ± 80	1310 ± 50	0.316
2-D LV Ejection Fraction (%)	39 ± 3	44 ± 9	45 ± 3	0.470
3-D LV Ejection Fraction (%)	61 ± 4	60 ± 7	57 ± 5	0.881
tau (ms)	48 ± 2	54 ± 3	64 ± 6 *	0.034
LV End Diastolic Pressure (mmHg)	12 ± 2	16 ± 2	13 ± 2	0.365
Titin isoform composition N2BA/N2B				
Titin N2BA/N2B	1.70 ± 0.26	1.36 ± 0.08	0.75 ± 0.02 *†	0.015
Collagen				
Total (%)	3.1 ± 0.4	3.0 ± 0.4	5.8 ± 0.8 *†	0.006
Type I (%)	2.3 ± 0.4	2.7 ± 0.4	5.0 ± 0.6 *†	0.005

LV = left ventricular; LV $dP/dt_{P=40}$ = rate of rise in LV pressure at 40 mmHg; tau = time constant of LV pressure decay during early diastole; LV End Diastolic Lumen Area and 2-D LV Ejection Fraction measured with open chest (n=6, n=4 and n=9 respectively for each group); Titin and collagen were measured in a subset within each group (n=7/4, n=7/5 and n=4/4 respectively for each group); Data are mean±SEM; *P<0.05 vs <75 kg-group; †P<0.05 vs 75-150 kg-group

To test the hypothesis that cardiac weight, dimensions and performance of modern growing pigs are proportional to body weight (BW), as predicted by the quarter scaling laws, we plotted various parameters as a function of BW. HW and LVW increased with BW according to the formulas $HW = 9.97 \times BW^{0.78}$ and $LVW = 6.00 \times BW^{0.78}$ (Figure 1), with the predicted value of exponent b of 0.75[9,

14] falling well within the 95% confidence limits (Table 2), indicating that both HW and LVW scale proportionally with body mass according to the natural scaling laws.

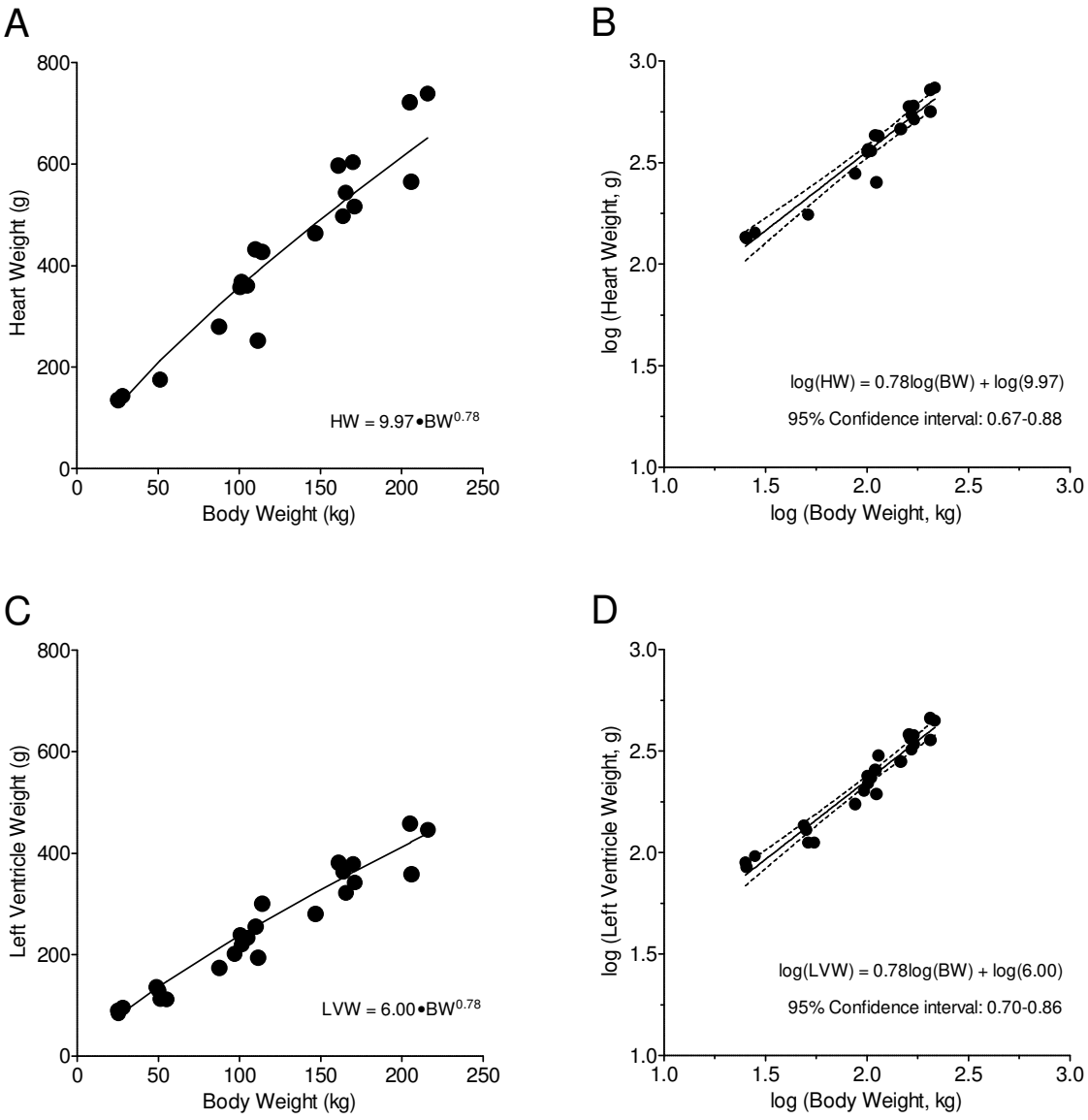

Figure 1 Relations between heart weight and body weight (panels A and B) and left ventricular weight and body weight (panels C and D).

Table 2. Confidence intervals of Coefficient b

	Lower	Mean	Upper	Predicted
Heart Mass	0.67	0.78	0.88	0.75
LV Mass	0.70	0.78	0.86	0.75
Cardiac Output#	0.40	0.52	0.65	0.75
Stroke Volume#	0.49	0.66	0.82	1.00
LV EDA (2-D)#	0.27	0.40	0.52	0.67
LV EDV (3-D)#	0.57	0.78	0.99	1.00

Shown are the 95% confidence intervals for coefficient b for anatomical and hemodynamic parameters of the pig heart; LV = left ventricle; EDA = end diastolic area; EDV = end diastolic volume, # is significantly different from predicted.

In contrast, CO and SV increased with BW described by the formulas: $CO = 0.55 \times BW^{0.52}$ and $SV = 3.51 \cdot BW^{0.66}$ (Figure 2), with the predicted exponents b of 0.75 for CO, and 1.00 for SV[9, 14] falling outside the 95% confidence intervals (Table 2), indicating that CO and SV failed to follow natural scaling laws.

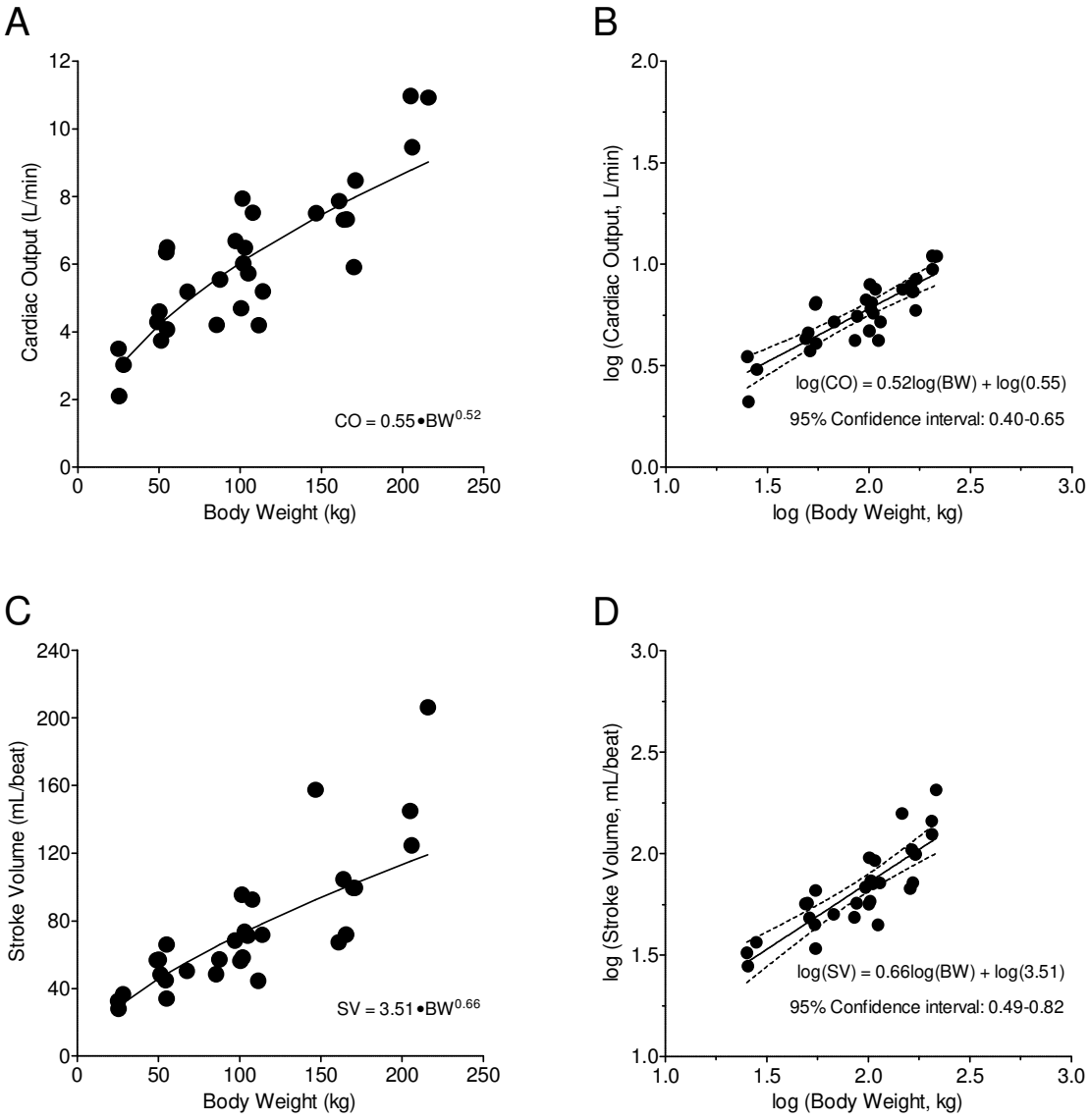

Figure 2 Relations between cardiac output and body weight (panels A and B) and stroke volume and body weight (panels C and D).

These observations were paralleled a similar disproportionality of LV dimensions. Thus, LV-EDA (measured with echocardiography) and LV-EDV (measured with the conductance catheter), increased with body weight according to the formula $LVEDLA = 370 \cdot BW^{0.40}$ and $LVEDLV = 4.24 \cdot BW^{0.78}$ (Figure 3), with the confidence limits failing to encompass the predicted exponents b of 0.67 for LV-EDA[15] and 1.00 for LV-EDV[8, 16] (Table 2).

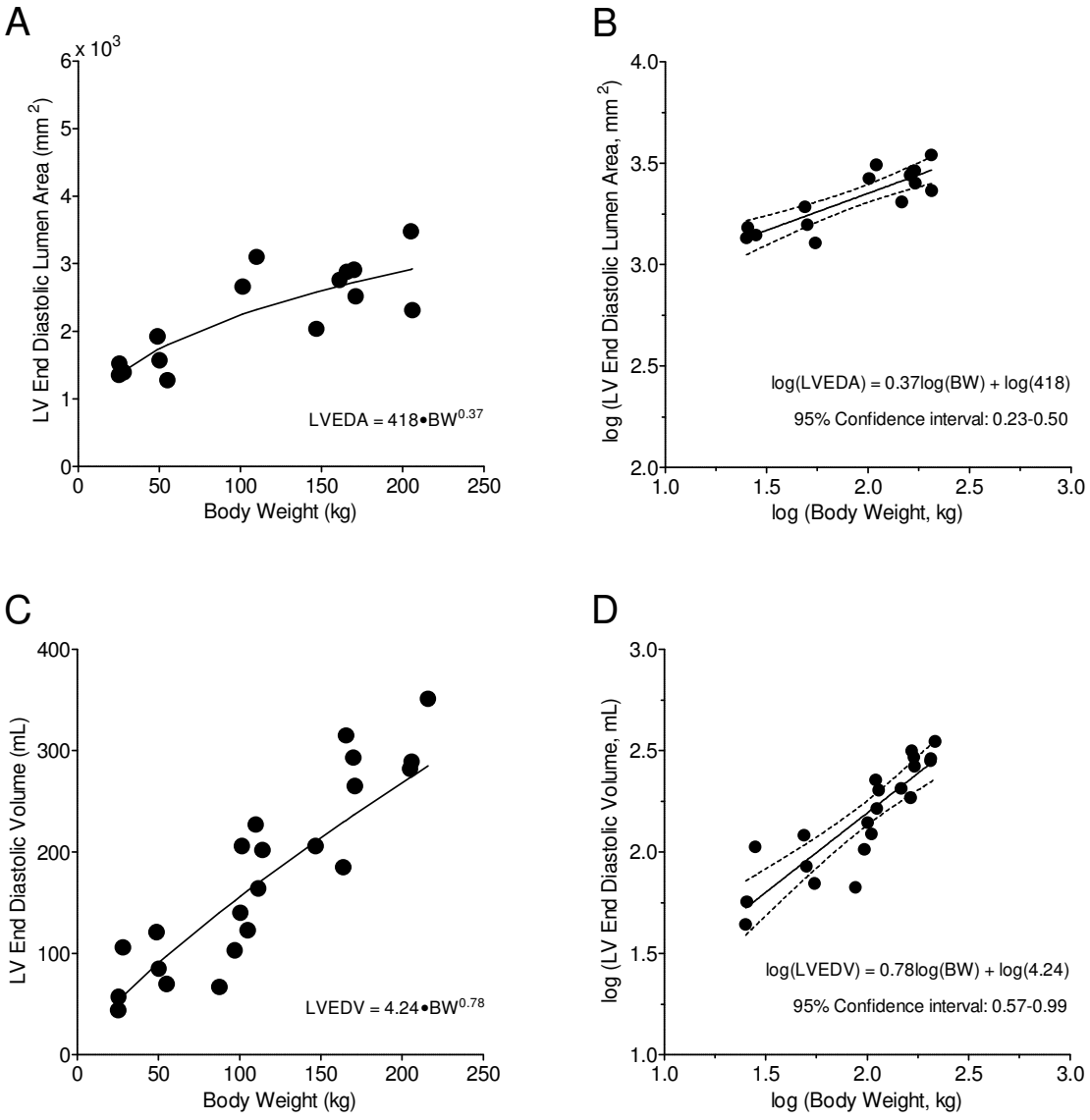

Figure 3 Relations between left ventricular end-diastolic lumen area and body weight (panels A and B) and left ventricular end-diastolic volume and body weight (panels C and D).

DISCUSSION

The present study was performed in modern farm pigs, covering a wide range of body weights, to test the hypothesis that cardiac weights, dimensions and performance obey quarter power scaling laws. The main findings were that (*i*) HW and LVW increase commensurately with BW according to the quarter power scaling laws; (*ii*) CO and SV failed to increase proportionally to BW, which (*iii*) was not due to impaired LV systolic function, but rather (*iv*) appeared due to a similar lack of proportional increase in LV dimensions, measured

with either echocardiography or conductance catheter. (*v*) Hemodynamic analysis revealed marked increases in arterial blood pressure, while analysis of myocardial tissue revealed increases in interstitial collagen content and in the stiff N2B titin isoform of the myocytes in animals with the highest BW. The wide range of implications of these findings will be discussed as follows.

Allometric scaling of the cardiovascular system in modern pigs

In 1932, Max Kleiber published a seminal paper, showing that metabolic rates among mammals varied with three–quarter power of body mass. Since then, many scientists have reported similar allometric scaling phenomena in mammals[17]. Allometric scaling is defined as a simple power law relation between body weight and various anatomical and physiological variables. West and Brown have proposed that metabolic rate plays a key role in determining the scale of biological phenomena and that the existence of a fourth spatial dimension in mammals explains why allometric scaling is quarter –power scaling[10, 14]. These biological quarter power scaling laws indicate that many biological variables scale to body weight to the power of b and the exponent b almost invariably approximates a simple multitude of ¼. Quarter power scaling of cardiovascular parameters is widely used in cardiovascular medicine, for example in pediatric clinical practice[18] and in veterinary practice[15, 19].

Using the quarter-power scaling laws we have investigated the cardiovascular proportionality of modern pigs[11, 12]. In those previous studies we found that while in growing and in adult pigs the heart weight was proportional to body weight, SV and CO were proportional only in pigs up to 75 kg[11], but were disproportionally low in sows at the end of their gestation[12]. However, in those studies pigs were included that had different genetic background, were either male or female, were subjected to different anesthesia regimen and were studied in different laboratory settings. Consequently, in the present study we included pigs of a single (female) sex, with similar genetic background, over a wide range of body weights, subjected to studies in a single laboratory using a uniform anesthesia regimen. The present study not only confirms our previous observations of disproportionally low values of SV and CO, but also investigated several mechanisms that could contribute to the low values of SV.

Mechanisms underlying the low stroke volume in adult modern pigs

A low SV can be caused by a decrease in systolic function. However, indices of LV systolic function, including $LVdP/dt_{P=40}$, and 2-D and 3-D ejection fractions were maintained suggesting that LV systolic function was well maintained in animals in the highest weight group. Importantly, a reduction in SV can also result from perturbations in diastolic function. Indeed, using two independent techniques to assess LV volume (conductance catheter) and LV short axis lumen area (echocardiography), we observed disproportionally low values of LV-EDV and LV-EDA. The exact mechanism for the disproportionally small end-diastolic volume in adult animals remains to be established, but several potential mechanisms could be proposed. First, the time constant of relaxation (tau) was increased indicating slowing of LV relaxation and impeding filling in the early rapid LV filling phase. Moreover, LV myocardial interstitial levels of total collagen and the thick fiber collagen type I were significantly higher in adult pigs, which acts to increase passive LV stiffness[20]. Moreover, we observed a shift from the compliant titin isoform N2BA to the stiff titin isoform N2B in LV tissue samples, reflected in a lower ratio of N2BA/N2B in the highest weight category (> 150 kg), which also acts to increase passive LV stiffness, and hence impair LV diastolic filling[21].

Importantly, we observed 40-60% higher LV systolic and mean arterial pressures in the highest body weight category. Chronic LV pressure overload can explain the increase in interstitial collagen levels and the impaired LV relaxation that we observed in the present study[22]. However, the 41% increase in LV systolic pressure would also be expected to result in LV hypertrophy. Yet, LV weight did not appear to be disproportionally increased according to the quarter power scaling law. Although an explanation for this unexpected finding is not readily found it could be speculated that LV weight in fact scaled proportionally to body weight just because of a hypertrophic response that may have acted to mask a disproportionally low LV weight in the heavy pigs. Future studies, using vasodilator drugs to reduce blood pressure, are required to test this hypothesis and investigate the scaling of LV weight to body weight in adult pigs in the presence of a normal blood pressure.

The present study provides further evidence for the concept that LV dimensions and performance do not scale with body weight in modern pigs according to allometric scaling laws, and shows that this is associated with pathological changes within the LV myocardium. The question then arises how domestication has led to structural and functional changes in the heart of modern pigs. Von Engelhardt[5] suggested that the small heart in relation to body

weight appears to be the result of selective breeding for growth rate, so that cardiac growth cannot keep up with body growth. Huisman[6] proposed that the relative low heart weight of modern pigs the result is of selective breeding and efficient diets. However, these suggestions were contradicted by studies that found that selection on reduction of back fat thickness and an increase of growth rate during eight generations in Yorkshire pigs resulted in a significant increase of organ weights including heart weight and a significant positive correlation between heart weight and food conversion efficiency[23] as well as a significant negative correlation between heart weight and back fat thickness in pure bred and cross bred pigs[24]. The latter authors proposed that the bigger relative heart size resulting from intensive selection for leanness may not necessarily reflect a returning to a natural form, but could also be an indication of pathophysiological changes such as hypertrophic cardiomyopathy[24]. Interestingly, the hypertension associated pressure-overload in our heavy domestic pigs may also have contributed to a higher heart weight, indeed suggesting that the return of heart weights towards "normal" may have a pathological origin and may not reflect natural form. Domestication of wild boar has clearly resulted in a decrease of relative heart weight while rigorous selection on performance characteristics in modern farm pigs , may instead have led to an increase in heart weight.

In addition to the influence of genetic selection, it is also likely that lifestyle of modern pigs contributes to the lack of scaling of LV structure and performance to body weight. Indeed, in humans lifestyle and physical (in)activity in particular is an important determinant of cardiovascular health[25]. The lifestyle of free living wild boar versus modern pigs in intensive pig industry differs markedly. Thus, wild boars are active about 12h per day to forage food (figure 1) and escape from predators, and can reach a speed of up to 40 km per hour. In contrast, modern pigs in intensive pig farming live a predominantly sedentary lifestyle, with a minimum of physical activity and a permanent load of the gastrointestinal tract to maximize body growth. We found a significant increase of both mean arterial pressure (MAP) with increasing body mass and a significant increase of the LV systolic pressure in the heaviest pigs (BW >150 kg). It is highly plausible that the sedentary lifestyle of modern pigs, with a marked decrease of physical activity during lifetime can cause physical deconditioning that contributes to the elevated arterial pressure with increasing bodyweight, resulting in LV pathology characterized by diastolic dysfunction and reduced LV compliance[26]. Increased systemic blood pressure is also well-known to be one of the most important risk factors for

human cardiovascular health, and associated strongly with overweight and obesity, but the possible pathophysiological changes due to increased body weight in humans are still scarce particularly at the level of the myocardium. From the cardiac perspective, the findings of the present study of disproportionally smaller LV size and increased LV wall stiffness associated with higher myocardial collagen content and titin isoform shift in adult modern farm pigs may therefore shed light on the mechanisms underlying cardiovascular risk due to lack of physical activity[27], overweight and obesity[28]. Altogether, further studies into lifestyle influences on blood pressure and ultimately on the myocardium of adult pigs are important to improve cardiovascular health of pigs in the farming industry, but also important to clinical medicine for the use of adult pigs as an animal model to study cardiovascular disease in humans.

Conclusions

The present study provides further evidence for the concept that cardiac dimensions and performance do not scale with body weight in modern domestic pigs, according to allometric scaling laws. The disproportionally low stroke volume was not the result of systolic dysfunction, but the result of diastolic perturbations, likely as a result of an increased myocardial interstitial collagen content in conjunction with marked elevations in aortic blood pressure. These findings underpin the growing concerns about intrinsic cardiovascular factors in modern domestic pigs, as well that of human inactivity and obesity, that may affect the animal's and individual's health. Future studies to evaluate possible health risks due to reduced performance of the porcine heart are warranted.

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CHAPTER 5

Summary and General Discussion

Based on G.J. van Essen, D. Merkus, D.J. Duncker.

Cardiovascular proportionality of modern pigs.

In preparation

5.1 Cardiovascular characteristics and proportionality of modern pigs

The cardiovascular system of modern pigs differs in many aspects, and probably in an unfavourable way, from the system of their ancestor, the wild boar. Some fifty years ago Von Engelhardt (1966) and Huisman (1969) reported that the relative porcine heart weight , the relative blood volume and the haemoglobin levels of modern pigs were all relatively low, as compared with wild boar. Consequently, Von Engelhardt (1966) and later Niewold *et al.* (2000) expressed concerns about the cardiovascular capacity, performance and adaptability to stress of modern domestic pigs. The question whether these differences are disadvantageous or even harmful to the health status of modern pigs, could at that time not be thoroughly investigated because knowledge of porcine cardiac physiology was fragmentary and reliable measurements of cardiac output in conscious pigs were lacking (Von Engelhardt, 1966).

One of the most important consequences of domestication and selective pig breeding has been the remarkable increase of body weight (**Chapter 1**). Thus, new-born piglets usually have a body weight of 1 – 1.5 kg and adult sows and boars can reach a body weight of 300 to 400 kg which is 3 – 4 times the body weight of adult wild boars (Müller *et al.*, 1999). In order to comprehensively assess the proportionality of the cardiovascular system of modern growing pigs from piglet to adult pig, it is not sufficient to merely compare relative heart weight (i.e. normalized to body weight) and normalized cardiac output and stroke volume values to those of wild boar. Clearly, a more generic and widely accepted biological reference is needed, which takes into account that many physiological variables are not linearly related to body mass, but rather appear to obey the quarter-power scaling laws (West *et al.*, 1997). The observed scaling is typically a simple power law: $Y = a\,BW^{b}$, where Y is an observed variable for example CO, “a” is a constant, BW is body weight of the animal and the exponent b almost invariably approximates a multitude of 1/4. Thus, in mammals, heart weight and cardiac output (CO) have been shown to scale with body weight (BW) to the power of 0.75, blood volume scales with BW to the power of 0.25, heart rate (HR) scales with the power of – 0.25 and stroke volume (SV) scales with the power of 1.00 (Holt *et al.*, 1968; West *et al.*, 1997 and 1999).

In this thesis, we investigated whether the heart of modern domestic pigs is proportional to body weight, by applying the allometric quarter scaling laws for cardiovascular variables. In our first study (**Chapter 2**), we investigated cardiovascular proportionality of conscious instrumented pigs at rest and during strenuous exercise, in pigs weighing less than

100 kg. To assess cardiovascular proportionality, we measured cardiac output, stroke volume and heart weight and plotted these variables as a function of body weight. We found that heart weight, cardiac output and stroke volume in these pigs scaled with body weight according the three-quarter power scaling laws, not only under quiet resting conditions, but even during strenuous exercise.

However, adult breeding sows and boars can reach a body weight of 300 – 400 kg and the results of our first study of pigs with body weights below 100 kg cannot be simply extrapolated to heavier pigs. Therefore we conducted a second study to investigate cardiovascular proportionality in heavy pigs, by measuring – under general anaesthesia – cardiac output, stroke volume and heart weight, pigs with body weights ranging from 20 to 350 kg (**Chapter 3**). The results of this second study demonstrated that heart weight was proportional to body weight, according the three-quarter power scaling laws, even in adult sows at the end of their gestation. In contrast, we observed disproportionally low values of cardiac output and stroke volume in the anesthetized adult sows. Admittedly, this second study suffered from a number of methodological limitations. Thus, we included pigs with a different genetic background, we used different anesthesia regimens, we studied adult pigs at the end of their gestational period, and animals were studied in different laboratories, using partly different techniques for assessing cardiac output and stroke volume. Moreover, as the study was in part retrospective, we were not able to further investigate the mechanisms underlying the disproportionally low levels of cardiac output and stroke volume in adult pigs. To circumvent these limitations, we designed a third study, in which we investigated the proportionality of the cardiovascular system over a wide range of body weights (25 – 225 kg) in pigs of a single (female) sex, with a similar genetic background, undergoing invasive hemodynamic studies in a single laboratory using a uniform anesthesia regimen and uniform methods for measurement of cardiac output and stroke volume (**Chapter 4**). In addition, we further explored the mechanisms underlying the disproportionally low stroke volume by assessing left ventricular dimensions as well as left ventricular systolic and diastolic function, and by performing myocardial tissue analysis. In accordance with the observations in **Chapter 3** we also found in **Chapter 4** that heart (and left ventricular) weight increased commensurately with body weight, according to the quarter power scaling laws, and that both cardiac output and stroke volume failed to increase proportionally to body weight. We found evidence that disproportional low CO and SV was not due to impaired left ventricular systolic

function, but instead appeared due to a lack of proportional increase in left ventricular dimensions, as measured with either echocardiography or conductance catheter. To investigate the mechanism(s) underlying the smaller left ventricular dimensions, we subsequently performed histological analysis of left ventricular myocardial tissue. Histological analysis revealed increases in interstitial levels of total collagen and type I collagen in the heaviest pigs (body weights greater than 150 kg). Moreover, protein analysis of myocardial tissue revealed an isoform shift in titin expression from the compliant isoform N2BA in pigs with body weights less than 150 kg to the stiffer isoform N2B in pigs with body weights greater than 150 kg. The increases in collagen and titin isoform shift (through an increased stiffness of the left ventricular wall) likely contributed to the disproportionally small left ventricular end-diastolic volume. Interestingly, hemodynamic analysis revealed marked increases in mean arterial pressure in pigs with body weights greater than 150 kg. The resultant increase in left ventricular afterload may have contributed to the observed stiffness promoting alterations in collagen and titin isoform shift, and may have contributed to the increase in time constant of relaxation (tau), and hence the disproportionally low left ventricular dimensions. In addition, it could be speculated that the increase in afterload produced concentric left ventricular hypertrophy, that not only masked a disproportionally low left ventricular weight in pigs with body weights greater than 150 kg, but also contributed to further increase in left ventricular chamber stiffness and hence the small left ventricular dimensions.

Taken together, the results described in Chapters 2-4 demonstrate that heavy adult pigs display disproportionally low levels of stroke volume and cardiac output, that are likely caused by early (relaxation) and late (stiffness) diastolic dysfunction resulting in disproportionally low left ventricular dimensions.

5.2 Physiological implications of cardiovascular disproportionality of modern farm pigs.

Heavy fattening pigs live a distinct sedentary lifestyle with barely any physical exercise. In contrast, the gastrointestinal tract is almost continuously active and filled with food, which serves to realise the incredibly rapid growth that occurs in modern fattening pigs. So most of the time pigs are in a parasympathetically dominant state, with focus on feed digestion and blood supply to the gastro-intestinal tract. The disproportionally low values of stroke volume and cardiac output in these heavy, often lethargic, pigs are, under these predominantly

parasympathetic conditions, unlikely to cause systemic circulatory insufficiency and inadequate perfusion of the loaded gastro-intestinal tract.

However, when a pig is exposed to external stressors it will respond with a stress response, which requires abrupt adjustments of the cardiovascular system. Pigs generally respond with a rapid flight reaction (the fight- flight response) to sudden threats in their environment, but individuals may differ in their coping capacities and style (Koolhaas *et al.*, 1999). Such a flight response causes a large increase of oxygen demand of skeletal muscles, that requires significant cardiovascular adaptations in order to increase oxygen transport and supply to the active skeletal muscle groups. The sympathetic nervous system rapidly becomes dominant over the parasympathetic nervous system and through adrenergic receptor stimulation ensures increases in both respiration, frequency and tidal volume and circulation, increasing of cardiac output mainly through increasing of heart rate. Moreover the increases in noradrenaline (released from the sympathetic nerve endings) and adrenaline (by the adrenals into the blood), contribute to the redistribution of cardiac output. Thus, blood is redistributed away from the skin and abdominal organs towards the active skeletal muscle groups to facilitate increases in muscle oxygen supply. Within a very short period of time, the pig has ended up in a completely different and partly opposite physiological situation in which the relatively low heart volumes can be a risk of the onset of circulatory insufficiency or failure. The necessary increase of cardiac output will be almost exclusively realised by increasing heart rate (HR). We observed in our exercise studies (**Chapter 2**) that heart rate increase is limited to approximately a twofold or threefold of the HR at rest, so tachycardia induced by strenuous exercise is in the order of 275 – 325 beats per minute. Because pigs, compared to other species, have an unfavourable diastole/systole time ratio 0.6 – 0.8 versus 1.26 for the wild boar (Huisman, 1969), ventricular diastolic filling time is limited at high heart rate, resulting in a slight decrease of stroke volume. In addition to the unfavourable effect of shortening of diastole on stroke volume, shortening of diastole could be detrimental to subendocardial blood flow. However more recent research conducted by Duncker and Bache(2008) has shown that diastole/systole time ratio is less abnormal than suggested buy Huisman (1969). Duncker and Bache (2008) indicates that in pigs performing strenuous exercise, the left ventricular transmural blood flow distribution reflected in the subendocardial to subepicardial blood flow ratio was not different from that in dogs and horses. Moreover pigs demonstrate residual subendocardial vasodilator capacity even during maximal heart rates, suggesting that

the risk of myocardial ischemia due to, exercise-induced, tachycardia is minimal, also in the pig. Importantly in this thesis we observed a pronounced decrease of stroke volume in heavy pigs, that could be attributed to smaller left ventricular dimensions, that were likely a result of pathophysiological alterations within the left ventricular chamber wall. It could be speculated that these pathophysiological phenomena contribute to reduced cardiovascular capacity and reduced adaptability to stress in heavy farm pigs.

In addition to exercise associated with an acute stress situation, it should be noted that any disruption of homeostasis or health threat in mammals, like an infection with pathogenic agents require an adequate cardiovascular response or adaptation. Also many physiological processes like gestation in sows or physical activity or exercise require cardiovascular adaptations. Limited cardiovascular performance and adaptive capacity of heavy pigs can therefore be considered a threat of animal health and welfare.

A proven and accurate method to assess cardiovascular performance and capacity of pigs is to exercise instrumented pigs on a treadmill like we did in our exercise studies with pigs up to 75 kg (van Essen *et al.*, 2009) but to carry out these kind of investigations in pigs with body weight of 100 – 300 kg is extremely difficult and probably unfeasible. We do not have a reliable alternative for this exercise test causing an acute physical load of the cardiovascular system but we did investigate stroke volume and cardiac output of anesthetized sows at the end of their gestation, which is a natural physical stressor of the cardiovascular system. Although this load is gradually imposed over a prolonged period of time (± 115 days) and results in pregnancy-induced increases of total blood volume (Anderson *et al.*, 1970) and cardiac output (Reynolds *et al.*, 1985), we found disproportional low values of cardiac output and stroke volume in these adult heavy sows, again suggesting inadequate capability to cope with a natural stressful situation.

In conclusion a well-functioning and adequately responsive cardiovascular system is crucial for all animal species – including pigs –to maintain homeostasis and to stay healthy, whereas our findings suggest that this is unlikely to be the case in heavy pigs. From that point of view our findings support and underscore the concerns regarding porcine cardiovascular adaptability expressed by Von Engelhardt (1966) and Niewold *et al.* (2001). However, further research is needed to further clarify the consequences of disproportional low values of stroke volume and cardiac output in heavy pigs.

5.3 Social implications of cardiovascular disproportionality of modern farm pigs.

Our Western society has become increasingly critical of intensive pig farming which is primarily related to issues such as animal welfare, food safety, animal health, the excessive use of antibiotics and the risk of transmission of pathogens from animals to humans. Importantly cardiovascular disproportionality of heavy pigs can hamper animal welfare, animal health and thereby may compromise food safety for humans.

5.3.1 Welfare and cardiovascular disproportionality.

Defining animal welfare is not as easy as it seems. In response to concerns raised in Ruth Harrison's 1964 book, Animal Machines the British government commissioned in 1965 an investigation, led by Professor Roger Brambell, into the welfare of intensively farmed animals The Brambell Committee first formulated 'the five freedoms of animals', animals has to be free of obvious causes of distress such as hunger, thirst, pain, anxiety etc. This Brambell report was updated by the British Farm Animal Welfare Council (BFAWC) in 1993. The BFAWC added recommendations on an appropriate environment including a comfortable berth and conditions to exhibit natural behaviour such as sufficient space and the presence of conspecifics. Despite the longstanding recognition of these 'five freedoms of animals', the available living space for pigs in the regular intensive pig farming continues to be very limited, thereby reducing the ability to exhibit natural behaviour and physical exercise. These environmental restrictions combined with the lethargic nature of adult modern domestic pigs result in an extremely sedentary lifestyle. Overweight and lack of exercise are seen as risk factors for humans in the development of hypertension and left ventricular diastolic dysfunction. Perhaps in a similar way, the sedentary lifestyle of pigs can be held responsible (at least in part) for the significantly increased arterial blood pressure, increased myocardial interstitial collagen and stiffer titin isoform in heavy pigs, as observed in **Chapter 4**. The increased pressure-load on the left ventricle will finally result in concentric hypertrophy, which together with the increased collagen deposition and stiffening of titin, will result in increased stiffness of the left ventricular wall, decreased left ventricular end-diastolic volume and hence decreased stroke volume and cardiac output.

5.3.2 Animal health, food safety and cardiovascular disproportionality.

Niewold *et al.* (2000) first suggested a pathophysiology-based approach to pig diseases by examining the role of intrinsic animal factors in multi-factorial pig diseases with unclear pathogenesis. They proposed that insufficient cardiac capacity of modern domestic pigs could contribute to the pathogenesis of Oedema Disease and transport-related diseases.

Oedema Disease is mostly found in piglets two weeks after weaning with the highest incidence in the fastest-growing piglets. The pathogenesis is not fully clarified but enterotoxaemic strains of E. Coli producing both adhesive fimbriae an the shiga-like (SLT-IIv) toxin are considered to be the cause of Oedema Disease (Bertschinger *et al.*, 1990). But this 80,000 MW toxin can only pass the intestinal wall to be absorbed when intestinal barrier function is impaired and permeability is increased. Impairment of intestinal barrier will occur when intestinal pH decreases due to insufficient perfusion of rapidly growing intestines of very fast growing piglets. The balance between increasing perfusion needs of the intestines and blood supply to the intestines appears to be very delicate and thereby vulnerable to perturbations (Niewold *et al.*, 2000). Following weaning a 'pig's life task' begins, eat to grow. Food intake, intestinal surface and intestinal blood supply increase rapidly during first weeks after weaning. At the age of approximately 4-6 week old piglets are socially interactive and still physically active, spending a considerable amount of time playing and fighting. From a physiological point of view, physical exercise with filled intestines is not an ideal combination because the increased oxygen demand and blood supply of active skeletal muscle will go at the expense of intestinal blood supply so that prolonged exercise can result in intestinal ischaemia, followed by intestinal acidosis and finally in impaired gut barrier function and increased permeability of the intestinal wall. This pathophysiological cascade may explain the observed absorption of the major toxin molecules like SLT–IIv toxin causing vascular damage and oedema and finally Oedema Disease. Given explanation of increased intestinal permeability in piglets post weaning seems to be plausible because heat stress can cause in a similar way in growing pigs increased intestinal permeability. Heat stress induces increased perfusion of the skin to increase heat dissipation and this redistribution of blood flow caused intestinal ischemia resulting in impaired intestinal barrier integrity (Pierce *et al.*, 2013). But the suggestion that high intestinal activity in mutable post weaning piglets is a risk for intestinal barrier integrity is contradicted by the results of the study conducted by Spreeuwenberg *et al.* (2001). They showed that especially the low food intake during the first four days post weaning together

with stress, as evidenced by elevated cortisol levels, caused the decrease of mucosal barrier integrity end an increase in intestinal paracellular transport.

Niewold et al (2010) suggested that the cardiovascular system of these piglets may become overloaded due to the high perfusion demand of the intestines. However, overloading of the cardiovascular system is, given our cardiovascular proportionality findings, not likely to occur in these newly-weaned piglets whose cardiovascular system is still in proportion to their body weight. Yet, this may play a role in transport related diseases in significantly heavier pigs.

Transport of fattening pigs from farm to slaughterhouse is invariably associated with stress, which is also demonstrated by Bradshaw *et al.* (1996) who found elevated cortisol serum levels in slaughter pigs from loading until delivery eight hours later. Transport also represents a major physical effort, particularly for animals that are not used to physical exercise due to their sedentary lifestyle. Preslaughter fasting of slaughter pigs is a common practise. The Belgian centre for applied pig research recommends, on the basis of a study of Frobose *et al.* (2014), a withdrawal of feed period of not more than 24 hours to limit losses in carcass weight (www.varkensloket.be, 2015). Stress and an empty gastrointestinal tract in slaughter pigs mimics the conditions in piglets post-weaning and similar pathophysiological mechanisms may occur that will result in impaired intestinal epithelial barrier function. But limited cardiovascular capacity of these heavy pigs may worsen intestinal ischaemia, thereby increasing intestinal permeability and causing absorption of toxic substances or intestinal bacteria. This is not only a threat to the individual animal but it is also a threat to food safety because gut bacteria like Salmonellae may end up in muscle tissue and can finally cause food poisoning in consumers.

In summary, cardiovascular disproportionality of heavy pigs can contribute to increased susceptibility to disease and reduced robustness of the animals, and to an increased risk of food poisoning for consumers. Both aspects go against the interests of society, because robust pigs reduce the burden of disease – which increases animal well-being – and reduces the use of antibiotics in pig industry which improves the safety of food of animal origin.

5.4 Implications of cardiovascular disproportionality for the pig industry.

The care for healthy animals and safe pork is the primary responsibility of the pig industry, which must deliver robust animals with sufficient resistance to diseases. Cardiovascular disproportionality can be seen as an intrinsic health risk that requires a solution. However, to

solve this problem we need to gain a deeper understanding of its aetiology. Is it a genetic disorder or an acquired abnormality: is this nature or nurture? Is it caused by selective breeding, strongly dominated by selection on performance traits like growth rate, carcass composition and meat quality?

Von Engelhardt (1966) argued that the small heart weights of domestic pigs appears to be the result of selective breeding because domestic pigs at the end of the 19th century had a greater relative heart weight (0.45%) than modern pigs (0.3%). He concluded that the rate of heart growth has failed to keep up with the rate of body growth. According to Huisman (1969), there are clear indications that the relatively low heart weight of modern pigs resulted from genetic selection and improved feed. The relative heart weight of modern pigs, (0.3%) is less than half of the relative heart weight of wild boar (0.64%) and Huisman (1969) proposed that this could result in a constantly overloaded heart during the growth of domestic pig. In short, the heavier the pig, the heavier the burden on the heart.

However, studies conducted by Cliplef and McKay (1993) and Yang and Lin (1997) showed that strong selection on economically relevant performance traits like growth rate, food conversion efficiency and reduced back fat resulted in significantly heavier hearts. This could be interpreted to suggest that perhaps these recent changes have resulted in mitigation of the cardiovascular pathology. However, Yang and Lin (1997) concluded that the bigger relative heart size caused by recent intensive selection for leanness should not be interpreted as returning to a natural form. These authors suggested that heavy selection pressure may have exceeded innate physiological limits and this unfitness leading the animal to exhibit pathophysiological changes, perhaps including hypertrophic cardiomyopathy. Interestingly, the hypertension associated pressure-overload in our heavy domestic pigs may also have contributed to a higher heart weight, indeed suggesting that the return of heart weights towards “normal” may have a pathological origin and may not reflect natural form.

Domestication of wild boar has clearly resulted in a decline of relative heart size, while intensive selection on modern performance traits in turn has led to an increasing heart size in modern pigs. Huisman (1969) and Yang and Lin (1997) assumed pathophysiological alterations of the porcine heart due to overload and heavy selection pressure, but they did not have sufficient evidence to substantiate their assumptions. In this thesis we have provided evidence for pathophysiological alterations in hearts of heavy modern pigs over 150 kg in body weight that are associated (likely through stiffening of the left ventricular myocardial wall), with a

reduction in left ventricular end-diastolic volume and hence reduced stroke volume and cardiac output. We also discovered elevated levels of arterial blood pressure, resulting in pressure-overload of the left ventricle in these heavy pigs, which is a stimulus for the development of concentric hypertrophy (Duncker *et al.*, 1993; van Deel *et al.*, 2011), The latter can further contribute to increased stiffness and decreased relaxation of the left ventricle chamber. It is in the interest of the pigs as well as the pig industry to continue this cardiovascular research in heavy pigs, in order to elucidate the etiology of increased myocardial wall stiffness and elevated arterial pressure. Recommendations for future research are provided below in chapter 5.6.

5.5 Relevance of our findings to human cardiovascular disease

It is likely that not only genetic selection but also the lifestyle of modern farm pigs contributes to the lack of scaling of LV structure and performance to body weight. Indeed in humans, lifestyle – particularly physical activity – is an important determinant of cardiovascular health (Booth *et al.*, 2012; Sharma *et al.*, 2015). The lifestyle of free living wild boar versus modern pigs in intensive pig farming differs dramatically. Thus, wild boars are active about 12h per day to forage food and escape from predators Figure 1), and can reach a speed of up to 40 km/h. In contrast, modern pigs in intensive pig farming live a predominantly sedentary lifestyle, with a minimum of physical activity and a permanent load of the gastrointestinal tract to maximize body growth.

Figure 1. Foraging wild boars

We found a significant increase in both arterial blood pressure with increasing body weight and a significant increase of the left ventricular systolic pressure in the heaviest pigs weighing over 150 kg. It is highly plausible that the sedentary lifestyle of modern pigs, with a marked decrease in physical activity during their entire lifetime causes physical deconditioning that predisposes to elevated arterial pressure with increasing bodyweight, resulting in LV pathology characterized by left ventricular diastolic dysfunction and reduced left ventricular compliance (Bhella *et al.*, 2014). Increased systemic blood pressure is also well-known to be one of the most important risk factors for human cardiovascular health, and associated strongly with overweight and obesity, but the possible pathophysiological changes due to increased body weight in humans are still scarce particularly at the level of the myocardium. From the cardiac perspective, the findings of the present study of disproportionally smaller LV size and increased LV wall stiffness associated with higher myocardial collagen content and titin isoform shift in adult modern farm pigs may therefore shed light on the mechanisms underlying cardiovascular risk due to lack of physical activity (Booth *et al.*, 2012), overweight and obesity (Aune *et al.*, 2016). Altogether, further studies into lifestyle influences on blood pressure and ultimately on the myocardium of adult pigs may not only aid in improving cardiovascular health of pigs in the pig farming industry, but may also prove a relevant animal model to study cardiovascular disease in humans.

5.6 Future directions

Our studies have shown that modern domestic pigs are characterized by a disproportionally small left ventricular chamber volume, leading to a small stroke volume and cardiac output. Our studies also indicate that an elevated arterial blood pressure, in conjunction with increases in collagen content and a shift towards the stiffer titin isoform N2B, which are well known substrates for increased stiffness properties of the left ventricular wall, are potential contributors to the disproportionally low values of ventricular volumes in heavy pigs. These findings raise several new research questions. For example, it is presently unclear what the prevalence and progression is of myocardial pathological changes in adult sows and boars of different ages. Moreover, the exact pathogenesis of increased collagen and isoform shift remain incompletely understood, as is the exact role of the elevation in arterial pressure in this process. Finally, it remains to be determined what the influence of lifestyle is on the cardiac abnormalities found in adult pigs. Answering these questions will yield more

knowledge about the abnormalities at the ventricular chamber and myocardial tissue level, and will allow evaluation of potential measures to improve cardiovascular performance and adaptability and ultimately the well-being of adult domestic pigs.

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CHAPTER 6

Nederlandse samenvatting

Het cardiovasculaire systeem van moderne varkens verschilt in veel opzichten van dat van hun voorvader, het wilde zwijn. Zo'n vijftig jaar geleden melden Van Engelhardt (1966) en Huisman (1969) dat zowel het relatieve hartgewicht als ook het relatieve bloedvolume en het hemoglobinegehalte van moderne varkens laag zijn in vergelijking met het wilde zwijn. Redenen voor van Engelhardt (1966) en later Niewold et al. (2000) om hun bezorgdheid te uiten over de cardiovasculaire capaciteit van moderne varkens en de adaptatiecapaciteit aan stress. Maar op de vraag of de geconstateerde veranderingen in het cardiovasculaire systeem nadelig of zelfs schadelijk voor de gezondheid van de moderne varkens zijn, kon op dat moment nog geen antwoord worden gegeven. Er was toentertijd onvoldoende bekend over de fysiologie van het varkenshart. Betrouwbare metingen van het hartminuutvolume in wakkere dieren bijvoorbeeld ontbraken toen nog (Van Engelhardt, 1966).
Een van de belangrijkste gevolgen van de domesticatie van het wilde zwijn en later van de strenge selectie in de varkensfokkerij is de opmerkelijke toename van het lichaamsgewicht (**Hoofdstuk 1**). Pasgeboren biggen wegen gemiddeld 1-1,5 kg terwijl volwassen zeugen en beren een lichaamsgewicht van 300 tot 400 kg kunnen bereiken. Dat is ca. 4 maal het lichaamsgewicht van volwassen wilde zwijnen (Muller et al., 1999). Om de proportionaliteit van het cardiovasculaire systeem van moderne vleesvarkens van biggetje tot volwassen varken te kunnen beoordelen, is het niet toereikend om uitsluitend het relatieve hartgewicht (dat wil zeggen genormaliseerd naar lichaamsgewicht) en de genormaliseerde hartminuutvolume - en slagvolume waarden te vergelijken met die van de wilde zwijnen omdat veel fysiologische variabelen niet lineair gerelateerd zijn aan het lichaamsgewicht. Het is duidelijk dat een meer generieke en breed geaccepteerde referentie nodig is zoals de allometrische schalingswetten die wel correct de relatie weergeven tussen een fysiologische variabele en het lichaamsgewicht. Allometrische schaling wordt beschreven als een eenvoudige macht: $Y = a\ BW^{b}$, waarin “Y” een waargenomen variabele is, bijvoorbeeld het slag volume, "a" een constante is en “BW” het lichaamsgewicht van het dier is waarbij de exponent b altijd een veelvoud van ¼ (0.25) is. In zoogdieren, schalen het hartgewicht en het hartminuutvolume met het lichaamsgewicht (BW) tot de macht 0.75, het bloedvolume schaalt met BW tot de macht 0.25, de hartslag (HR) schaalt met BW tot de macht - 0.25 en het slagvolume (SV) schaalt met BW tot de macht 1.00 (Holt et al., 1968; West et al., 1997 en 1999).
In dit proefschrift hebben we, door allometrische schalingswetten toe te passen op

cardiovasculaire variabelen, onderzocht of het hart van moderne varkens proportioneel is ten opzichte van het lichaamsgewicht. In onze eerste studie (**Hoofdstuk 2**), onderzochten we de cardiovasculaire proportionaliteit van wakkere geïnstrumenteerde varkens, met een lichaamsgewicht van minder dan 100 kg, in rust en tijdens zware lichamelijke inspanning. Om de cardiovasculaire proportionaliteit te kunnen bepalen, maten we het hartminuutvolume, slagvolume en hartgewicht en vervolgens werden deze variabelen uitgezet als functie van het lichaamsgewicht. We vonden dat het hart gewicht, het hartminuutvolume en het slagvolume in deze varkens, in overeenstemming met de allometrische schalingswetten, schaalden met het lichaamsgewicht en niet alleen in rust maar ook tijdens zware inspanning. Echter, volwassen fokzeugen en beren kunnen een lichaamsgewicht van 300 - 400 kg bereiken en de resultaten van deze eerste studie met varkens beneden de 100 kg kunnen niet zonder meer worden geëxtrapoleerd naar de veel zwaardere volwassen varkens. Daarom hebben we een tweede studie uitgevoerd om ook de cardiovasculaire proportionaliteit te onderzoeken van zware volwassen zeugen aan het einde van de draagtijd (3 maanden + 3 weken + 3 dagen) en van niet drachtige dieren die lichter waren dan 100 kg. Onder algehele narcose is bij al deze varkens, het hartminuutvolume, het slagvolume en het hartgewicht bepaald (**Hoofdstuk 3**). De resultaten van deze tweede studie toonden aan dat het hartgewicht, conform de allometrische schalingswetten, zelfs bij volwassen zeugen aan het einde van hun zwangerschap proportioneel is ten opzichte van het lichaamsgewicht. Daarentegen vonden wij bij de volwassen zeugen disproportioneel lage waarden voor het slagvolume en het hartminuutvolume. Daarbij dient wel te worden vermeld dat deze tweede studie een aantal methodologische beperkingen kende. Zo hebben we varkens met verschillende genetische achtergrond gebruikt, gebruikten we verschillende anesthesie regimes, werden de dieren bestudeerd in verschillende laboratoria, werden hartminuutvolume en slagvolume met verschillende technieken gemeten en daarbij hebben we deze cardiovasculaire parameters bij de zeugen gemeten aan het einde van de draagtijd. Bovendien, was de studie gedeeltelijk retrospectief, en waren we niet in staat om de mechanismen die ten grondslag liggen aan de disproportionele lage waarden van slagvolume en hartminuut volume bij zware varkens verder te onderzoeken. Om deze methodologische beperkingen te vermijden, hebben we een derde studie uitgevoerd waarin wederom de proportionaliteit van het cardiovasculaire systeem bij varkens met uiteenlopende lichaamsgewichten, van 25 tot 225 kg, is bepaald. Alle dieren waren van hetzelfde (vrouwelijke) geslacht, ze hadden dezelfde genetische

achtergrond, ondergingen, onder een uniform anesthesie regime, invasieve hemodynamische studies in hetzelfde laboratorium en daarbij werden uniforme methoden voor het meten van het slagvolume en het hartminuutvolume gebruikt (**Hoofdstuk 4**). Bovendien hebben we in deze studies wel de mechanismen, die mogelijk ten grondslag kunnen liggen aan de disproportionele lage slagvolume waarden, kunnen onderzoeken door, linker ventrikel afmetingen te bepalen, de linker ventriculaire systolische en diastolische functie te onderzoeken en door hartspierweefsel te analyseren. Overeenkomstig de resultaten in **Hoofdstuk** 3 vonden wij ook in **Hoofdstuk 4** dat het hart (en linker ventrikel) gewicht, conform de allometrische schalingswetten, evenredig toeneemt met het lichaamsgewicht. Daarentegen neemt het slagvolume en het hartminuutvolume, volgens de allometrische schalingswetten niet proportioneel toe bij een toenemend lichaamsgewicht. We hebben bewijs gevonden dat het disproportioneel lage slagvolume bij zware varkens niet het gevolg is van een verminderde systolische (linker) ventrikel functie, maar dat er sprake bleek te zijn van een disproportionele ontwikkeling van de linker ventrikel afmetingen en volumes zoals we die gemeten hebben met zowel echocardiografie alsook met de geleiding katheter. Om de mechanismen, die ten grondslag zouden kunnen liggen aan de kleinere linker ventrikel dimensies, nader te kunnen onderzoeken hebben we een histologische analyse van de linker ventrikel wand uitgevoerd. Deze weefsel analyse toonde aan dat er in het interstitiële weefsel in de harten van de zwaarste varkens (lichaamsgewicht van meer dan 150 kg) een significante toename was van totaal collageen en van type I collageen. Bovendien leverde eiwitanalyse, van hartspierweefsel van deze zware varkens, duidelijk bewijs op van een intracellulaire titine isovorm verschuiving, de vervormbare isovorm N2BA neemt af terwijl de stijvere isovorm N2B juist toeneemt bij toenemend lichaamsgewicht. Deze toename van collageen en de titin isovorm verschuiving dragen, door een verhoogde stijfheid van de linker ventriculaire wand, waarschijnlijk bij aan het disproportionele kleine linker ventriculaire eind-diastolische volume. Interessant is ook de waargenomen, zeer uitgesproken toename van de gemiddelde arteriële druk bij varkens met een lichaamsgewicht van meer dan 150 kg. De resulterende toename van linker ventrikel afterload kan ook hebben bijgedragen aan de extracellulaire collageen toename en de intracellulaire titin isovorm verschuiving, en aldus hebben bijgedragen aan toename van de tijdconstante van relaxatie (tau) en uiteindelijke aan de disproportionele kleine linker ventrikel dimensies. Bovendien kan ook worden gespeculeerd dat de stijging van de afterload bij varkens, met een lichaamsgewicht van meer dan 150 kg, zal leiden tot

concentrische linker ventriculaire hypertrofie, dat niet alleen een onevenredig lage linker ventrikel gewicht zal maskeren, maar ook zal bijgedragen aan een verdere toename van de linker ventrikel stijfheid en wat uiteindelijke zal resulteren in kleinere linker ventrikel afmetingen.

Samenvattend, de in de hoofdstukken 2-4 beschreven resultaten tonen aan dat zwaardere varkens (>150 kg), volgens de allometrische schalingswetten, een disproportioneel klein eind-diastolisch volume en - slagvolume vertonen hetgeen waarschijnlijk wordt veroorzaakt door vroege (ontspanning) en late (stijfheid) diastolische dysfunctie.

List of Publications

1. Van Essen G.J., M Blom and J Fink Gremmels-Gehrmann. **1995.** Ryegrass cramps in horses**.** Tijdschrift voor Diergeneeskunde, 120 (24), pp. 710-711.

2. Theunissen G.T., G.J. van Essen, C. van Maanen and R.S. Schrijver. **1995**. Field trial with a subunit rhinopneumonie vaccine. Tijdschrift voor Diergeneeskunde, 120 (3), pp. 72-74.

3. Niewold T.A., G.J. van Essen, M.J. Nabuurs, N. Stockhofe- Zurwieden and J. van der Meulen. **2000**. A review of porcine pathophysiology: a different approach to disease. Veterinary Quarterly, 22 (4), pp 209-212.

4. Van Essen G.J. and J.M. Leeuwen. **2000.** Assessment of health risks of large semi-wild herbivores in urbanized areas. Veterinary Quarterly, 22 (2), pp. 112-116.

5. Nabuurs M., G.J. van Essen, P. Nabuurs, T.A. Niewold and J. van der Meulen. **2001**. Thirty minutes transport causes small intestinal acidosis in pigs. Research in Veterinary Science, 70 (2), pp. 123-127.

6. Ure, B.M., T.A. Niewold, N.M.A. Bax, M. Ham, D.C. Zee and G.J. van Essen. **2002.** Peritoneal, systemic and distant organ inflammatory responses are reduced by a laparoscopic approach and carbon dioxide versus air. Surgical Endoscopy, 16 (5), pp. 836-842.

7. Van Maanen C., G.J. van Essen, J. Minke, J.M. Daly and P.J. Yates, **2003.** Diagnostic methods applied to analysis an outbreak of equine influenza in a riding school in which vaccine failure occurred. Veterinary Microbiology, 93 (4), pp. 291-306.

8. Berkeveld M., S.W.M. Hendriksen, H.M.G. van Beers-Schreurs, A.P. Koets, P. Langendijk, G.J. van Essen, M.A.M. Taverne, J.H.M. Verheijden. **2007**. Measuring intestinal blood flow in group-housed weaner pigs using Physiogear™ I: a pilot study. Livestock Science,108 (1-3), pp. 159-162.

9. Van Essen G.J., J.C.M. Vernooij, J.A.P. Heesterbeek, D. Anjema, D. Merkus and D.J. Duncker. **2009**. Does cardiovascular performance of modern fattening pigs obey allometric scaling laws? Journal of Animal Science 87: pp. 1991- 1997.

10. Van Essen G.J., J.C.M. Vernooij, J.A.P. Heesterbeek, D. Anjema, D. Merkus and D.J. Duncker. **2011.** Cardiovascular performance of breeding sows fails to obey allometric scaling laws. Journal of Animal Sciences 89: pp. 376 – 382.

11. Van Essen G.J., M. te Lintel Hekkert, O. Sorop, J. van der Velden, D. Merkus and D.J. Duncker. **2016**. Cardiovascular performance of modern pigs does not comply with allometric scaling laws. Paper in preparation.

12. Van Essen G.J. **2016.** Cardiovascular proportionality of modern pigs. Are we breaking the allometric scaling laws? Thesis in preparation.

PhD Portfolio

Name PhD student: Gerard Jan van Essen

Erasmus MC department: Experimental Cardiology

PhD period: 2006- 2016

Promotor: Prof. dr. D.J. Duncker

Copromotor: Dr. D. Merkus

Educational Activity	Code[#]	Date	Days	ECTS
Post-Grad Education in Veterinary Science				
16 courses/training	1	1988 – 1996	17	5.1
Courses				
Statistiek voor biomedisch onderzoek	1	Autumn, 1998	5	1.5
Scientific Writing	1	Spring 1999	5	1.5
Basiscursus Microchirurgie	1	13-15 October, 1999	3	0.9
Proefdierkunde (Art. 9)	1	19-30 June, 2000	10	3.0
English writing and communication	1	2014	12	3.6
Congresses				
Equine medicine and surgery (Genève)	3	Winter, 1996	4	1.2
Experimental Biology (Washington)	3	Spring, 2002	3	0.9
Experimental Biology (San Diego)	3	Spring 2005	3	0.9
Poster Presentation				
Symposium "The pig in biomedical research" (Lelystad)	3	September, 2010	1	0.3
Oral presentation				
ASAS congress (Salt Lake City)	3	19 -23 July, 2016	5	1.5
Total				**20.4**

[#]Activity Codes: 1 = followed education, 2 = given education; 3 = congresses and symposia.

About the Author

Curriculum Vitae

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Loopbaan overzicht/ professionele ervaring.

Afdelingshoofd Animal Research Department (2011 – 2015)

MSD Animal Health, Boxmeer.

- Het managen van een afdeling met 46 medewerkers bestaande uit zes dierenartsen, vijf groepsleiders, zes staf medewerkers en 29 biotechnici
- Verantwoordelijk voor de bedrijfsvoering van 11 dier faciliteiten op drie verschillende locaties inclusief een bedrijfsboerderij, een sectiezaal en een histologie laboratorium
- Afgevaardigde van de vergunninghouder (MSD - AH) in het kader van de WoD, verantwoordelijk voor het werk van de lokale Dier Experimenten Commissie (DEC) en de oprichting en werkzaamheden van de Instantie voor Dierenwelzijn (IvD)
- Implementatie van een nieuwe organisatie structuur voor de afdeling
- Implementeren van een genetische fok strategie ter vermijding van inteelt in een geïsoleerde honden kolonie
- Ontwikkeling en implementatie van een uitgebreide revitalisatie strategie voor de katten kolonie
- Ontwikkeling en implementatie van een volledig nieuw ‘feed safety monitoring system’ voor GLP doeleinden waarmee diervoeders en voedermiddelen worden gecontroleerd op contaminanten zoals mycotoxinen, zware metalen e.d.
- Ter nagedachtenis aan mijn verdronken voorganger heb ik de ‘Egbert Hanenberg Memorial Run’ stichting bedacht en opgericht (www.ehmrun.nl)

Afdelingshoofd (interim) afdeling Rapid Manufacturing van TNO Industrie en Technologie, Eindhoven (2009 – 2011)

- Het managen van een afdeling met 33 hoog opgeleide medewerkers zoals (gepromoveerde) natuurkundigen, werktuigbouwkundigen en ICT experts.
- Voor een omvangrijk ontwikkel project voor een externe opdrachtgever dat volledig was ontspoord qua financiën en voortgang heb ik een stuurgroep geïnstalleerd met mijzelf als voorzitter en met heel veel inspanning en intensieve samenwerking kregen we dit project weer op koers.

Part-time **onderzoeker** Precision Livestock Farming (2007 – 2009) Animal Sciences Group van Wageningen UR

Oprichter en **General Manager** van TeleMetronics biometry B.V. (2001 – 2009)

TeleMetronics biometry B.V., een (hightech) startup bedrijf was een spin-off van Wageningen UR

Als general manager was ik verantwoordelijk voor de integrale bedrijfsvoering inclusief de uitvoering en realisatie van diverse complexe technische ontwikkelprojecten die wij uitvoerden in opdracht van de European Space Agency (ESA), zoals:

- *S.T.A.R.: Space Telemetry for Animal Research, development project of a biotelemetry system for mice,*
- *M.O.T.: MOuse Telemetry, development of an accelerometer test board for the Dutch Delta Mission of astronaut André Kuipers,*
- *TIR: Tele-injection in Rodents, a feasibility study to explore opportunities to develop an in mouse implantable micro-injection systems with telemetric control,*
- *IPM: Invasive Physiology Monitoring of Mice, a follow-up order of the ESA for the development of a highly innovative mouse telemetry system for remote monitoring of ECG, blood pressure and body temperature of each individual mouse in collaboration with Technical University of Delft.*

Dieronderzoeker pathofysiologie van dierziekten (1997 – 2001) Animal Sciences Group van Wageningen UR

Zelfstandig praktiserend dierenarts, specialisatie paard (1990 – 1997)

Dierenarts in loondienst, in een gemengde praktijk (1986 – 1990)

Andere professionele activiteiten

- Promotie onderzoek **(PhD)** bij het Erasmus MC, Vakgroep Experimentele Cardiologie in Rotterdam. De titel van het proefschrift is: " Cardiovascular proportionality of modern pigs" *Are we breaking the scaling laws*? Promotor is Prof. Dr. Dirk J. Duncker en de verdediging staat gepland op 23 februari 2017.
- Veterinair **adviseur** van TeleMetronics biomedical B.V. (Wageningen) over de implementatie van biotelemetrie in de veehouderij als een 'remote monitoring system' van diergezondheid en dierwelzijn.
- **Voorzitter** Dier Experimenten Commissie **(DEC)** van het LUMC en de Universiteit Leiden.
- **Bestuurslid** (penningmeester) van de Nederlandse Vereniging van Dierexperimenten Commissies NVDEC).
- Als practicus, dieronderzoeker en promovendus (Co) **Auteur** van wetenschappelijke publicaties.

Opleiding

1979 - 1986 Universiteit Utrecht, Faculteit Diergeneeskunde

1970 - 1978 Atheneum-B, Prof. Mr. S. Vissering Scholengemeenschap, Velsen

Post academisch verschillende cursussen zoals:

- Artikel-9 cursus (Faculteit Diergeneeskunde)
- Micro chirurgie (Erasmus MC)
- Financieel management (MKB Nederland), Accounting(Exact)
- Engels door ‘native speakers’ (MSD – AH)

Dankwoord en terugblik.

Gedurende de ruim tien jaar waarin het onderzoek naar de proportionaliteit van het varkenshart is uitgevoerd hebben velen een bijdrage geleverd. Sterker, zonder die inbreng had dit onderzoek niet uitgevoerd kunnen worden en was deze thesis nooit verschenen.
De kiem werd al twintig jaar geleden gelegd nadat ik in 1997 de praktijk had verruild voor een baan als dieronderzoeker in de pathofysiologie groep van Marius Nabuurs, onderdeel van het ID-DLO In Lelystad. Binnen deze groep was twijfel ontstaan of de vitale organen van het varken, het hart en de longen, wel capabel genoeg waren in het geval de varkens meer beweging zouden krijgen bijvoorbeeld bij de overgang naar een extensievere bedrijfsvoering. Deze twijfels waren deels gestoeld op de reeds bekende hart problemen bij snelgroeiende vleeskuikens waar zogenaamde 'doodgroeiers' al voor de slachtleeftijd van zes weken dood gingen aan hartfalen. Daarbij was in de onderzoeksgroep de hypothese ontstaan dat onvoldoende hartcapaciteit bij het varken, met name tijdens stress, zou kunnen leiden tot verhoogde doorlaatbaarheid van de darmwand als gevolg van onvoldoende bloedtoevoer naar de darm. Vandaar dat hartonderzoek deel uit ging maken van een groot onderzoeksprogramma naar darmaandoeningen bij varkens. Ik werd, zonder enige onderzoekservaring, verantwoordelijk voor het hartonderzoek en Marius werd mijn enthousiaste leermeester en coach in de omscholing van paardenarts naar dieronderzoeker. Daar ben ik hem nog steeds dankbaar voor.

Nadat ik mij georiënteerd had op het uitvoeren van inspanningsonderzoek bij varkens bij de vakgroep Experimentele Cardiologie van het Erasmus MC begonnen we in 1999 In Lelystad met inspanningsonderzoek bij geïnstrumenteerde varkens en daarin vervulde biotechnicus en collega Dirk Anjema een hoofdrol. Dirk was niet alleen de man met 'de gouden handjes' maar hij regelde alles rond en tijdens de operaties. Zo bewaakte hij de anesthesie, de recovery en de postoperatieve verzorging van de geïnstrumenteerde varkens. Dirk ik wil je hartelijk bedanken voor jouw inzet en collegiale steun, je was bij het ID DLO de onmisbare pijler in al het dierexperimentele werk.

Ondanks al het interessante onderzoekswerk had ik veel moeite met de overgang van zelfstandig paardenarts, waar ik zeven dagen per week 'lekker buiten' op pad was, naar een bureaubaan. Ik voelde mij opgesloten in het kleine kantoor dat ik met Marius deelde. De overgang van een 1-mans praktijk naar een groot ambtelijk onderzoeksinstituut was zo groot

dat ik, ondanks het comfort van vrije weekends en echte vakanties, wilde 'ontsnappen aan het peloton'. Toen ik, per toeval, de mogelijkheden van biotelemetrie bij dieren, het draadloos meten van fysiologische signalen, ontdekte zag ik een kans om mijn loopbaan een nieuwe wending te geven. Met behulp van financiers richtte ik TeleMetronics biometry BV op. In deze spin-off van Wageningen UR gingen we 'het telemetrie erfgoed' van collega Mans Jansen 'door ontwikkelen' om uiteindelijk innovatieve biotelemetrie producten op de markt te kunnen brengen. Ik zag veel potentie in biotelemetrie met name in de veehouderij, en ik droeg dit uit met de volharding van een Jehova getuige en was door geen mens van 'mijn geloof' af te brengen. Helaas was er toentertijd in de veehouderijsector weinig tot geen belangstelling voor draadloze sensortechnologie en dat was op zijn zachtst gezegd nogal frustrerend voor 'een bevlogen evangelist'. Daarbij moest ik leiding geven aan de ontwikkeling van geavanceerde elektronica en een ASIC (een soort chip) waar ik plat gezegd 'de ballen verstand van had'. Feitelijk was ik een volkomen leek op dit terrein terwijl de TeleMetronics technici echte elektronica specialisten waren. Dat voelde niet alleen ongemakkelijk maar het was, zakelijk en organisatorisch, een uiterst onhandige en bovendien onverstandige constructie. Wellicht heeft dit bijgedragen aan mijn zoektocht naar mogelijkheden om vanuit die lastige situatie (deels) terug te kunnen keren naar de dierwetenschap. De (Prof.) Hendrik Casimir subsidie die, de uitwisseling van kenniswerkers met het bedrijfsleven moest stimuleren en door NWO in 2006 beschikbaar werd gesteld voor kansrijke bilaterale onderzoeksvoorstellen bood wellicht een mogelijkheid om dit te gaan realiseren. In samenwerking met (Prof.) Hans Heesterbeek van de faculteit Diergeneeskunde schreef ik een onderzoeksvoorstel dat, aanvankelijk net buiten de prijzen viel maar nadat enkele andere voorstellen afvielen alsnog, 1 van de 15 uitverkoren voorstellen werd. Ons voorstel werd gehonoreerd met een subsidiebedrag van 160.000 euro voor een periode van vier jaar. Dat was buitengewoon riant en Hans en ik gingen samen naar Den Haag om deze Casimir subsidie in ontvangst te nemen bij de kick-off bijeenkomst op het Ministerie van Economische Zaken.

Hiermee kon ik mijn (dier) onderzoekersloopbaan een tweede kans geven en werd de salaris verplichting van TeleMetronics substantieel ontlast. Ik moest, zonder relevante schrijfervaring de inmiddels belegen onderzoeksresultaten uit de ID-DLO periode (1997 – 2001) gaan opschrijven en dat viel niet mee. In die periode verkaste ik regelmatig voor een lang weekend naar een appartementje in het Noord Hollandse Andijk om 'schrijfmeters' te maken. Ondanks

de vele uren en pagina's en de deskundige begeleiding door Hans lukte het niet om er een goed wetenschappelijk artikel uit te distilleren. De statisticus van de faculteit Diergeneeskunde vond het niet verantwoord om de verschillende Lelystad studies in één artikel te stoppen en daardoor stagneerde het hele verhaal. Dat was het moment om vers bloed in te brengen. Ik vroeg (Prof.) Dirk-Jan Duncker, hoogleraar Experimentele Cardiologie aan het Erasmus MC, om samenwerking in dit varkenshart onderzoeksproject. Dat leverde een doorbraak in de impasse op. Dirk-Jan stelde een nieuwe, wetenschappelijk verantwoorde, opzet voor waarin zowel resultaten van inspanningsonderzoek bij varkens uit Rotterdam alsook uit Lelystad werden samengevoegd. Met een nieuwe statisticus, Hans Vernooij, die met engelengeduld mijn diep weggezakte statistiek kennis opvijzelde, kwam er eindelijk licht in de tunnel. Het eerste artikel (hoofdstuk 2) werd aangeboden aan - en uiteindelijk in 2009 gepubliceerd door 'The Journal of Animal Science' (JAS).

Daarna wilden we persé metingen gaan doen aan harten van zwaardere varkens en ik kreeg de unieke mogelijkheid deze metingen te doen bij zeugen die voor een verlossing middels een keizersnede onder anesthesie werden gebracht. Deze operaties werden, onder regie van Dirk Anjema, uitgevoerd in de dierfaciliteiten van de Animal Science Groep (ASG) van Wageningen UR in Lelystad. In die periode (2009- 2010) had ik een 50% aanstelling als onderzoeker 'Precision Livestock Farming' bij ASG en kon ik 's morgens assisteren bij de keizersnede operaties en 's middags op de laatste zeug de metingen doen. Dat leverde een tweede doorbraak op. Met dezelfde groep coauteurs schreven we onder begeleiding van Dirk-Jan Duncker en Hans Heesterbeek, een tweede artikel waarin opnieuw resultaten van zowel Rotterdamse varkensstudies alsook die van de zeugenstudies in Lelystad waren opgenomen. Met de opgedane ervaring van het eerste artikel kwam het tweede nu veel sneller tot stand en in 2011 publiceerde JAS ons tweede artikel (hoofdstuk 3) waarin, voor het eerst werd beschreven dat de hartvolumina van de zwaarste varkens, de zeugen aan het einde van de dracht, niet schaalden met het lichaamsgewicht zoals beschreven door de schalingswetten.
Ik wil beide Utrechtse onderzoekers, Hans Heesterbeek en Hans Vernooij, hartelijk bedanken voor hun waardevolle bijdrage aan het eerste deel van het onderzoek en de daaruit voortgekomen twee (JAS) publicaties.

Medio 2009 moest ik op zoek naar een andere inkomsten omdat TeleMetronics de bodem van haar schatkist had bereikt, er door de recessie nauwelijks meer geld binnen kwam en de Casimir subsidie inmiddels ook 'verstookt' was. Toen kwam ik in Brabant te werken allereerst als interim manager bij TNO Techniek in Eindhoven en in 2011 werd ik afdelingshoofd bij MSD – Animal Health in Boxmeer. Dat was een baan die niet alleen (te)veel reistijd kostte maar ook heel veel 'vrije tijd' opslokte. Er was eigenlijk geen ruimte meer om door te werken aan het onderzoek behalve dat ik regelmatig een dag vrij nam om bij de experimenten in Rotterdam aanwezig te zijn. Want met Dirk-Jan en Daphne Merkus was er een derde studie opgezet die, om eerdere methodologische beperkingen te vermijden, geheel in Rotterdam moest worden uitgevoerd. Omdat we in deze studie vooral weer metingen wilde doen bij zware varkens, wat men in Rotterdam niet gewend was, moesten er speciale voorzieningen worden getroffen. Er werd een takel in de OK opgehangen, er kwam een grotere transportkar en een 'draagzijl' waarop de dieren van ca. 200 kg, door een compleet peloton van de Experimentele Cardiologie, de stal uit werden gedragen nadat ze door Maaike te Lintel Hekkert vakkundig in slaap waren gebracht. Vervolgens werd het varken, met vereende krachten, in de kar gehesen en dan met een luid snurkend varken de lift in op naar de 23ste etage, waar de onderzoeksruimtes zijn. Die grote varkens trokken veel bekijks en vrijwel iedereen van de afdeling wipte even de ok in om te zien hoe wij die zware dieren onder anesthesie instrumenteerden. Deze varkens werden primair geopereerd voor onderzoek naar hartziekten bij de mens en daarnaast werden de metingen gedaan die voor mijn onderzoek van belang waren. Er zijn in totaal 31 varkens geopereerd en geïnstrumenteerd waarvan ik veruit de meeste wel heb bijgewoond en met eigen ogen heb kunnen zien hoe 'duizendpoot' Maaike deze klussen klaarde. Maaike heeft, net zoals Dirk Anjema, 'gouden 'handjes' en ze beschikt daarbij ook over veel organisatietalent. Zij was de uitvoerder, de regisseur, de instructeur van assistenten, de proef-protocol bewaker, data analist enzovoort, enzovoort. Daphne ontfermde zich meestal over de druk – volume metingen en zij werd als eerste achter haar bureau vandaan getrokken als er een crisis in de ok ontstond. Ze schoot in een witte jas, dook als 'experimenteel crisis interventie cardioloog', met een grijns van oor tot oor, in het varken en wist gelukkig vaak het tij te keren.

Beste Maaike en Daphne jullie zijn beiden van cruciale betekenis geweest vooral in het experimentele gedeelte maar ook in het traject daarna. Ik wil nogmaals proberen jullie

hiervoor te bedanken door thuis een lekkere visje voor jullie en Dirk-Jan te bakken. Dat betekent wel een reisje naar IJmuiden en dat is voor jullie alle drie 'wel een dingetje'. Naast Maaike en Daphne hebben nog vele andere stafmedewerkers belangrijke bijdragen geleverd. Zoals Oana Sorop die solo de histologie uitvoerde en daarmee de collageen data verzamelde en Monique Hanegraaff die al die administratieve klussen, waar ik zelf 'een broertje aan dood heb', uitvoerde. Ik wil alle andere leden van de vakgroep die fysiek meegeholpen hebben om die 'snurkende zware varkens boven te krijgen' en of hebben geassisteerd bij de operaties hartelijk bedanken voor hun inspanningen.

Toen in juli 2015 mijn dienstverband bij MSD in Boxmeer eindigde kreeg ik opeens een zee van tijd en daarmee de ultieme gelegenheid om (eindelijk) het promotie traject af te kunnen ronden. Als onverbeterlijke optimist vertelde ik in mijn omgeving dat ik het nu **even** ging afmaken. Maar gaandeweg bleek dat ik het leeuwendeel nog moest doen en dat het nog lang geen 'kat in het bakkie' was. Promotor Dirk-Jan zette de hoofdlijnen van de thesis uit, gaf heldere instructies over de invulling en wist mij te stimuleren en te motiveren om door te pakken.

Beste Dirk-Jan wij kennen elkaar al meer dan 10 jaar maar nog steeds doe jij mij versteld staan van jouw bijzondere kwaliteiten. Je bent een buitengewoon begaafde en productieve wetenschapper met het geheugen van een kudde olifanten. Tijdens besprekingen viel er alleen een stilte als jij een slok koffie nam. Als je op je stokpaardje zat, de cardiofysiologie, dan begonnen je ogen te glinsteren, raakte je in een wetenschappelijke trance en steeg je op naar een buitenaards niveau. Ik liet die woordenvloed, die tsunami van gedetailleerde kennis met vermelding van alle relevante literatuur en auteurs, rustig over mij heen komen wetende dat alleen een klop op de deur van jouw volgende afspraak jou weer terug op aarde zou brengen. Dirk-Jan jij bent niet alleen een verbale geweldenaar maar je beschikt ook over een fenomenaal schrijftalent. Daar heb ik uitgebreid van geprofiteerd want alle teksten zijn door jou geredigeerd, gecorrigeerd en gestileerd. Jij hebt van mijn 'schrijf brei' verhalen gemaakt die zoals jij zelf laatst aangaf 'lezen als een trein'. Voor al jouw hulp, steun, begeleiding en enthousiasmerende coaching ben ik je heel veel dank verschuldigd. Zonder jouw veelomvattende inbreng was ik nooit tot dit eindresultaat gekomen; mijn hartelijke dank daarvoor!

Daphne Merkus, mijn copromotor die, laverend tussen haar gezin en haar drukke baan bij de experimentele cardiologie, een flinke bijdrage aan het gehele traject heeft geleverd ben ik ook veel dank verschuldigd. Daphne was op operatiedagen meestal simultaan met meerdere studies en experimenten bezig. Tussendoor gaf ze nog even onderwijs, schreef ze (mee aan) artikelen, begeleide ze (andere) PhD studenten en deed ze de metingen bij de zware varkens. Daphne ik heb groot respect voor jouw 'multi tasking kwaliteiten', stress bestendigheid en je onverwoestbaar goede humeur. Hartelijk bedankt voor alle inbreng, ondersteuning en goede adviezen!

Dan is het manuscript eindelijk gereed om door de leescommissie te worden beoordeeld. Ik wil de hoogleraren van de kleine commissie, Robert Jan van Geuns, Bas Kemp en Leo van Leengoed hartelijk bedanken voor hun inspanningen en geïnvesteerde tijd in de promotie van deze overjarige PhD student. Daarnaast wil ik de overige hoogleraren, Martien Groenen en Jolanda van der Velden hartelijk bedanken voor hun rol in de promotie commissie. Jolanda en haar collega Wies Lommen wil ik tevens bedanken voor de titine bepalingen die in belangrijke mate hebben bijgedragen aan de extra dimensie van onze derde paper. Tezamen met de collageen data werpt het licht op het mogelijke mechanisme achter de disproportionele hartvolumina.

En dan 'the inner circle'. Madeleine wil ik heel erg bedanken omdat ze mij, ondanks verlies van vertrouwen in een goede afloop, wel 'vrijaf gaf' om in Andijk of in Driehuis te kunnen werken aan die artikelen. Daarmee bleef ze mijn 'never ending story' wel steunen. Voor Milan en Luca, mijn nageslacht, is het tot op heden nog volkomen onduidelijk waar dit onderzoek over gaat en waarom ik daar zoveel tijd in heb gestoken. Ik hoop dat ik hen met de presentatie, voorafgaand aan de verdediging, toch nog een idee kan geven waar ik al die tijd mee bezig ben geweest. Mijn beide paranimfen, Rob van der Woude, mijn oudste vriend en Leo van Essen, mijn jongste broer(tje) wil ik hartelijk bedanken voor hun waardevolle vriendschap en ondersteuning in het eindtraject.

Tenslotte denk ik terug aan mijn overleden ouders. Mijn vader, hovenier in hart en nieren die mij altijd op het belang van praktijkervaring wees en dat duidelijk veel belangrijker vond dan

‘al dat doorleren’. Ik was van jongs af aan ‘zijn tuinknechtje’ en heb, gedurende al die uren die ik met hem heb doorgebracht op zijn volkstuin en in al die andere tuinen van particulieren die hij onderhield voor ‘een rokertje’, veel van hem geleerd. Mijn moeder was leergierig en assertief, zij had thuis ‘de broek aan’. Zij was een bolleboos in haar lagere schoolklas maar vervolgonderwijs zat er toentertijd niet in, ze ging op haar veertiende als ‘melkmeid’ werken bij Verbeek, de melkboer in Langbroek. Dat heeft haar levenslang dwarsgezeten. Samen hebben ze zes kinderen groot gebracht en om mij te laten studeren moesten ze alle zeilen bij zetten. Mijn moeder wond daar geen doekjes om, ze was glashelder als ze zei: “wij hebben er jaren krom voor moeten liggen”. Maar wat waren ze apentrots toen ik geslaagd was voor veearts en als ze hadden kunnen meemaken dat ik nu een redelijke kans maak om ook het vervolgdiploma, de doctors bul, te bemachtigen zouden ze nog trotser dan een pauw zijn geweest. Mijn moeder was vaak onder behandeling van medisch specialisten en als mijn vader daar vragen over stelde dan kapte ze dat resoluut af door te zeggen: “Hoor eens Leen, hij heeft wel dé – èr (Dr.) voor zijn naam staan hoor, denk daar goed aan”.