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Organ data from the developing Göttingen minipig: first steps towards a juvenile PBPK model

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Abstract

The Göttingen minipig is the most commonly used pig breed in preclinical drug development in Europe and has recently also been explored for physiologically based pharmacokinetic (PBPK) modelling. To develop such a model, not only physiological data from adult animals but also data from juvenile animals are required, especially when using this model for paediatric drug development. Therefore, the aim of our study was to document body and organ weights (brain, heart, lungs, liver, gastrointestinal tract, spleen and kidney), lengths of the small and large intestines and pH values of the gastrointestinal tract in Göttingen minipigs from the foetal stage until the age of 5 months. Postnatal organ and body weights were fitted to regression models to find suitable equations that could be used to estimate organ weights as a function of body weight in the neonatal and juvenile Göttingen minipig. Most organs followed a non-linear growth curve during the first 5 months of life. In general, relative organ weights were the highest during the first week of life, during which the gastric pH was more alkaline than at 28 days of age.

Keywords: Göttingen minipig – growth – organ and body weights – prediction – gastrointestinal pH values

Introduction

Physiologically Based Pharmacokinetic Modelling (PBPK) is a mathematical modelling technique, which aims to predict absorption, distribution, metabolism and excretion (ADME) of drugs in man and animals. These models take the drug specific characteristics as well as the physiological and anatomical characteristics of the body into account, resulting in a more reliable prediction of pharmacokinetic data [1]. The different compartments in a PBPK model represent organs with inherent volumes and blood flows [2, 3]. Oral dosing is often the preferred route of drug administration and therefore the main focus is on PBPK gastrointestinal absorption models, but models of topical and parenteral administration are also important as they represent common routes of administration [1].

With regard to orally administered drugs, several physiological and anatomical factors in the gastrointestinal tract influence drug absorption. Local pH has a significant effect on the ionisation of weakly acidic and basic compounds, which will affect their absorption [4, 5]. The total absorptive surface area of the small intestine at the luminal side, which is a factor of length, circular folds and villi with microvilli on the enterocytes, plays a key role [6, 7]. Besides the surface area, the presence of phase I- and phase II-metabolism and drug transport in enterocytes, also influence drug absorption and exposure [8, 9]. However, first-pass metabolism largely takes place in the liver, after transportation via the portal vein. Secretion of drugs and their metabolites in the bile is largely species - and size-dependent and many compounds are secreted in the bile, resulting in a secretion back into the intestinal lumen. Here, they may be reabsorbed by intestinal cells and made available for recycling in a process known as 'enterohepatic cycling'. When drugs enter the systemic

circulation via the hepatic vein or via the lymphatic system, the target organs will be exposed to them prior to their elimination [10].

Both human data and laboratory animal data can be included in PBPK models. There are several advantages to using the Göttingen minipig in toxicity studies. They share important anatomical and physiological characteristics with humans, they can be bred under controlled conditions (e.g. specific pathogen-free, genetically coherent, transgenic animals), and are easy to handle. As such they represent a valuable alternative to dogs and nonhuman primates [11-13]. Including physiological data from sexually mature minipigs has already proven to be valuable in PBPK models, but additional data are still required [3]. To create and optimize a Göttingen minipig PBPK model, an extensive database with anatomical and physiological data is a prerequisite.

PBPK modelling is not only useful for prediction of adult PK data, but it has recently also gained much attention in paediatric drug development since the use of children in clinical studies is limited by ethical and practical concerns [2, 14, 15]. The prediction of pharmacokinetics and pharmacodynamics is very challenging in children, and particularly in the neonate [16]. In a developing body, the ADME of drugs is constantly changing due to the dynamic changes in absolute and relative organ weights, body composition, blood flow, maturation of organs and the presence and activity of metabolic enzymes and transporters [2, 5, 17]. As a result of the dynamic character and complexity of the ADME processes, and given the fact that the clearance mechanisms are not fully mature in very young children, scaling paediatric doses of drugs from adult doses on the basis of body weight, height or age only may result in under- or overdosing and adverse drug reactions [15, 17]. Integrating both anatomical and physiological data on the developing body in a PBPK model will be helpful to more accurately predict the outcome of pharmacokinetics. Nevertheless, especially for orally ingested drugs in children, these predictions remain difficult [2].

The aim of the study was to share morphometric organ data and pH-values of the gastrointestinal tract from foetal, neonatal, juvenile, and sexually mature Göttingen minipigs. These data can be used to frame a minipig PBPK model, but may also be useful as reference data in other scientific fields. Data from 1-day-old till 5-month-old animals were used to create equations, allowing for estimation of organ weight as a function of body weight in neonatal and juvenile animals.

Materials and Methods

Animals

Organs were obtained from healthy Göttingen minipigs at the University of Antwerp and at Sequani Limited (Ledbury, United Kingdom). Ellegaard Göttingen minipig A/S (Dalmose, Denmark) kindly donated the animals to the University of Antwerp. The following age groups were investigated at the University of Antwerp: 84-86 days of gestation (N = 18), 108 days of gestation (N = 8), Day 1 (within 24 hours after birth) (N = 10), Day 3 (N = 11), Day 7 (N = 12), Day 14, Day 28 (N = 14) and at Sequani Limited Day 1 (N = 10), Day 7 (N = 10), Day 14 (N = 7), Day 28 (N = 9), 3 months (N = 29), 4 months (N = 10) and 5 months (N = 10) of age. Day 28 is

the age at which Göttingen minipigs are usually weaned in juvenile studies. The higher ages were of interest since male and female Göttingen minipigs reach sexual maturity at 3-4 months and 4-5 months of age, respectively. In general, minipigs enter toxicological studies at 3.5-4.5 months of age when they are almost or already sexually mature [11].

Both sexes were included in each age group. Euthanasia of these animals was performed as part of other research goals [18, 19]. Adult sows were euthanized by electrocution, followed by exsanguination either before or after delivery, according to the desired age of their offspring. The fetuses were harvested and immediately placed on ice until further processing. The neonatal and juvenile piglets were naturally delivered and housed with the sow until euthanasia. The piglets were randomly allocated to a specific postnatal age group. They were anaesthetized by an intraperitoneal injection of sodium pentobarbital 20% (Kela NV, Hoogstraten, Belgium) (90 mg/kg), followed by exsanguination. Animals older than 28 days of age were sedated with Rompun (xylazine hydrochloride, Baeyer, Leverkusen, Germany) and Ketamine (ketamine hydrochloride, Ceva S.A., Libourne, France), prior to an intravenous injection with sodium pentobarbital. Animals were not fasted prior to euthanasia. The Ethical Committee of Animal Experimentation from the University of Antwerp (Belgium) (Dossier 2012-30) and the Animal Health Care Committee of Sequani Limited approved the protocol and use of the animals.

Morphometry and pH assessment

Body weight was assessed prior to euthanasia and exsanguination. Brain, lungs, heart, left kidney, liver, spleen, stomach, small intestine, large intestine and caecum were removed after exsanguination and weighed (Precision Balance BA3100P, Sartorius, Vilvoorde, Belgium). Determination of weights, lengths and pH values of the gastrointestinal tract was only performed at the University of Antwerp and consequently these data are limited to the first month of life. These animals were not fasted prior to euthanasia. The pH values of the stomach (anatomical region not specified) and the intestines including the proximal part (Zone 1) of the duodenum, the middle part (Zone 2) of the jejunum and the ileum, which is defined as the terminal part (Zone 3) of the small intestine that attached to the caecum via the plica ileocaecalis (World Association of Veterinary Anatomists, 2012, <http://www.wava-amav.org/>), the middle part (Zone 2) of large intestine and the caecum were determined by bringing Dosatest® pH 1-11 indicator paper (VWR international, Fontenay/Bois Cedex, France) into contact with the luminal side of the gastrointestinal wall. After determination of pH, all intestinal segments were subsequently rinsed with cold 0.1 M phosphate buffered saline (pH 7.4) before weighing.

Statistical analysis

The Kruskal-Wallis (Mean Rank) test was used to detect potential statistically significant differences. A p -value < 0.05 was considered statistically significant. For organ and body weights, differences between 3-, 4- and 5-month-old animals were evaluated. Differences in pH among intestinal regions and for each separate gastrointestinal region were evaluated

for the Day 1, Day 3, Day 7 and Day 28 animals. Bonferroni correction for pairwise comparisons adjusted the p - value for statistical significance to 0.017 for organ and body weights, and to 0.01 for the age-dependent differences in pH for each separate gastrointestinal region. Statistical analyses were performed with IBM SPSS statistics (version 20; IBM, Armonk, NY, USA).

Mathematical analysis

Calculation of descriptive values was performed with IBM SPSS statistics (version 20; IBM, Armonk, NY, USA). For each organ, we fitted the data on organ weights (in g, on the Y-axis) as a function of body weight (in g, on the X-axis) by nonlinear curve fitting (first, second, third or fourth order polynomial) in GraphPad Prism 6.0 f (GraphPad Software, Inc., La Jolla, CA, USA). First, each set of organ weights was split by sex. Then, we checked whether the best-fit values of all the unshared parameters for a certain order of polynomial equation differed significantly between both datasets by F-test ($p < 0.05$). If not, one single curve was used to fit the organ data and the data from both sexes were compiled. If the parameters differed significantly, individual equations were calculated for each sex. In the case of two apparently valid models, the difference in adapted Akaike Information Criteria (AICc) was used to decide which model fitted the best. Outlier identification was performed by the ROUT-method. Additionally, the observed *versus* predicted values (and *vice versa*) for each best-fit equation were plotted in a linear regression model to check for goodness of fit, and proportional and systematic bias in the prediction of the organ weights to validate the chosen model. The same procedure was followed to fit body weights as a function of age in days.

F-test, D'Agostino-Pearson normality test, Runs test, AICc calculations and outlier detection were performed in GraphPad Prism 6.0 f (GraphPad Software, Inc., La Jolla, CA, USA). Plotting and evaluation of linear relationships between observed and predicted data was performed in Microsoft Excel® (Version 14.3.1, Microsoft Corporation, Redmond, WA, USA).

Preliminary validation of the model

A publicly available database, at the website of Ellegaard Göttingen minipig A/S (<http://minipigs.dk/the-goettingen-minipig/background-data/>), with mean body weights and mean organ weights from 2-month- and 6-month-old Göttingen minipigs, was used to perform a preliminary validation of the generated best-fit equations for brain, heart, lungs, spleen, kidney and liver as a function of body weight.

Results

Total body weight and absolute organ weight

The absolute weights (in g) of the brain, lungs, heart, left kidney, spleen, liver and total body weights (in g) are listed in Table 1. Between 3- and 4-month-old animals, statistically significant differences were present for body weights ($p < 0.001$) and for all organ weights ($p < 0.005$), except for the spleen ($p = 0.024$). Between 3-month- and 5-month-old animals,

statistically significant differences were present for all organ and body weights ($p < 0.001$). No statistically significant differences were present between 4- and 5-month-old animals ($p > 0.17$ for all organ weights and $p = 0.06$ for body weights). These results indicate that body weight is still increasing between 4 and 5 months of age, but the increase is less pronounced than in younger animals. Organ growth is clearly slowing down after 4 months of age. The absolute gastrointestinal organ weights (in g) are depicted in Table 2.

Table 1 Organ and body weights in the developing Göttingen minipig. Data represent mean organ weights (in g) and total body weights (in g) $\pm$ standard deviation from female and male Göttingen minipigs from late foetal until 5 months of age. Results for spleen weights may be biased by drug-induced (barbiturates, ketamine) splenomegaly or by splenic contraction due to exsanguination

/ missing data

d G.A. days of gestational age

Table 2 Gastrointestinal weights and lengths in the developing Göttingen minipig. Data represent mean weights (in g) of the stomach, small intestine, caecum and large intestine and lengths (in cm) of the small and large intestines $\pm$ standard deviation from female and male Göttingen minipigs as a function of age during suckling period

/ missing data

d G.A. days of gestational age

Length and pH of the gastrointestinal tract

The lengths of the small and large intestines (in cm) are listed in Table 2. The pH values are presented in Table 3. Statistically significant differences in pH were detected among the three small intestinal regions at Day 3 ($p = 0.001$) and Day 7 ($p = 0.003$) with increasing pH along the small intestinal tract, but not at Day 1 ($p = 0.78$) and Day 28 ($p = 0.20$). Only in 7-day-old animals, a weakly significant difference was noted between the ileum, caecum and colon ($p = 0.044$). No significant differences were found between the caecum and colon ($p > 0.362$ for all age groups). Gastric pH differed among postnatal age groups, with a significantly different and more acidic pH at Day 28 compared to Day 1 ($p = 0.002$) and Day 7 ($p = 0.001$). The intestinal pH values remained at a more alkaline level compared to the gastric pH during the first month of life. No significant differences were found among the observed postnatal age groups for the proximal, middle or distal part of the small intestine and for the colon ($p \geq 0.05$). For the pH in the caecum, a statistically significant difference was present among the age groups ($p = 0.023$) with a significantly lower pH at Day 1 compared to Day 28 ($p = 0.005$).

Table 3 Gastrointestinal pH values in the developing Göttingen minipig. Data represent mean pH values $\pm$ standard deviation at the gastrointestinal wall from female and male Göttingen minipigs as a function of age during suckling period

Zone 1, 2 and 3 represent the proximal, middle and distal zone of the intestine, respectively.

d G.A. days of gestational age

Body weight and organ weight prediction

The increase in body weight as a function of age in days was best described by a linear growth curve in male and female animals (Figure 1 and Table 4). The best-fit equations describing the body weight-dependent changes for the brain, heart, lungs, spleen, kidney, liver, stomach, small intestine and large intestine in female and male developing Göttingen minipigs are shown in Table 4. The body weights used to generate these equations ranged from 200g to 13,000g and from 300g to 12,400g for females and males, respectively, and covered the first 5 postnatal months for brain, heart, lungs, kidney, spleen and liver. For stomach, small intestine and large intestine, the body weights ranged from 301 to 3,821 g and from 378 to 3,636g for females and males, respectively, and covered the first month of life (Table 4). Best-fit lines, as described by the best-fit equation, and their 95% confidence interval are presented for the brain, lungs, spleen, liver, heart and kidney in Figure 2, and for the stomach, and small and large intestines in Figure 3. Residuals for spleen were not normally distributed. Among the female heart, male heart, and spleen weights, 1, 3 and 6 outliers, respectively, were detected and were excluded to create the equation.

For each best-fit equation, the observed *versus* predicted data (and *vice versa*) were plotted in a linear regression model (graphs not shown). Evaluation of these linear regressions resulted in a very good fit (mean $R^2 \pm$ S.D.: 0.998 ± 0.002), a very low proportional bias (mean slope $\pm$ S.D.: 0.999 ± 0.0003) and a very low systematic bias (Y-intercept ≤ 0.0657) for all the best-fit equations, validating these models.

Fig. 1 Growth curve of the Göttingen minipig till 5 months of age. Blue dots and red triangles represent body weights (in g) at different postnatal ages in male and female Göttingen minipigs, respectively. The solid line represents the best-fit line obtained by the best-fit equation with their 95% confidence intervals (dashed line)

Table 4 Best-fit equations to predict organ weight as a function of body weight and body weight as a function of age in the developing Göttingen minipig

*Equations cover body weights ranging from 200 to 13,000 g or first 5 months of life

** Equations cover body weights ranging from 300 to 3,800 g

°Prediction of spleen weight may be biased by drug-induced (barbiturates, ketamine) splenomegaly or by splenic contraction due to exsanguination.

a body weight in g

t age in days

e mathematical constant, Euler's number

Fig. 2 Organ weights as a function of body weight in the developing Göttingen minipig until 5 months of age. The blue dots and red triangles represent the observed organ weights (in g) in male and female animals, respectively, for brain, lungs, spleen, liver, heart and kidney. The empty dots represent excluded outliers (by ROUT-method). The solid line represents the best-fit line obtained by the best-fit equation with their 95% confidence intervals (dashed line)

Fig. 3 Gastrointestinal weights as a function of body weight in the developing Göttingen minipig until 28 days of age. The blue dots and red triangles represent the observed organ weights (in g) in male and female animals, respectively, for stomach, small intestine and large intestine. The solid line represents the best-fit line obtained by the best-fit equation with their 95% confidence intervals (dashed line)

Relative organ weights

Organ weights as a percentage of body weight are plotted to visualize age-related changes in the relative size of each organ (Figure 4). Relative intestinal weights and relative intestinal lengths, expressed as length in cm per 100g body weight, are shown in Figure 5.

Except for the spleen and the gastrointestinal tract, the highest relative organ weights were noted during the first week of life. One-day-old piglets showed a very high relative brain weight (5%), which exponentially decreased immediately after birth to level off at 0.5% of the body weight from 3 months of age onwards. The highest relative organ weights for the heart, lungs, kidney and liver were present during the first week of life. Later, a decrease with increasing age was present, which levelled off from 3 months of age onwards. In contrast, the relative spleen weight was very low in Day 1 piglets, but had been raised 2.6-fold during the first week of life to remain at this level. The relative organ weights for the stomach, small intestine and large intestine varied between 0.85 and 0.99%, 3.43 and 5.42%, and 0.82 and 0.96%, respectively, during the first month of life, suggesting no clear age-related trend. In contrast, the relative intestinal lengths were clearly decreasing during this time period, indicating a higher increase in intestinal weight compared to intestinal length.

Fig. 4 Relative organ weights during development of the Göttingen minipig. Graphs show the age-related changes in organ weight as a percentage of body weight for the brain, heart, lungs, spleen, kidney and liver. Blue dots and error bars represent the mean value $\pm$ standard deviation for each age group. The dashed line represents the age-related trend during the first 5 months of life

BW body weight

Fig. 5 Relative intestinal weights and lengths during the first month of life of the Göttingen minipig. Graphs on the left show the age-related changes in relative weight of small intestine and large intestine. Graphs on the right show the age-related changes in relative length of small intestine and large intestine in cm per 100 g body weight. Blue dots and error bars represent the mean value $\pm$ standard deviation for each age group. The dashed line represents the age-related trend
BW body weight

Preliminary validation of the models

The differences in predicted and observed organ weights as a percentage of the observed organ weights (<http://minipigs.dk/the-goettingen-minipig/background-data/>) are presented in Tables 5, with body weight as a dependent variable. For brain and lungs, the percentage over- or under-prediction was below 10% for both age groups. For kidney and liver, the percentage over- or under-prediction was below 17 and 13%, respectively, for both age groups. For the heart, there was an over-prediction of about 20% for both age groups. Overestimation of predicted spleen weight was clearly present for both age groups, varying between 139 and 264%.

Table 5 Differences in predicted (as a function of body weight) and observed organ weights as a percentage of the observed organ weight

* data from <http://minipigs.dk/the-goettingen-minipig/background-data/>

Discussion

The aim of this study was to provide morphometric data of particular organs and gastrointestinal pH values in the developing Göttingen minipig. Furthermore, equations were generated to predict organ weight as a function of body weight and body weight as a function of age.

These data may be useful for integration in PBPK models, in the translation of toxicokinetic and pharmacokinetic data from the juvenile minipig to the human infant, and in pharmaceutical research using the minipig as an animal model. However, some attention should be paid to possible limitations when the presented data are used in biomedical research. The created equations were based on a limited range of not full-grown organ and body weights. Hence, they do not reflect the entire development till adulthood, limiting the applicability of the equations to the used range of body weights. Organ weights were determined after exsanguination, while the recorded body weights included the weight of the blood. Estimation of lost and residual blood volume was not performed since collection of the presented data was part of other research goals than PBPK modelling, but would be recommended in future experiments. However, organ- and age-dependent factors are known to complicate the estimation of residual blood volumes. At necropsy in rats, Kanerva et al. detected 23 and 15% lower liver and kidney weights, respectively, in an exsanguinated

group compared to a non-exsanguinated group [20]. In contrast, no significant differences in absolute weights of heart, spleen and brain were observed between both groups [20]. Linderkamp et al. reported a tendency to reduction of blood volumes per g of tissue with increasing age in 1-day-, 7-day- and 14-day-old piglets, especially for the liver, lungs and skeleton, while the blood volume in the great vessels outside the organs increased during this time period [21].

Most organs did not follow a linear growth curve during the first five months of life and relative organ weights varied over time. In general, the relative organ weights were the highest during the first week of life, while the major increases in absolute organ weights were observed during the first 3 to 4 months of life. In contrast to the other organs, the spleen showed a low relative organ weight in neonates, which strongly increased during the first month of life. The absolute spleen weight followed a linear increase as a function of body weight during the first five months of life. However, the observed spleen weights, and consequently also the equation to predict spleen weight from body weight, should be interpreted with caution because the spleen is a highly vascular organ and its size is largely dependent on its distension [22]. Barbiturates and ketamine, used as anaesthetics in this study, can result in congestive splenomegaly, which is mainly described in dogs. This sequestration of blood is probably due to smooth muscle relaxation in the splenic capsule, following administration of these anaesthetics [23, 24]. Indeed, the preliminary validation of the equations with external data from 2- and 6-month-old animals showed a large overestimation of the spleen weight. Exsanguination, on the other hand, will result in a contraction of the spleen in an attempt to compensate for the loss of blood and lack of oxygen [25]. Given the aforementioned interfering factors, it is difficult to quantify the possible discrepancy between the pre-mortem and post-mortem spleen weights. Nevertheless, a linear relationship between spleen volume and body weight was also found in children aged between 1 day and 18 years [26].

Not only the juvenile Göttingen minipig, but also the human infant possesses relatively large organs. In human beings, pronounced increases in the organ weights occur in the first years of their life. The largest increase in human brain weight occurs during the first 3 years of life with the highest relative brain weight in neonates [27, 28], while the largest increase in kidney volume already takes place in the first year of life [29]. In a study with 5,036 children, the median relative liver weight was 3.5% between 0 and 2 years of age, which had decreased to 2.2% for young adults of 18 years and older [30]. Noda et al. reported a similar age-related trend for the liver [31]. As very young individuals have relatively large organs, the body weight normalized clearance of drugs could be expected to be fast. However, the functional immaturity of metabolising and excreting organs, including metabolic Phase I- and Phase II- enzymes and drug transporters, and their absolutely smaller organ size compared to adults, result in an impaired response to drug exposure in these individuals [4, 5, 15]. This statement is further supported by our recent observation of the postnatal increase in abundance and activity of CYP3A, one of the most abundant and important Phase I- enzymes, in the liver of the developing Göttingen minipig [19].

During the first 28 days of life, no clear age-related trend in the relative weight of the different intestinal segments was noted despite a clear decrease in relative intestinal length, indicating a higher increase in intestinal weight compared to intestinal length. Nevertheless, lengths of the small intestines at Day 1 and Day 28, recorded in our study were already about 27 and 60%, respectively, of the lengths that were measured in 6-month-old Göttingen minipigs by Suenderhauf and Parrott [3]. This suggests an accelerated development of the small intestine during the first month of life compared to some other organ systems.

In the examined piglets (not fasted), the gastric pH was more acidic at 28 days of age compared to the younger animals. Snoeck et al. have also described this age-related decreasing trend in gastric pH in domestic piglets, when observing age groups at different time points around weaning [32]. For suckling piglets (17 days of age) and at weaning (24 days of age), they found no gastric pH values below 2.5, but at 1 and 2 weeks post weaning, they detected pH values as low as 1.6-1.7 [32]. Suenderhauf and Parrott compiled gastrointestinal pH values from 5 different studies (animals aged from 6 months to 2 years) and reported highly variable gastric pH values, ranging from 1.15 to 6, indicating the influence of feeding status and anatomical region [3]. Also in human beings, the trend of a more alkaline gastric pH in very young children is present with adult levels of gastric acidity by 6 months till 2 years of age [6]. The maturational change in gastric pH is likely to be a critical factor with regard to orally administered drugs in paediatric populations, since acid-labile compounds and weak bases will be easily absorbed from the more basic neonatal stomach while the absorption of more acidic compounds will be slowed down [5, 6]. The presence of milk in the stomach may also influence the absorption of protein or lipid soluble drugs [6]. The pH values that we determined at weaning age in different segments of the intestine, were in agreement with values reported by Snoeck et al [32]. As described in human beings, the intestinal pH values in our study were also more alkaline than the gastric pH values [33]. Although the gastrointestinal pH values were determined in dead animals, time lapse between euthanasia and sampling was limited to a maximum of 30 minutes. Hence, we believe that the post-mortal gastro-intestinal pH values reflect the in vivo situation.

Göttingen minipigs reach adult body weight around two years of age (35-45 kg) and show a nearly linear increase in body weight during the first 5 months of life [3, 11, 34]. During the first year of life, they gain approximately 2 kg per month, assuming that they are fed a restriction diet to prevent obesity [3]. The 4-month-old female animals, included in our study, however, showed relatively high body weights compared to the observed body weights at 5 months of age and they were 2 kg heavier compared to the mean 4-month-old body female weight of 9.18 kg, reported in the Growth Data Base from Ellegaard Göttingen Minipig A/S (<http://minipigs.dk/the-goettingen-minipig/background-data/>). This may have misleadingly suggested that the increase in body weight is already slowing down after 4 months of age. Nevertheless, the evolution of body weight as a function of age was best described by a linear growth curve.

In conclusion, this paper provides organ and body weights, intestinal lengths and pH-values of the gastrointestinal wall in the developing Göttingen minipig, which may be of use in the construction of a PBPK model. Based on the compiled data, we have developed best-fit equations to predict brain, heart, lungs, kidney, liver, stomach, small intestine and large intestine weights as a function of body weight and body weight as a function of age.

This information could be useful for researchers who use Göttingen minipigs in their biomedical studies.

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Age group	Sex	N	Weight in g Mean $\pm$ Standard Deviation						
			Brain	Heart	Lungs	Spleen	Kidney	Liver	Body Weight
84-86 d G.A.	F	9-10	9.57 $\pm$ 1.68	1.56 $\pm$ 0.28	6.55 $\pm$ 1.65	0.35 $\pm$ 0.10	1.09 $\pm$ 0.31	4.89 $\pm$ 0.90	174 $\pm$ 37
	M	6-8	9.53 $\pm$ 1.03	1.59 $\pm$ 0.29	6.42 $\pm$ 1.75	0.36 $\pm$ 0.07	1.02 $\pm$ 0.20	4.71 $\pm$ 0.94	171 $\pm$ 32
108 d G.A.	F	3-4	18.2 $\pm$ 3.13	3.65 $\pm$ 0.78	11.3 $\pm$ 2.91	0.59 $\pm$ 0.21	1.73 $\pm$ 0.41	11.3 $\pm$ 2.17	356 $\pm$ 89
	M	3-4	18.2 $\pm$ 3.56	3.46 $\pm$ 0.45	13.9 $\pm$ 3.71	0.55 $\pm$ 0.15	1.67 $\pm$ 0.34	11.3 $\pm$ 1.56	358 $\pm$ 52
Day 1	F	9	20.3 $\pm$ 1.28	3.26 $\pm$ 0.67	5.35 $\pm$ 1.53	0.62 $\pm$ 0.20	1.87 $\pm$ 0.54	10.2 $\pm$ 2.37	372 $\pm$ 101
	M	10-11	20.4 $\pm$ 1.70	4.08 $\pm$ 0.93	6.58 $\pm$ 1.61	0.72 $\pm$ 0.22	2.17 $\pm$ 0.66	11.8 $\pm$ 4.04	445 $\pm$ 102
Day 3	F	4	21.4 $\pm$ 3.80	5.74 $\pm$ 1.56	11.5 $\pm$ 4.05	1.37 $\pm$ 0.54	3.43 $\pm$ 0.52	22.6 $\pm$ 8.90	600 $\pm$ 150
	M	4-6	22.3 $\pm$ 2.00	6.57 $\pm$ 0.86	13.4 $\pm$ 2.18	1.87 $\pm$ 0.50	4.37 $\pm$ 0.67	25.2 $\pm$ 4.08	758 $\pm$ 126
Day 7	F	8-9	21.8 $\pm$ 2.23	6.54 $\pm$ 2.23	12.6 $\pm$ 4.23	3.59 $\pm$ 1.41	3.63 $\pm$ 1.30	24.7 $\pm$ 9.45	726 $\pm$ 253
	M	10-13	24.7 $\pm$ 2.29	7.47 $\pm$ 1.83	14.4 $\pm$ 4.24	3.37 $\pm$ 1.39	4.61 $\pm$ 1.58	28.5 $\pm$ 8.44	856 $\pm$ 233
Day 14	F	4	28.7 $\pm$ 2.13	10.8 $\pm$ 2.62	19.1 $\pm$ 5.31	5.93 $\pm$ 3.04	6.80 $\pm$ 1.49	45.4 $\pm$ 12.7	1,450 $\pm$ 480
	M	3	27.2 $\pm$ 0.95	10.8 $\pm$ 0.81	20.1 $\pm$ 1.47	8.73 $\pm$ 0.99	5.83 $\pm$ 1.07	49.5 $\pm$ 9.07	1,567 $\pm$ 252
Day 28	F	9	35.3 $\pm$ 2.09	19.2 $\pm$ 3.96	32.9 $\pm$ 6.31	11.8 $\pm$ 2.78	11.6 $\pm$ 2.74	71.1 $\pm$ 18.3	2,842 $\pm$ 540
	M	12-14	34.9 $\pm$ 3.25	21.2 $\pm$ 4.42	31.6 $\pm$ 6.39	12.9 $\pm$ 3.44	11.2 $\pm$ 2.55	69.4 $\pm$ 13.6	2,681 $\pm$ 480
3 Months	F	15	50.5 $\pm$ 3.05	32.8 $\pm$ 3.78	51.7 $\pm$ 6.37	38.6 $\pm$ 14.3	18.5 $\pm$ 3.55	153 $\pm$ 20.0	6,960 $\pm$ 1,120
	M	14	51.0 $\pm$ 4.84	37.0 $\pm$ 5.45	51.0 $\pm$ 7.47	38.0 $\pm$ 11.6	20.5 $\pm$ 3.46	154 $\pm$ 21.4	7,136 $\pm$ 985
4 Months	F	5	55.9 $\pm$ 5.49	48.7 $\pm$ 4.50	73.4 $\pm$ 9.46	55.1 $\pm$ 17.8	25.3 $\pm$ 2.47	229 $\pm$ 35.0	11,160 $\pm$ 1,282
	M	4-5	56.7 $\pm$ 6.22	53.5 $\pm$ 8.78	73.2 $\pm$ 6.29	44.8 $\pm$ 8.45	26.9 $\pm$ 3.85	202 $\pm$ 27.4	9,400 $\pm$ 731
5 Months	F	5	57.6 $\pm$ 2.78	48.9 $\pm$ 4.14	67.8 $\pm$ 4.81	55.4 $\pm$ 17.2	23.2 $\pm$ 3.1	205 $\pm$ 12.2	11,140 $\pm$ 1,519
	M	4-5	60.2 $\pm$ 2.56	61.7 $\pm$ 5.94	78.3 $\pm$ 5.87	60.2 $\pm$ 11.4	29.2 $\pm$ 2.63	240 $\pm$ 21.6	11,800 $\pm$ 572

Table 1 Organ and body weights in the developing Göttingen minipig. Data represent mean organ weights (in g) and total body weights (in g) $\pm$ standard deviation from female and male Göttingen minipigs from late foetal until 5 months of age. Results for spleen weights may be biased by drug-induced (barbiturates, ketamine) splenomegaly or by splenic contraction due to exsanguination
/ missing data
d G.A. days of gestational age

Age group	Sex	N	Weight in g Mean $\pm$ Standard Deviation				Length in cm Mean $\pm$ Standard Deviation	
			Stomach	Small Intestine	Caecum	Large Intestine	Small Intestine	Large Intestine
84-86 d G.A.	F	6-10	1.34 $\pm$ 0.375	3.77 $\pm$ 1.07	/	1.59 $\pm$ 0.673	100 $\pm$ 21.3	33.5 $\pm$ 4.51
	M	6-8	1.36 $\pm$ 0.230	3.09 $\pm$ 1.11	/	1.51 $\pm$ 0.668	101 $\pm$ 30.4	32.1 $\pm$ 3.94
108 d G.A.	F	4	3.48 $\pm$ 0.706	10.3 $\pm$ 1.57	/	3.55 $\pm$ 0.522	155 $\pm$ 11.9	41.1 $\pm$ 6.01
	M	4	3.40 $\pm$ 0.568	10.7 $\pm$ 2.84	/	4.14 $\pm$ 2.06	167 $\pm$ 10.2	46.5 $\pm$ 6.45
Day 1	F	3-4	4.14 $\pm$ 0.846	20.9 $\pm$ 4.27	0.53 $\pm$ 0.139	3.52 $\pm$ 0.66	219 $\pm$ 12.0	43.4 $\pm$ 5.07
	M	3-6	4.04 $\pm$ 0.727	19.6 $\pm$ 5.37	0.713 $\pm$ 0.387	4.53 $\pm$ 0.658	230 $\pm$ 38.1	51.3 $\pm$ 1.08
Day 3	F	3-4	5.88 $\pm$ 1.63	33.8 $\pm$ 9.71	0.643 $\pm$ 0.185	5.19 $\pm$ 1.27	260 $\pm$ 33.4	50.8 $\pm$ 8.26
	M	3-7	7.61 $\pm$ 1.75	40.9 $\pm$ 4.84	0.823 $\pm$ 0.274	6.61 $\pm$ 0.309	295 $\pm$ 23.5	59.8 $\pm$ 4.44
Day 7	F	3-4	7.94 $\pm$ 2.40	39.2 $\pm$ 13.4	1.48 $\pm$ 0.468	9.75 $\pm$ 2.66	294 $\pm$ 34.9	71.3 $\pm$ 11.0
	M	6-8	9.13 $\pm$ 3.02	45.1 $\pm$ 12.3	1.16 $\pm$ 0.349	8.79 $\pm$ 2.89	318 $\pm$ 37.9	65.7 $\pm$ 8.67
Day 28	F	5	28.5 $\pm$ 6.48	105.5 $\pm$ 14.5	4.96 $\pm$ 0.859	28.5 $\pm$ 7.38	480 $\pm$ 34.5	86.1 $\pm$ 13.7
	M	8-9	20.9 $\pm$ 3.73	93.4 $\pm$ 16.3	5.51 $\pm$ 2.29	20.4 $\pm$ 5.67	493 $\pm$ 52.5	71.3 $\pm$ 10.8

Table 2 Gastrointestinal weights and lengths in the developing Göttingen minipig. Data represent mean weights (in g) of the stomach, small intestine, caecum and large intestine and lengths (in cm) of the small and large intestines $\pm$ standard deviation from female and male Göttingen minipigs as a function of age during suckling period

/ missing data

d G.A. days of gestational age

Age group	Sex	N	pH					
			Mean $\pm$ Standard Deviation					
			Stomach	Small intestine Zone 1	Small intestine Zone 2	Small intestine Zone 3	Caecum	Large intestine Zone 2
84-86 d G.A.	F	10	7.7 $\pm$ 0.5	7.5 $\pm$ 0.5	7.4 $\pm$ 0.5	7.8 $\pm$ 0.6	8.0 $\pm$ 0.5	7.8 $\pm$ 0.4
	M	8	7.1 $\pm$ 0.4	7.5 $\pm$ 0.5	7.1 $\pm$ 0.6	7.4 $\pm$ 0.5	7.8 $\pm$ 0.5	7.6 $\pm$ 0.5
108 d G.A.	F	3-4	5.5 $\pm$ 1.3	7. $\pm$ 0.0	7.4 $\pm$ 0.5	7.8 $\pm$ 0.6	7.7 $\pm$ 0.6	7.3 $\pm$ 0.6
	M	1-4	6.5 $\pm$ 1.0	6.7 $\pm$ 0.6	7.1 $\pm$ 0.6	7.4 $\pm$ 0.5	8.0 $\pm$ 0.0	6.7 $\pm$ 1.2
Day 1	F	4	5.3 $\pm$ 0.5	6.3 $\pm$ 0.5	6.5 $\pm$ 0.6	6.0 $\pm$ 0.0	6.8 $\pm$ 0.5	6.8 $\pm$ 0.5
	M	6	4.8 $\pm$ 0.8	6.5 $\pm$ 0.8	6.5 $\pm$ 0.8	7.2 $\pm$ 1.0	7.5 $\pm$ 0.6	7.2 $\pm$ 0.8
Day 3	F	4	4.3 $\pm$ 0.5	5.8 $\pm$ 0.5	6.3 $\pm$ 0.5	7.3 $\pm$ 0.9	7.3 $\pm$ 0.5	6.5 $\pm$ 0.6
	M	6	5.2 $\pm$ 1.2	5.8 $\pm$ 0.4	6.0 $\pm$ 0.0	6.8 $\pm$ 0.8	6.5 $\pm$ 0.8	6.5 $\pm$ 0.8
Day 7	F	3-4	5.0 $\pm$ 0.0	5.8 $\pm$ 0.5	6.0 $\pm$ 0.0	7.3 $\pm$ 0.9	6.0 $\pm$ 0.0	6.0 $\pm$ 0.0
	M	6-7	5.0 $\pm$ 0.6	6.1 $\pm$ 0.4	6.0 $\pm$ 0.6	7.1 $\pm$ 0.9	6.6 $\pm$ 0.8	6.3 $\pm$ 1.0
Day 28	F	5	3.8 $\pm$ 0.8	5.8 $\pm$ 0.5	5.8 $\pm$ 0.5	6.4 $\pm$ 0.6	6.4 $\pm$ 0.5	6.8 $\pm$ 0.5
	M	8	3.6 $\pm$ 1.2	6.1 $\pm$ 0.6	6.0 $\pm$ 0.0	6.1 $\pm$ 0.4	6.4 $\pm$ 0.5	6.3 $\pm$ 0.5

Table 3 Gastrointestinal pH values in the developing Göttingen minipig. Data represent mean pH values $\pm$ standard deviation at the gastrointestinal wall from female and male Göttingen minipigs as a function of age during suckling period

Zone 1, 2 and 3 represent the proximal, middle and distal zone of the intestine, respectively.
d G.A. days of gestational age

Organ	Sex	Equations to predict organ and body weight in g (Y)
Brain*	F+M	$Y = 17.10 + 0.008051a - 5.585e^{-007}a^2 + 1.469e^{-011}$
Heart*	F	$Y = 0.3466 + 0.008592a - 8.089e^{-007}a^2 + 3.730e^{-011}a^3$
	M	$Y = 0.3543 + 0.008637a - 7.525e^{-007}a^2 + 4.057e^{-011}a^3$
Lungs*	F+M	$Y = -1.092 + 0.02029a - 3.857e^{-006}a^2 + 3.803e^{-010}a^3 - 1.287e^{-014}a^4$
Spleen*°	F+M	$Y = -1.292 + 0.005224a$
Kidney*	F	$Y = 0.1025 + 0.005256a - 5.127e^{-007}a^2 + 2.095e^{-011}a^3$
	M	$Y = -0.4873 + 0.006445a - 1.062e^{-006}a^2 + 1.075e^{-010}a^3 - 3.774e^{-015}a^4$
Liver*	F+M	$Y = -4.332 + 0.04251a - 7.151e^{-006}a^2 + 8.395e^{-010}a^3 - 3.328e^{-014}a^4$
Stomach**	F+M	$Y = 0.4527 + 0.008595a$
Small intestine**	F+M	$Y = -1.037 + 0.05446a - 6.518e^{-006}a^2$
Large intestine**	F	$Y = -0.216 + 0.009642a$
	M	$Y = 1.417 + 0.007046a$
Total body weight*	F+M	$Y = 343,9 + 77,18t$

Table 4 Best-fit equations to predict organ weight as a function of body weight and body weight as a function of age in the developing Göttingen minipig

*Equations cover body weights ranging from 200 to 13,000 g or first 5 months of life

** Equations cover body weights ranging from 300 to 3,800 g

°Prediction of spleen weight may be biased by drug-induced (barbiturates, ketamine) splenomegaly or by splenic contraction due to exsanguination.

a body weight in g

t age in days

e mathematical constant, Euler's number

Age group	Sex	N	Observed data from Ellegaard Göttingen minipigs*	Over- or underestimation of predicted organ weights as a percentage of observed organ weights					
			Mean Body Weight (in g)*	Brain	Heart	Lungs	Spleen	Kidney	Liver
2 Months	F	20	3,900	-5.09	+21.1	+9.48	+139	+16.7	-3.52
	M	23	4,300	-1.87	+18.6	+1.11	+153	+8.86	-3.86
6 Months	F	20	14,600	-1.62	+16.2	-7.98	+264	+11.7	-11.8
	M	20	14,200	-2.36	+18.6	-6.72	+218	-6.51	-12.2

Table 5 Differences in predicted (as a function of body weight) and observed organ weights as a percentage of the observed organ weight

* data from <http://minipigs.dk/the-goettingen-minipig/background-data/>

Fig. 1 Growth curve of the Göttingen minipig till 5 months of age. Blue dots and red triangles represent body weights (in g) at different postnatal ages in male and female Göttingen minipigs, respectively. The solid line represents the best-fit line obtained by the best-fit equation with their 95% confidence intervals (dashed line)

Fig. 2 Organ weights as a function of body weight in the developing Göttingen minipig until 5 months of age. The blue dots and red triangles represent the observed organ weights (in g) in male and female animals, respectively, for brain, lungs, spleen, liver, heart and kidney. The empty dots represent excluded outliers (by ROUT-method). The solid line represents the best-fit line obtained by the best-fit equation with their 95% confidence intervals (dashed line)

Fig. 3 Gastrointestinal weights as a function of body weight in the developing Göttingen minipig until 28 days of age. The blue dots and red triangles represent the observed organ weights (in g) in male and female animals, respectively, for stomach, small intestine and large intestine. The solid line represents the best-fit line obtained by the best-fit equation with their 95% confidence intervals (dashed line)

Fig. 4 Relative organ weights during development of the Göttingen minipig. Graphs show the age-related changes in organ weight as a percentage of body weight for the brain, heart, lungs, spleen, kidney and liver. Blue dots and error bars represent the mean value $\pm$ standard deviation for each age group. The dashed line represents the age-related trend during the first 5 months of life
BW body weight

Fig. 5 Relative intestinal weights and lengths during the first month of life of the Göttingen minipig. Graphs on the left show the age-related changes in relative weight of small intestine and large intestine. Graphs on the right show the age-related changes in relative length of small intestine and large intestine in cm per 100 g body weight. Blue dots and error bars represent the mean value $\pm$ standard deviation for each age group. The dashed line represents the age-related trend
BW body weight