A novel method optimizing the normalization of cardiac parameters in small animal models

Hagdorn, Quint A. J.; Bossers, Guido; Koop, Anne-Marie C.; Piek, Arnold; Eijgenraam, Tim R.; van der Feen, Diederik E.; Sillje, Herman H. W.; de Boer, Rudolf A.; Berger, Rolf M. F.

Published in:
AMERICAN JOURNAL OF PHYSIOLOGY-HEART AND CIRCULATORY PHYSIOLOGY

DOI:
10.1152/ajpheart.00182.2019

Publication date:
2019

Am J Physiol Heart Circ Physiol 316: H1552–H1557, 2019.
First published April 12, 2019; doi:10.1152/ajpheart.00182.2019.

RAPID REPORT | *Translational Physiology*

A novel method optimizing the normalization of cardiac parameters in small animal models: the importance of dimensional indexing

Quint A. J. Hagdorn,[1] Guido P. L. Bossers,[1] Anne-Marie C. Koop,[1] Arnold Piek,[2] Tim R. Eijgenraam,[2] Diederik E. van der Feen,[1] Herman H. W. Silljé,[2] Rudolf A. de Boer,[2] and Rolf M. F. Berger[1]

[1]*Center for Congenital Heart Diseases, Beatrix Children's Hospital, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; and* [2]*Department of Cardiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands*

Submitted 20 March 2019; accepted in final form 11 April 2019

Hagdorn QA, Bossers GP, Koop AC, Piek A, Eijgenraam TR, van der Feen DE, Silljé HH, de Boer RA, Berger RM. A novel method optimizing the normalization of cardiac parameters in small animal models: the importance of dimensional indexing. *Am J Physiol Heart Circ Physiol* 316: H1552–H1557, 2019. First published April 12, 2019; doi:10.1152/ajpheart.00182.2019.—For indexing cardiac measures in small animal models, tibia length (TL) is a recommended surrogate for body weight (BW) that aims to avoid biases because of disease-induced BW changes. However, we question if indexing by TL is mathematically correct. This study aimed to investigate the relation between TL and BW, heart weight, ventricular weights, and left ventricular diameter to optimize the current common practice of indexing cardiac parameters in small animal models. In 29 healthy Wistar rats (age 5–34 wk) and 116 healthy Black 6 mice (age 3–17 wk), BW appeared to scale nonlinearly to TL^1 but linearly to TL^3. Formulas for indexing cardiac weights were derived. To illustrate the effects of indexing, cardiac weights between the 50% with highest BW and the 50% with lowest BW were compared. The nonindexed cardiac weights differed significantly between groups, as could be expected ($P < 0.001$). However, after indexing by TL^1, indexed cardiac weights remained significantly different between groups ($P < 0.001$). With the derived formulas for indexing, indexed cardiac weights were similar between groups. In healthy rats and mice, BW and heart weights scale linearly to TL^3. This indicates that not TL^1 but TL^3 is the optimal surrogate for BW. New formulas for indexing heart weight and isolated ventricular weights are provided, and we propose a concept in which cardiac parameters should not all be indexed to the same measure but one-dimensional measures to $BW^{1/3}$ or TL^1, two-dimensional measures to $BW^{2/3}$ or TL^2, and three-dimensional measures to BW or TL^3.

NEW & NOTEWORTHY In healthy rats and mice, body weight (BW) scales linearly to tibia length (TL) to the power of three (TL^3). This indicates that for indexing cardiac parameters, not TL^1 but TL^3 is the optimal surrogate for BW. New formulas for indexing heart weight and isolated ventricular weights are provided, and we propose a concept of dimensionally consistent indexing. This concept is proposed to be widely applied in small animal experiments.

allometry; hypertrophy/remodeling; indexing; normalization; tibia length

INTRODUCTION

In the study of human cardiovascular diseases and in preclinical cardiovascular disease models, cardiac dimensions and weight are important measures for disease severity, cardiac adaptation, and the effect of interventions. These measurements are commonly normalized for subject size, to correct for variations in body size. This is called indexing and is usually performed with body weight (BW), body surface area (BSA), or body mass index. Despite that indexing by BSA is common practice in both clinical and research settings, the most commonly used method to calculate BSA is still based on century-old formulas, derived from only nine subjects (3). In animal experiments, BW is generally considered the preferred measure to index cardiac measures. However, BW might be directly affected or confounded by the disease under study, for example by cachexia or fluid retention in heart failure. Furthermore, treatments may induce alterations in body composition. In such cases, indexing organ size for BW may be misleading and may introduce significant error in normalized values and comparisons between groups. To avoid such error, tibia length (TL) has been recommended and widely used for indexing cardiac measures in small animal models. Even though BW and TL are related, there is a conceptual difference between indexing by BW and by TL. When using BW, disease- or treatment-induced changes in BW will affect the indexing, whereas TL normalizes for subject size, irrespective of alterations in body composition. For instance, in animal heart failure studies, fluid retention (e.g., edema, ascites, pleural effusion) will obscure actual BW and thus affect the indexing of cardiac variables by BW. Because of the increased BW resulting from fluid retention, indexed cardiac measurements are underestimated when compared with controls. In such cases, indexing these variables by TL would be preferable. On the other hand, when normalizing for altered body composition is desired, BW may be preferred for normalizing cardiac variables. For example, in the case of obese animals, if one considers some degree of cardiac hypertrophy to be normal for their body composition and subsequently prefers to normalize for this, the measure of choice to index by would be BW. However, if one considers this hypertrophy to be abnormal compared with animals of normal body composition, one should in such case choose TL. The choice of either BW or TL depends on the disease model under study and the research question that is being addressed.

Address for reprint requests and other correspondence: Q. A. J. Hagdorn, Ant. Deusinglaan 1, AB43, 9713AV Groningen, The Netherlands (e-mail: q.a.j.hagdorn@umcg.nl).

Despite the generally appreciated importance of indexing, remarkably little attention has been paid to the question if indexing by either BW or TL exerts the desired effects. The objective of indexing is to relate variables to a measure of animal size (e.g., BW, TL) so that in a group of normal, healthy animals of one species only different in body size, indexed values can be compared. To achieve this, the measure of body size used for indexing must scale proportionally and thus linearly to the to-be-indexed measure. A generally accepted concept is that a mammal's heart weight scales linearly to its BW, when different mammal species of a wide range of sizes are compared (1). With respect to natural growth within one species, the assumption is that cardiac growth also scales proportionally to body growth. This seems to apply to humans (5, 6). Various other cardiovascular parameters also scale to BW, although not necessarily linearly. For example, aortic length (from valve to bifurcation) scales to BW to the power of 0.32, aortic radius to the power of 0.36, aortic luminal area to BW to the power of 0.67, and ventricular volumes and stroke volume linearly to BW (4, 7). These relationships resemble the mathematical relationships of one- ($x^{1/3}$), two- ($x^{2/3}$), and three-dimensional (x^1) measures to mass and volume, following the straightforward mathematic formula, volume = length $\times$ width $\times$ height. The relationship between BW and TL, however, has not been explored previously. The former formula implies that TL, being a one-dimensional measure, scales to $BW^{1/3}$ and thus BW scales to TL^3. Therefore, we question if the use of TL^1 for indexing is physically and mathematically correct. We investigated the relationship between TL and BW, and the relation of these parameters to ventricular weights and diameter in a cohort of healthy rats and in a cohort of healthy mice of different ages and sizes to optimize the current common practice of indexing cardiac parameters in small animal models.

METHODS

Twenty-nine sham-operated, drug-naive male Wistar rats and 117 nonoperated or sham-operated, drug-naive male and female Black 6 mice, used in various animal experiments conducted in our laboratory, were included retrospectively. Age at termination of included rats ranged from 5 to 34 wk, and age of termination of included mice ranged from 3 to 17 wk. All animals were euthanized by exsanguination and extraction of the heart under inhalation of 2%–3% isoflurane. TL, BW, and heart weight were measured of both the mice and rats. In the rats, left ventricular (LV) weight (including septum), right ventricular (RV) weight, and LV end-diastolic internal diameter (measured using echocardiography before termination) were additionally measured. One mouse was excluded, as it was considered to be an outlier with a heart weight of 232 mg, leaving 116 mice included. All included rats and mice were divided, according to BW, into the heaviest 50% (i.e., above the median BW) and the lightest 50% (i.e., below the median BW). Furthermore, 15 full-grown, drug-naive Wistar rats (defined as TL $>$ 4 0 mm) with increased cardiac workload (by either pulmonary artery banding, $n = 8$ or aortocaval shunt, $n = 7$) were included. These rats were stratified according to the presence or absence of clinical symptoms of congestive heart failure (CHF) and fluid retention (defined as the presence of ascites, pleural effusion, or visible liver edema/cirrhosis at termination). Appropriate regression analyses were performed. Groups were compared using the Mann-Whitney *U*-test. Statistical analyses were performed using SPSS (version 23, 2015) and GraphPad Prism (version 7, 2016).

All animal experimental protocols have been approved by the Dutch Central Committee for Animal Experiments and the Animal Care Committee of the University Medical Center Groningen (permit nos. AVD105002015134, AVD10500201583, DEC6827A, DEC6920A). The experiments were conducted according to the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes and the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

RESULTS

Rats. BW correlated nonlinearly (cubically) with TL^1 but linearly with TL^3 ($R^2 = 0.996$ and 0.995, respectively; Fig. 1, *A* and *B*). Heart weight, LV weight, and RV weight showed a linear relationship with BW ($R^2 = 0.957$, 0.950, and 0.939, respectively; Fig. 1, *C*, *E*, and *G*). The linear equation of heart weight crossed the *x*-axis at BW = −143.2 [95% confidence interval (CI) −183; −109.2], whereas LV weight crossed the *x*-axis at BW = −127.9 (95% CI −169.8; −92.65) and RV weight at BW = −116.7 (95% CI −162.7; −78.62). This indicates that for optimal indexing by BW, the heart weight, LV weight, and RV weight should be indexed by dividing the weights by 143.2 + BW, 127.9 + BW, and 116.7 + BW, respectively. Heart weight, LV weight, and RV weight also followed a close linear relation with TL^3 ($R^2 = 0.938$, 0.931, and 0.931, respectively; Fig. 1, *D*, *F*, and *H*). The linear equation of heart weight crossed the *x*-axis at $TL^3 = -27.7^3$ (95% CI -30.6^3; -24.8^3), LV weight crossed the *x*-axis at $TL^3 = -26.7^3$ (95% CI -29.8^3; -23.4^3), and RV weight at $TL^3 = -26.5^3$ (95% CI -29.0^3; -22.2^3). This indicates that for optimal indexing by TL, the heart weight, LV weight, and RV weight should be indexed by dividing the weights by $27.7^3 + TL^3$, $26.7^3 + TL^3$, and $26.5^3 + TL^3$, respectively.

To illustrate the effects of indexing, heart weight and LV and RV weight were compared between the 50% with highest BW and the 50% with lowest BW. As expected, nonindexed heart weight and LV and RV weights differed significantly between groups ($P < 0.001$, Fig. 2, *A–F*). Heart weight and LV and RV weights still differed significantly between groups when they were indexed by BW or TL^1 ($P < 0.001$). After indexing by BW, indexed values appeared to be higher in the group with the lightest rats, whereas after indexing by TL^1, values remained lower when compared with the group with heaviest rats. When heart weight and LV and RV weights were indexed by the newly proposed formulas, indexed weights did not differ between groups (Fig. 2, *A–F*). LV diameter, a one-dimensional measure, differed significantly between groups without indexing ($P < 0.001$). However, after indexing by $BW^{1/3}$ or TL^1, indexed LV diameter did not differ between groups (Fig. 2*G*).

Rats that were subjected to increased loading conditions, either in the presence or absence of CHF (CHF^+ and CHF^-, respectively), were compared with healthy rats. Out of the 15 rats with increased loading conditions, 5 showed clinical CHF (ascites 4/5, pleural effusion 2/5, liver edema/cirrhosis 1/5). TLs of all these rats, and the control group, were within the range of 40–42 mm. Healthy rats had a median BW of 467 g, CHF^- rats had a median BW of 399 g ($P = 0.043$ compared with control), and CHF^+ rats had a median BW of 549 g ($P = 0.009$ compared with control, and $P = 0.002$ compared with CHF^-). Because BW appears to scale to TL^3, BW-to-TL^3 ratio

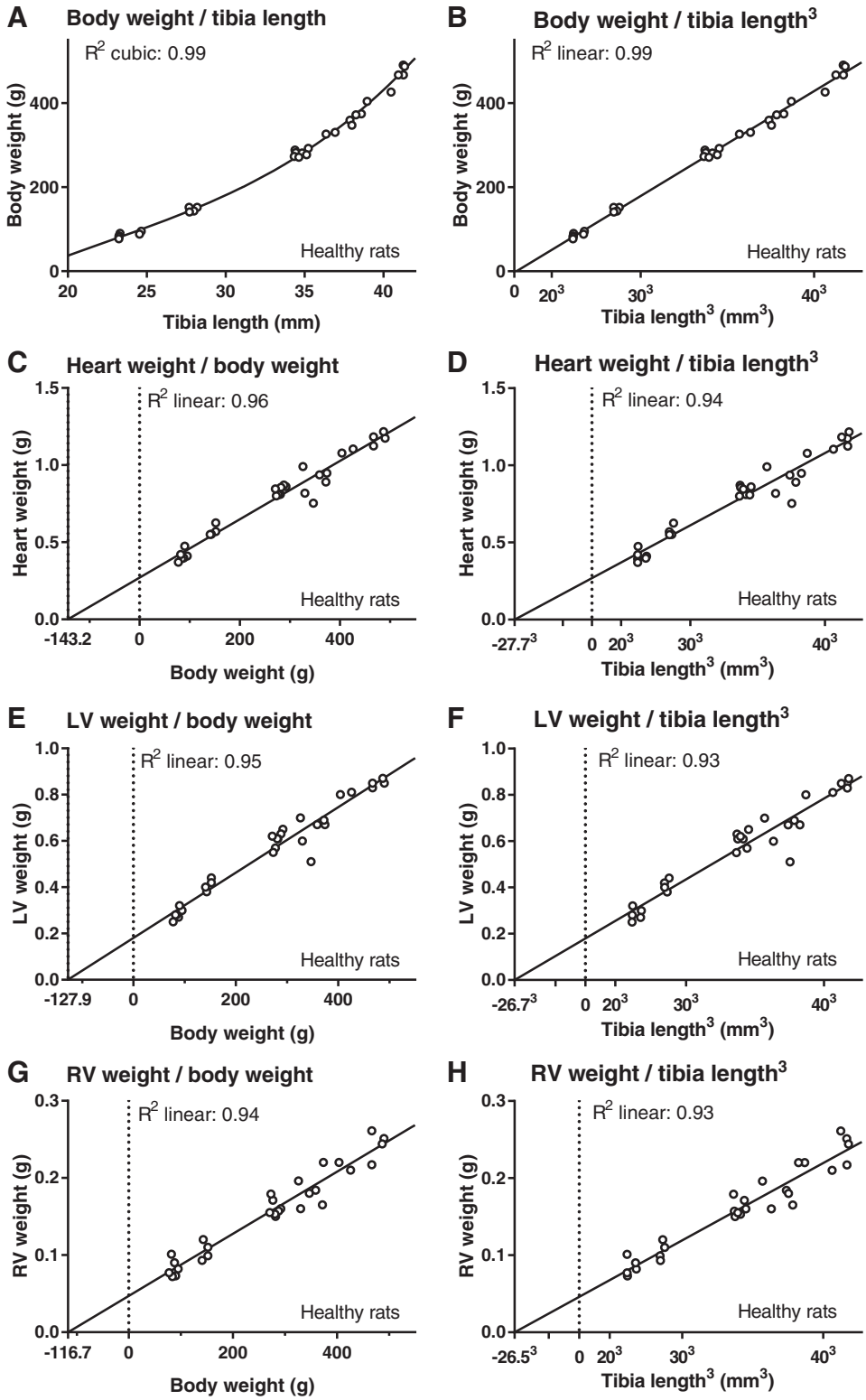

Fig. 1. BW and cardiac weights of the rat scale to TL^3. Scatter plot illustrating the cubic relationship between BW and TL (*A*), the linear relationship between BW and TL^3 (*B*), heart weight versus BW (*C*), heart weight versus TL^3 (*D*), LV weight versus BW (*E*), LV weight versus TL^3 (*F*), RV weight versus BW (*G*), and RV weight versus TL^3 (*H*). BW, body weight; LV, left ventricular; RV, right ventricular; TL, tibia length.

was calculated and used as a measure similar to body mass index, as it also reflects the relation of mass to length. Rats that were subjected to increased loading conditions without signs of CHF had a lower BW-to-TL^3 ratio and were thus relatively lighter when compared with healthy rats ($P = 0.012$, Fig. 2*H*). In contrast, rats with signs of clinical CHF (ascites, pleural effusion, or visible liver edema/cirrhosis) had increased BW-to-TL^3 ratios and were thus heavier compared with healthy rats ($P = 0.012$, Fig. 2*H*). To illustrate how the alterations in BW induced by CHF will affect indexing of cardiac variables, RV weight was indexed using the newly proposed formulas based on either BW or TL and compared between CHF^- and

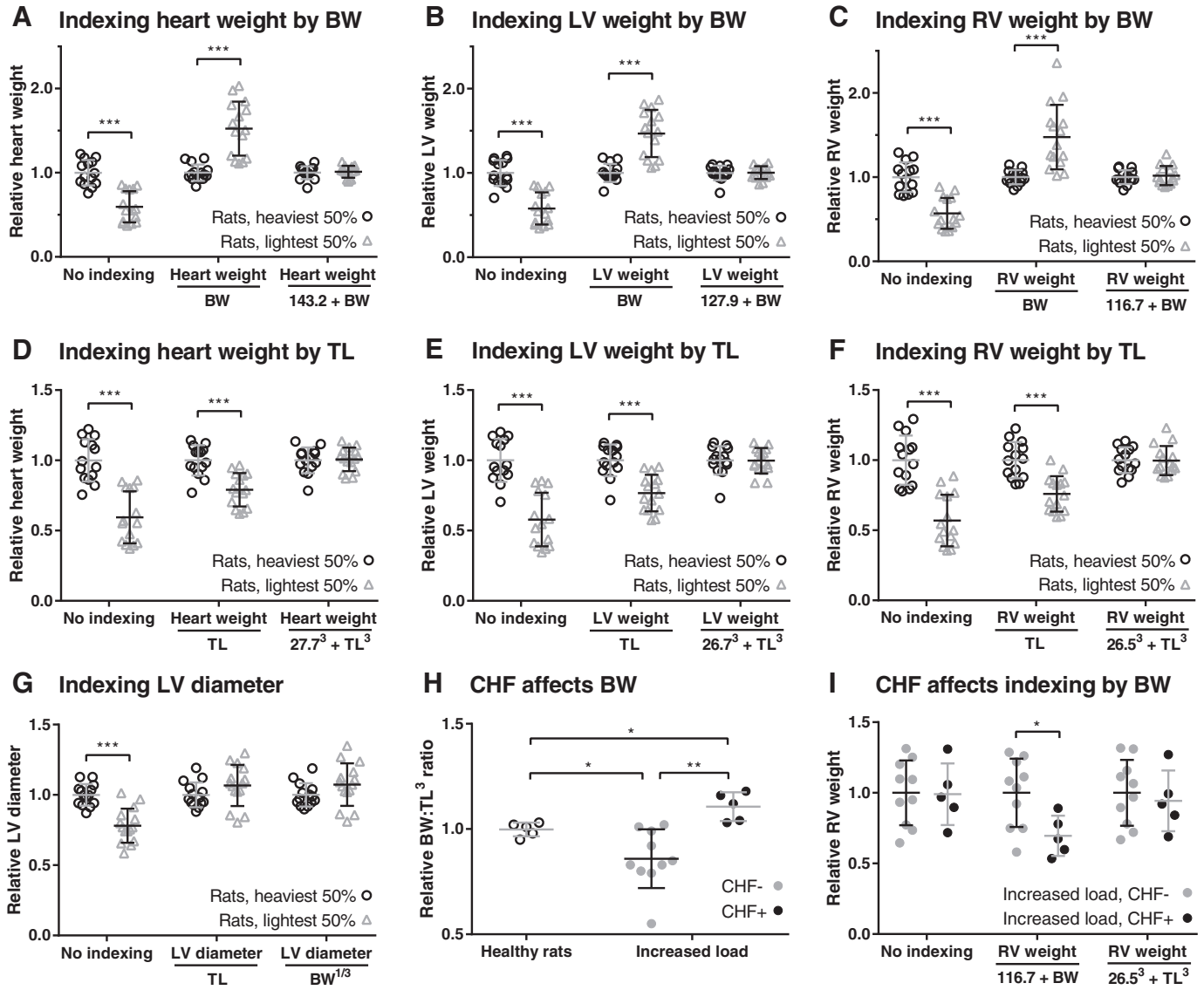

Fig. 2. Effects of indexing in rats. Comparison of heart weight (*A* and *D*), LV weight (*B* and *E*), and RV weight (*C* and *F*) (relative to mean of the heaviest 50% group) between the heaviest 50% (by BW) of the rats (○) with the lightest 50% of the rats (Δ) using either no indexing, indexing by dividing by TL or BW, or indexing using the newly proposed formulas. Comparison of the heaviest 50% (by BW) of the rats (○) with the lightest 50% of the rats (Δ) by LV diameter (relative to mean of the heaviest 50% group) using either no indexing, indexing by dividing by TL or $BW^{1/3}$(*G*). Comparison of the BW-to-TL^3 ratio between healthy full-grown rats (○) and full-grown rats subjected to increased cardiac workload, either in CHF^-/CHF^+ rats, relative to the mean of the healthy rat group (*H*). Comparison of RV weight (relative to mean of the CHF^- group) between CHF^- rats with CHF^+ rats using either no indexing, indexing by BW according to the proposed formula, or by TL^3 according to the proposed formula (*I*). Bars represent means ± SD; $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$. BW, body weight; CHF^-, absence of congestive heart failure; CHF^+, presence of congestive heart failure; LV, left ventricular; RV, right ventricular; TL, tibia length.

CHF^+ rats (Fig. 2*I*). Because the RV is subjected to increased load both in the pulmonary artery banding and the aortocaval shunt models in this series, RV weight was chosen for this comparison. Nonindexed RV weight did not differ between CHF^- and CHF^+ rats. RV weight indexed for BW differed significantly between groups ($P = 0.037$) whereas RV weight indexed for TL did not differ between groups (Fig. 2*I*).

Mice. Similar to rats, BW in mice appeared to scale linearly to TL^3 ($R^2 = 0.887$, Fig. 3*A*). Heart weight showed a linear relationship with both BW and TL^3 ($R^2 = 0.913$ and 0.849, respectively, Fig. 3, *B* and *C*). The linear equations for heart weight with BW and TL^3 crossed the *x*-axis at BW = −0.36 (95% CI −1.83; 0.96) and $TL^3 = -5.8^3$ (95% CI -8.4^3; 5.4^3), respectively. Because zero falls within both CIs, the origins of the equations are not significantly different from zero. This implies that for optimal indexing, heart weight should be indexed by dividing by BW or TL^3 without additional correction factor. Similar to rats, heart weight was compared between the 50% of the mice with highest BW and the 50% with lowest BW. Nonindexed heart weight differed significantly between groups ($P < 0.001$). As in rats, heart weight in mice still differed significantly between groups when indexed by TL^1 ($P < 0.001$). However, when indexed by TL^3 or BW, indexed heart weight did not differ between groups (Fig. 3*D*).

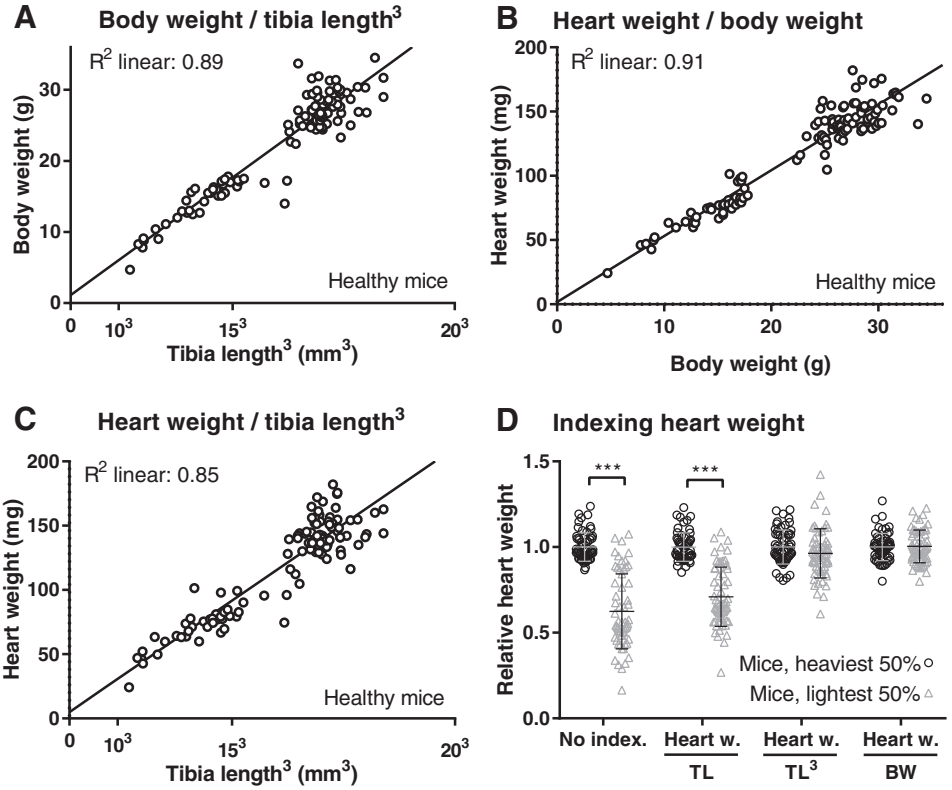

Fig. 3. BW and heart weight of the mouse scale to TL^3. Scatter plots illustrating the linear relationship between BW and TL^3 (*A*), heart weight and BW (*B*), and heart weight and TL^3 (*C*) in 116 mice. Comparison of the heaviest 50% (by BW) of the mice (○) with the lightest 50% of the mice (**Δ**) by heart weight (relative to mean of the heaviest 50% group), using no indexing, indexing by dividing by TL, indexing by dividing by TL^3, or indexing by dividing by BW (*D*). Bars represent means ± SD; ***$P < 0.001$. BW, body weight; LV, left ventricular; RV, right ventricular; TL, tibia length.

DISCUSSION

The present study shows that in healthy rats and mice of a broad range of ages, BW scales to TL^3. This implies that when TL is used as an alternative for BW for indexing cardiovascular measures, one should use TL^3 instead of TL^1. Indexing cardiac weights in rats directly by BW appears to induce significant error, and indexing by TL^1 appears to be insufficient. The proposed formulas for indexing by BW with correction factor in rats appear to be superior to indexing by uncorrected BW or by TL^1, as the differences in cardiac weights between the groups of rats with different BW disappeared when indexed with the proposed formulas. In mice, indexing by BW directly seemed to be appropriate. However, similarly to rats, indexing heart weight in mice by TL^1 is insufficient, whereas indexing by TL^3 results in similarly indexed heart weights between groups. The authors therefore issue a warning that both in rats and in mice, indexing cardiac weights by TL^1 should be considered pseudo-indexing.

As previously discussed, the choice to use either BW or TL for indexing remains dependent of the desired concept of indexing, whether indexing for subject size, incorporating disease-induced body weight alterations (BW), versus indexing for normal subject size (TL). The present study provides formulas for either approach in both rats and mice, summarized in Table 1.

We propose new methods for indexing cardiac weights using either BW or TL. The current study did not address the question of how to index other cardiovascular measures optimally, such as cardiac output, but the authors propose to stick to the mathematic formula, volume = length × width × height. Accepting this concept would mean that cardiac parameters should not be all indexed to the same measure but one-dimensional measures (diameter) to $BW^{1/3}$ or TL^1, two-dimensional measures (area) to $BW^{2/3}$ or TL^2, and three-dimensional measures (volume or mass) to BW or TL^3. This concept, summarized in Table 1, is strengthened by the finding that LV diameter in rats, being a one-dimensional measure, was accurately indexed by $BW^{1/3}$ and TL^1 (Fig. 2*D*). The use of parameter-specific and dimensionally consistent methods of indexing, similar to this concept, have also been proposed in humans (2).

The data from the current study underscore the substantial knowledge gaps regarding indexing of experimental cardiac measures. The observation that the equations of cardiac weights with BW and TL^3 in rats do not cross the $x = 0$ and $y = 0$ point, in contrast to the equations in mice, illustrates that indexing may be more complex than often appreciated. As the youngest ages of inclusion (rat 5 wk, mouse 3 wk) are rela-

Table 1. *Formulas for indexing cardiac weights*

	BW Formula	TL Formula
Rat		
Heart weight	HW/(143.2 + BW)	HW/(27.7^3 + TL^3)
LV weight	LVW/(127.9 + BW)	LVW/(26.7^3 + TL^3)
RV weight	RVW/(116.7 + BW)	RVW/(26.5^3 + TL^3)
Mouse	HW/BW	HW/TL^3
Dimensionally consistent concept		
1-D measures, diameter	*x*/$BW^{1/3}$	*x*/TL^1
2-D measures, area	*x*/$BW^{2/3}$	*x*/TL^2
3-D measures, volume/mass	*x*/BW^1	*x*/TL^3

Values for weight are in grams. Values for TL are in millimeters. *x* represents the to-be-indexed parameter. 1-D, one-dimensional; 2-D, two-dimensional; 3-D three-dimensional; BW, body weight; HW, heart weight; LVW, left ventricular weight; RVW, right ventricular weight; TL, tibia length.

tively similar in relation to the respective life spans, it seems unlikely that different ages of inclusion account for this difference between rats and mice. One could speculate that this could be because of differences between rats and mice in either fetal cardiac growth rate or cardiac growth rate in the neonatal period, before the youngest included animals in this study.

Finally, the effects of increased cardiac workload on BW in relation to TL^3 were studied in rats. The decreased BW in rats without CHF, presumably because of a certain degree of cachexia, and the increased BW in rats with CHF, presumably resulting from fluid retention, underscore that the choice of indexing by BW or TL depends on the research question, goal of indexing, and the animal model under study. Figure 2*I* shows that in this cohort of rats with increased cardiac loading conditions, alterations in BW can significantly influence indexing and thus induce error by underestimation of the indexed variable in the group with fluid retention. In case of fluid retention, the use of the proposed formulas containing TL instead of BW should be used to avoid substantial error.

CONCLUSION

The present study shows that in healthy rats and mice of a broad range of ages, BW scales to TL^3. Therefore, when using TL as surrogate for BW for indexing, one should thus use TL^3 to avoid pseudo-indexing. We provide new formulas for indexing cardiac weights in rats and mice and propose a concept in which cardiac parameters should not all be indexed to the same measure but one-dimensional measures to $BW^{1/3}$ or TL^1, two-dimensional measures to $BW^{2/3}$ or TL^2, and three-dimensional measures to BW or TL^3.

GRANTS

This work was supported by Sebald Fund, Stichting Hartekind, and The Netherlands Heart Foundation Grant NHS2013-T091, Cobra3.

DISCLOSURES

The University Medical Center Groningen (UMCG) has received fees for consultancy activities of R. Berger for Actelion and Lilly outside the content of this manuscript. The UMCG has received research grants and/or fees from AstraZeneca, Abbott, Bristol-Myers Squibb, Novartis, Novo Nordisk, Roche, Trevena, and ThermoFisher GmbH for projects of R. de Boer, outside the content of this manuscript. R. de Boer is a minority shareholder of scPharmaceuticals, Inc. and received personal fees from MandalMed Inc., AstraZeneca, Novartis, Servier, and Vifor. The other authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

Q.A.J.H. and R.M.F.B. conceived and designed research; Q.A.J.H., G.P.L.B., A.M.C.K., A.P., and T.R.E. performed experiments; Q.A.J.H. analyzed data; Q.A.J.H., G.P.L.B., A.M.C.K., A.P., T.R.E., D.E.v.d.F., H.H.W.S., R.A.d.B., and R.M.F.B. interpreted results of experiments; Q.A.J.H. and D.E.v.d.F. prepared figures; Q.A.J.H. drafted manuscript; Q.A.J.H., G.P.L.B., A.M.C.K., A.P., T.R.E., D.E.v.d.F., H.H.W.S., R.A.d.B., and R.M.F.B. edited and revised manuscript; Q.A.J.H., G.P.L.B., A.M.C.K., A.P., T.R.E., D.E.v.d.F., H.H.W.S., R.A.d.B., and R.M.F.B. approved final version of manuscript.

REFERENCES

1. **Brody S.** *Bioenergetics and Growth*. New York: Reinhold Publishing, 1945.
2. **Dewey FE, Rosenthal D, Murphy DJ JR, Froelicher VF, Ashley EA.** Does size matter? Clinical applications of scaling cardiac size and function for body size. *Circulation* 117: 2279–2287, 2008. doi:10.1161/CIRCULATIONAHA.107.736785.
3. **Dubois D, Dubois E.** Clinical calorimetry: tenth paper a formula to estimate the approximate surface area if height and weight be known. *Arch Intern Med (Chic)* 17: 863–871, 1916. doi:10.1001/archinte.1916.00080130010002.
4. **Li JK.** *Comparative Cardiovascular Dynamics of Mammals*. Boca Raton, FL: CRC Press, 1995.
5. **Seo JS, Lee SY, Won KJ, Kim DJ, Sohn DS, Yang KM, Cho SH, Park JD, Lee KH, Kim HD.** Relationship between normal heart size and body indices in Korean. *J Korean Med Sci* 15: 641–646, 2000. doi:10.3346/jkms.2000.15.6.641.
6. **de Simone G, Daniels SR, Devereux RB, Meyer RA, Roman MJ, de Divitiis O, Alderman MH.** Left ventricular mass and body size in normotensive children and adults: assessment of allometric relations and impact of overweight. *J Am Coll Cardiol* 20: 1251–1260, 1992. doi:10.1016/0735-1097(92)90385-Z.
7. **West GB, Brown JH, Enquist BJ.** A general model for the origin of allometric scaling laws in biology. *Science* 276: 122–126, 1997. doi:10.1126/science.276.5309.122.