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Architectural assessment of rhesus macaque pelvic floor muscles: comparison for use as a human model

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Abstract

Introduction and hypothesis—Animal models are essential to further our understanding of the independent and combined function of human pelvic floor muscles (PFMs), as direct studies in women are limited. To assure suitability of the rhesus macaque (RM), we compared RM and human PFM architecture, the strongest predictor of muscle function. We hypothesized that relative to other models, RM best resembles human PFM.

Methods—Major architectural parameters of cadaveric human coccygeus, iliococcygeus, and pubovisceralis (pubococcygeus + puborectalis) and corresponding RM coccygeus, iliocaudalis, and pubovisceralis (pubovaginalis + pubocaudalis) were compared using 1- and 2-way analysis of variance (ANOVA) with post hoc testing. Architectural difference index (ADI), a combined measure of functionally relevant structural parameters predictive of length-tension, force-generation, and excursions muscle properties was used to compare PFMs across RM, rabbit, rat, and mouse.

Results—RM and human PFMs were similar with respect to architecture. However, the magnitude of similarity varied between individual muscles, with the architecture of the most distinct RM PFM, iliocaudalis, being well suited for quadrupedal locomotion. Except for the pubovaginalis, RM PFMs inserted onto caudal vertebrae, analogous to all tailed animals. Comparison of the PFM complex architecture across species revealed the lowest, thus closest to human, ADI for RM (1.9), followed by rat (2.0), mouse (2.6), and rabbit (4.7).

Conclusions—Overall, RM provides the closest architectural representation of human PFM complex among species examined; however, differences between individual PFMs should be taken

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Compliance with ethical standards

Conflict of interest None.

into consideration. As RM is closely followed by rat with respect to PFM similarity with humans, this less-sentient and substantially cheaper model is a good alternative for PFM studies.

Keywords

Rhesus macaque; Pelvic floor muscles; Muscle architecture

Introduction

Pelvic floor disorders (PFDs) affect 25% of the US female population and are associated with decreased quality of life, depression, and social isolation [1]. The pelvic floor muscle (PFM) complex, comprised of contiguous muscles joined by connective tissue network, is essential for normal pelvic floor function in women, and PFM dysfunction plays a key role in the development of PFDs [2]. Despite this, little is known about tissue-level pathological transformations that account for PFM dysfunction. One of the main reasons is that these muscles are located deep in the pelvis, making them very difficult to access in living women, thus precluding direct studies. Furthermore, procurement of sufficient tissue for analyses is often not possible. Therefore, animal models are necessary for high-resolution investigations of PFMs. But how does one choose a suitable experimental model for studying intrinsic components of PFMs to determine muscle physiological and pathological properties? It is well established that neither the size of an organism nor gross muscle anatomy correlates well with muscle function and should not be used as measures of an animal model's reliability for predicting human conditions [3, 4]. In skeletal muscles, a set of structural parameters, referred to as muscle architecture, is the strongest predictor of in vivo muscle function and is best fitted to answer this question [5]. Therefore, determining PFM architectural similarity to humans is of utmost importance for animal model selection, meaningful interpretation of study findings, and translation to humans.

Nonhuman primates (NHP) have been promoted as the best animal model for studying PFDs and PFM dysfunction [6, 7] for three main reasons: (1) maternal and fetal body size of many members of this diverse taxonomic order are closer to humans than other experimental animals; (2) some NHP are partially bipedal [8]; (3) a proportion of parous NHP develop pelvic organ prolapse (POP) [7, 9]. However, anatomical and locomotive variances impact the extent to which PFMs of this animal model can represent human conditions. In particular are the preferred quadrupedal locomotion in NHP vs. obligate bipedalism in humans, distinct shape of the bony pelvis, and presence of a tail. Any of these conditions may lead to functionally important structural differences [6, 8] and result in overestimation of NHP relevance in the study of human PFMs. Thus, the primary objective of this research was to determine the architectural design of individual PFM complex components of rhesus macaque (RM), the most extensively studied NHP in PFD research, and to compare functionally relevant structural parameters for use as a human model. Second, we tested the hypothesis that RM PFMs best resemble humans by assessing RM and human muscles relative to other animal models commonly used in female pelvic medicine.

In addition to the architecture that determines muscle's active mechanical properties, intramuscular extracellular matrix (ECM), a network of connective tissue dominated by

collagen, provides structural stability to myofibers, accounts for muscle load-bearing capacity, and serves as an important biological mechanotransducer responsible for muscle stiffness [10]. Differential collagen content is observed in muscles with variable functional roles [11]. Furthermore, pathological increase in intramuscular collagen content, known as fibrosis, which often occurs with muscle injury and aging, profoundly impacts muscle function [10, 12]. Published histological studies suggest that collagen proportion of RM PFMs is substantially greater relative to other RM muscles. This is thought to be due to the supportive function of PFMs [6]. Therefore, our third objective was to quantify and compare intramuscular collagen content of RM and human PFMs.

Materials and methods

The study was exempt by the University of California, San Diego, institutional review board (IRB) and Institutional Animal Care and Use Clinic (IACUC), given it did not involve living subjects. Human PFM specimens were obtained from eight female cadaveric donors with no history of PFDs, in collaboration with the Bequest Body Donation Program at the University of Minnesota. Rhesus macaque (*Macaca mulatta*) cadaveric PFMs were procured from 15 animals without detectable POP in collaboration with the Oregon National Primate Research Center. PFMs were fixed in situ attached to the skeleton to preserve in vivo architecture. The human PFM complex consists of coccygeus (C) and components of the levator ani (LA) muscle: iliococcygeus (IC); pubovisceralis (PV), composed of pubococcygeus (PC) and puborectalis (PR). Individual muscles were identified by origin-insertion pairs and harvested, as previously described [12]. Similarly, RM PFM complex consists of C and muscles comprising the LA: iliocaudalis (ICa); PV, which includes pubovaginalis (PVa) and pubocaudalis (PCa). RM PFMs were identified by the following origin–insertion pairs: C: ischial spine–lower sacrum through third caudal vertebrae; ICa: ilium–second/third caudal vertebrae; PVa: pubic ramus–arcus tendinous fascia pelvis; PCa: inferior pubic ramus–second/ third caudal vertebrae. Each RM PFM was matched to its respective human analog based on anatomical and functional considerations (Fig. 1) [13]. We designated PVa to be the most analogous to PC because of its contribution to the arcus tendinous fascia pelvis that, similar to humans, serves as a lateral attachment for the RM vagina (Fig. 2).

Muscle architecture

The PFM complex was studied both as a whole and as individual muscles. For each muscle, mass and the following functionally relevant architectural parameters were determined and compared within and between species: muscle length (L_m), physiologic cross-sectional area (PCSA), normalized fiber length (L_{fn}), and sarcomere length (L_s). The relationship between PCSA and L_{fn} determines force generation relative to muscle excursion [5, 12]. One of the fundamental properties of skeletal muscle is that its contractile force depends on sarcomere length (L_s). The maximum force during active muscle contraction is produced at species-specific optimal sarcomere length (L_{so}), corresponding to the most favorable overlap of actin and myosin [5, 14]. However, operational in vivo L_s varies between muscles to best fit specific muscle function [15].

The percent contribution of each PFM to total mass and PCSA of the muscle complex was calculated by dividing individual muscle mass and PCSA by the corresponding parameter of the entire PFM complex. L_m was measured from the origin of the most proximal to insertion of the most distal fibers. Muscles were divided into three regions, and L_f was measured with digital calipers from small fiber bundles (10–20 myofibers) obtained from each region under a dissecting stereomicroscope. Three myofibers were then microdissected from each bundle for L_s determination by laser diffraction, as previously described [14]. To account for potential differences in L_m and L_f that can occur as a result of variable body position at the time of death or fixation, these parameters were normalized by L_{so}/L_s ratio using well-established methods [16]. Because the fixation process shortens L_s by <10%, L_s measurements were also used to determine in vivo operational L_s of each muscle [12]. PCSA was calculated, as previously described [3]. To account for differences in absolute size of human and RM and to facilitate meaningful comparisons, architectural parameters of LA components were normalized to C within each species.

Intramuscular extracellular matrix (ECM)

Surrounding and imbedding contractile myofibers is the intramuscular ECM, which offers dynamic adaptations in response to various mechanical and physiological demands placed on the muscle. To quantitate the amount of total collagen of human and RM PFMs, samples from anterior, mid, and posterior regions of each muscle were obtained at the center of the muscle belly to avoid origin–insertion sites. A well-established and previously validated in our laboratory hydroxyproline assay was used to determine intramuscular collagen content [12, 17].

Architectural difference index (ADI)

The ADI is a validated measure of skeletal muscle comparison across species that is extensively used to guide animal model selection for studies of appendicular and abdominal wall muscles [4, 18]. ADI was calculated for the entire PFM complex and its individual components using the best predictors of in vivo muscle function, specifically: L_{fn}/L_{mn} , L_s/L_{so} , and percent contribution to the total PCSA and mass of the entire complex. Historic data from our lab were used for rat, mouse, and rabbit [3]. PFMs from each species were compared with human specimens ($ADI = 0$), with smaller ADI indicating greater similarity to humans.

Statistical considerations

Consistent with architectural studies of the limb muscles, a sample size of seven specimens per group was calculated based on 14% coefficient of variation (CV) of L_{fn} , one of the main skeletal muscle comparative parameters, from our preliminary data and the effect size (δ) of 20% using the following equation: $N = [16 \times (CV^2) / (\ln(1 - \delta))]^2$ [19]. The power was set to 0.80 and significance level (α) to 0.05. Data were screened for normality and skew to ensure appropriateness of parametric tests. Demographic data were compared between groups using an unpaired Student *t* test. Comparisons within and between species were done with one- and two-way repeated measures analysis of variance (ANOVA) or Friedman test for normally and not-normally distributed data, respectively, followed by post hoc Tukey's or Dunn's pairwise comparisons, if indicated. Results are presented as mean $\pm$ standard error

of the mean (SEM), except where noted. All statistical analyses were performed using GraphPad Prism version 6.00 GraphPad Software, San Diego, CA, USA.

Results

Human ($N=8$) and RM ($N=15$) cadaveric donors were middle aged (51.3 ± 7.4 and 11.1 ± 4.5 years, respectively), which coincides with the onset of sarcopenia for each species [20]. Body condition score (BCS) in the RM is analogous to human body mass index (BMI), ranging from 1 (cachectic) to 5 (obese) [21]. Mean BMI was $19.8 \pm 1.5 \text{ kg/m}^2$ and mean BCS 2.4 ± 0.7 , both of which represent normal weight. The groups did not differ with respect to parity ($P=0.2$).

Human PFM formed a continuous sheet that spanned the pelvic outlet, with the most proximal C located in the same plane as LA (Fig. 1). Unlike humans and similar to other animal species, RM C was posterior to the plane of LA (Fig. 1). Also analogous to other tailed animals and in contrast to humans, RM PFMs were oriented in the same plane as the obturator internus muscle and inserted onto ipsilateral caudal vertebrae. With respect to RM PFM insertion, the exception was the PVa portion of the PV muscle, which, similar to human PC, originated at the pubic ramus and inserted onto the arcus tendinous fascia pelvis, forming a paravaginal supportive structure (Fig. 2). However, unlike human PC, RM PVa was distal to PCa. PVa, which previously has not been consistently identified as a separate muscle [13], was present in all 15 RM specimens. Gross disruption of PFM bellies or attachments was not observed in any human or RM specimen. There were no regional or left-to-right-side differences between PFMs within each species; therefore, these data were combined.

In skeletal muscles, PCSA is the best predictor of isometric force-generating capacity, while L_{fn} and L_{fn}/L_{mn} ratio determine important active muscle properties of excursion and contractile velocity [22]. Muscles with large PCSA, short L_{fn} , and small L_{fn}/L_{mn} ratio are optimized for stabilization. On the other hand, muscles with smaller PCSA and longer fibers are better suited for actions requiring greater range of motion (ROM) and faster contractile velocity [5]. Muscle operational L_s and the resultant ratio of operational to species-specific optimal sarcomere length (L_s/L_{s0}) determines the in vivo length-tension relationship of a resting muscle. Muscles with $L_s/L_{s0} = 1$ operate at optimal L_s . $L_s/L_{s0} < 1$ allows muscle to increase its contractile force when fibers elongate, while $L_s/L_{s0} > 1$ indicates that active force increases during muscle shortening. As expected, the absolute values of examined parameters varied between human and RM PFMs by up to an order of magnitude due to differences in body size (Table 1).

With respect to force-generating capacity, C had the largest PCSA among human PFMs ($P < 0.01$) while both PC and PR portions of PV had the smallest PCSA ($P < 0.04$) (Table 1). In RM, on the other hand, PCSA of ICa and PVa portion of PV were similar ($P = 0.9$) and substantially larger compared with PCa ($P < 0.01$) while approaching statistically significant difference relative to the smaller PCSA of C ($P = 0.06$). With respect to excursions capacity, in both humans and RM, C had the shortestest L_{fn} and therefore the smallest ROM relative to components of the LA muscle ($P < 0.05$), (Table 1).

Operational L_s of all four human PFMs was very similar ($P > 0.7$) and almost identical to human L_{s0} of $2.7 \mu\text{m}$ (Table 1). With the exception of ICa, RM operational L_s was also close to RM L_{s0} of $2.41 \mu\text{m}$ [23] (Table 1). ICa L_s ($2.18 \pm 0.04 \mu\text{m}$), on the other hand, was significantly shorter relative to C and PVa ($P < 0.02$) and compared with RM L_{s0} ($P < 0.0001$.) This indicates that ICa active force increases when this muscle is stretched.

Notably, PC and PR portions of human PV did not differ with respect to architectural parameters. PVa and PCa portions of RM PV were also similar with respect to L_{mn} , L_{fn} , and L_s , but PVa PCSA was significantly larger than that of PCa ($P = 0.002$).

Given the absolute size differences between humans and RM, the contribution of each muscle to total mass of the PFM complex was compared (Table 2). In humans, the mass of C made up $37.6 \pm 1.3\%$ of total mass, compared with $15.5 \pm 1.6\%$ in RM ($P < 0.0001$). In contrast, ICa dominated muscle mass in the RM PFM complex at $39.2 \pm 2.4\%$, which significantly exceeded mass fraction of human IC ($26.9 \pm 1.5\%$), ($P < 0.0001$). The mass contribution of RM PVa ($28.0 \pm 1.9\%$) exceeded that of human PC ($15.5 \pm 0.8\%$) by close to twofold, ($P < 0.0001$), while mass fraction of PCa ($17.6 \pm 1.1\%$) was comparable with human PR ($19.9 \pm 1.5\%$) ($P = 0.9$) (Table 2).

With the exception of PR/PCa homologues, RM and human PFMs differed in their relative contribution to total PCSA of the PFM complex (Table 2). As expected, C had the largest PCSA fraction among human PFMs, exceeding that of RM ($P < 0.0001$). In contrast, in RM, ICa had the largest PCSA fraction, which was substantially greater than the contribution of human IC ($P = 0.04$). L_{fn}/L_{mn} , which reflects muscle excursions capacity and contractile velocity, was similar between human and RM C, IC/ICa, and PC/PVa, $P > 0.1$, (Table 2). Comparison of L_{fn}/L_{mn} between PR and PCa demonstrated a trend toward a larger ratio in humans ($P = 0.05$). We also explored the functionally important PCSA- L_{fn} relationships of individual LA components normalized to respective C to take into account interspecies differences in absolute muscle size. Among muscles comprising LA, RM ICa showed the greatest departure from its human counterpart with respect to PCSA, which was significantly larger in RM ($P < 0.0001$); while L_{fn} was similar for all components of the LA between humans and RM (Fig. 3). Human and RM L_s/L_{s0} ratios were also similar for all muscles. There was a trend toward smaller L_s/L_{s0} of ICa relative to IC; however, the difference did not reach statistical significance (Table 2).

Intramuscular collagen content of 4.5–5% across all human PFMs (Table 1) was on the high end of ~3–5% normally present in skeletal muscles [11]. In contrast, collagen content of RM C ($19.6 \pm 2.1 \mu\text{g}/\text{mg}$, 2%), ICa ($7.9 \pm 1.4 \mu\text{g}/\text{mg}$, 0.8%), and PCa ($13.3 \pm 2.0 \mu\text{g}/\text{mg}$, 1.3%) was substantially lower than their human counterparts ($P < 0.0001$). Total collagen content of PVa ($48.4 \pm 8.5 \mu\text{g}/\text{mg}$, 4.8%) was significantly higher than that of other RM PFMs $P < 0.0001$ (Table 1) and analogous to human PC ($P > 0.9$).

Comparison of PFM architecture across species

Based on conventional nomenclature, the LA is composed of ICa and PCa in animal species [24]. With its relatively broad enthesal region at the pubic ramus [24, 25], filmy attachments to the vagina laterally [26], insertion below the rectum, and contribution to

urethral and anal closure pressures [25, 27], PCa of lower animal species, such as rabbit and rodent, combines anatomical and functional features of both PC and PR portions of human PV muscle. In RM, on the other hand, PCa is more analogous to human PR, while PVa corresponds better with human PC. Although RM PVa has been identified in some studies [13], this muscle has not been included in traditional nomenclature thus far, likely due to a lack of consistent description in the existing body of literature.

To compare PFMs across species commonly used as models in human female pelvic medicine research, we calculated the architectural difference index (ADI) using functionally relevant parameters described above. The lower ADI values indicate a greater similarity to humans [18]. PFM architectural data of mouse, rat, and rabbit were previously reported [3]. The two portions of PV muscle were combined for human and RM. Based on total ADI of the PFM complex, RM (1.9) was found to be the closest to humans (Fig. 4). Surprisingly, despite the close phylogenetic relationship between rat and mouse, total ADI of rat PFMs (2.0) differed more from the total ADI of mouse (2.6) than from RM. Rabbit (4.7) showed the greatest departure from humans among species examined. Notably, the extent to which laboratory animals matched humans varied among individual PFMs (Fig. 4). Despite its lower PCSA, RM C showed the highest similarity to humans when all functionally relevant structural parameters were considered together, while ICa was the least comparable. In fact, rat ICa was the closest to human IC, while the similarity of rat and RM to human PV was identical. ADI of LA components was lower in both rat and RM than in mouse and rabbit.

Discussion

Results of this first comparative study of NHP PFMs demonstrate that based on overall architecture of the PFM complex, RM is the most similar to humans among species examined, confirming our hypothesis. Furthermore, presence of the PVa portion of the PV muscle in RM, which was consistently and reliably identified in all specimens examined, provides an advantage over rodents and rabbits, which do not have this muscle. PVa corresponds to human PC and is the only RM PFM that inserts onto the arcus tendinous fascia pelvis and contributes to paravaginal attachments. PVa contribution to the PFM complex force-generating capacity exceeds that of PC, however PVa and PC are similar with respect to muscle excursion, force–length relationship, and collagen content. Computational models and imaging studies demonstrate that PC component of the LA muscle is the most vulnerable to excessive strain in humans [28]. Therefore, RM PVa is likely to be useful as a human model of simulated childbirth injury. In addition, a large fetal head size relative to maternal vaginal diameter of RM presents some of the same challenges observed in human parturition [6, 9]. However, several important differences exist between the other components of the human and RM PFM complex. Specifically, the design best suited for stabilizer muscles—namely, larger PCSA and shorter fiber length—is found in human C. In contrast, RM C PCSA is small compared with the other muscles, with PCSA of RM PFM complex dominated by ICa instead. The larger isometric force-generating capacity of ICa highlights a likely important role of this muscle in pelvic and spinal stabilization during postural changes. With respect to muscle excursion and contractile velocity, in humans, the longest fibers are found in PV, specifically the PR portion. Such design appears conducive to this muscle's role in maintaining continence, which requires a large ROM and fast speed of

contraction without significant loss of force. In RM, on the other hand, ICa has the longest fibers and, therefore, the largest excursion. In addition, unlike human PFMs, ICa operational L_s is shorter than L_{so} , which facilitates increased force production with muscle lengthening. These structural features likely developed to optimize quadrupedal locomotion. Overall, ICa has the lowest similarity to humans, and its architectural design appears to be specifically conducive for bipedal-to-quadrupedal positional changes. Such differences may overshadow similarity between species due to phylogenetic closeness and account for the disparate impact of induced PFM dysfunction, which, contrary to in humans, does not cause POP in NHP [7, 29]. In fact, the role of LA atrophy in the pathogenesis of POP was brought into question in a study of multiparous squirrel monkeys in which prolapse did not occur despite marked LA atrophy [7]. Rather than an indication of a faulty biological relationship, this discrepancy may represent a limitation of the animal model. If similar to RM, the LA force-generating capacity in squirrel monkeys is larger than in humans, a much greater extent of LA atrophy is likely necessary before these animals reach a clinical phenotype observed in humans. This discrepancy highlights the importance of PFM architectural comparisons for aiding in the interpretation of animal model studies.

In addition to variability in muscle architecture, differences in intramuscular ECM collagen content can greatly affect muscle function [10]. Previously, we demonstrated a substantial fibrotic response of human PFMs with aging [12]. Therefore, specimens for this study were purposefully procured from RM and human donors of similar relative age. Contrary to the published histological findings [6], collagen content of RM PFMs, with the exception of PVa, was minimal and significantly lower relative to human PFMs. Such disparities may predispose human and RM PFMs to a differential impact of aging [1, 12], which is one of the leading risk factors for PFM dysfunction in women. We plan on examining whether, similar to humans, aging is associated with PFM fibrosis in RM.

In our previous comparative study of PFM architectural design across small laboratory species commonly used in PFDs research, we found rat to be the nearest to human [3]. Interestingly, results of the current investigation indicate that PFM architecture of RM and rat are very close with respect to their similarity to humans. NHP accessibility and cost can be prohibitive, especially when the sample size needed to answer a novel research question is not known a priori. Thus, similarities between RM and rat PFM in comparison with humans make the rat model an excellent alternative for tissue-level direct PFM studies given its ready availability, cost effectiveness, and ease of handling.

Limitations of this study are the use of fixed cadaveric tissue, although differences in muscle tone do not affect major determinants of muscle architecture. Furthermore, any potential differences in muscle and fiber length at the time of death or fixation were accounted for by normalizing these parameters to species-specific optimal sarcomere length [3, 18]. Another limitation is that PFM specimens were obtained from middle-aged parous donors and may not be representative of muscles in younger, nulliparous women and RM. However, given similar relative age and parity of the two groups, our comparative results provide valuable information regarding RM PFMs relative to humans. This study is also limited only to the species examined.

Conclusions

Comparison of PFM architecture between laboratory animals and humans is an important consideration for experimental model selection and interpretation of study results. With respect to the architectural design of the PFM complex, RM is closest to humans. However, the similarity between RM and human PFMs differs only marginally from that between rat and human PFMs. Therefore, the less-sentient and substantially cheaper rat model is a good alternative to consider for PFM studies [30]. Future directions include determination of PFM architecture in other NHP, given the diversity of this large taxonomic order.

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Abbreviations

ADI	Architectural difference index
ATFP	Arcus tendinous fascia pelvis
BCS	Body condition score
C	Coccygeus
ECM	Extracellular matrix
H	Human
IC	Iliococcygeus
ICa	Iliocaudalis
LA	Levator ani
L_{fn}	Normalized fiber length
L_{mn}	Normalized muscle length
L_s	Sarcomere length
L_{so}	Optimal sarcomere length
NHP	Nonhuman primates
PC	Pubococcygeus
PCa	Pubocaudalis
PCSA	Physiologic cross-sectional area

PFM	Pelvic floor muscle
PR	Puborectalis
PV	Pubovisceralis
PVa	Pubovaginalis
RM	Rhesus macaque

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Fig. 1. Human and rhesus macaque (RM) en block homologous pelvic floor muscles, with individual muscle borders outlined on the *right side*. The *punctate line* demonstrates more posteriorly located coccygeus in RM

Fig. 2.
Rhesus macaque pelvis illustrating pubovaginalis (PVa) inserting onto arcus tendinous fascia pelvis (ATFP)

Fig. 3. Fiber length vs physiological cross-sectional area (PCSA) demonstrating relationship between excursions and force-generating properties of individual components of human (H) and rhesus macaque (RM) levator ani (LA) muscle, normalized to coccygeus of the respective species. *IC/ICa* Iliococcygeus/Iliocaudalis, *PC/PVa* pubococcygeus/pubovaginalis, *PR/PCa* puborectalis/pubocaudalis. **P* values derived from repeated measures two-way analysis of variance (ANOVA), followed by Tukey's pairwise comparisons

Fig. 4.

Total and individual architectural difference index of the pelvic floor muscle complex and of coccygeus (C), iliococcygeus/iliocaudalis (IC/ ICa), and pubovisceralis/ pubocaudalis (PV/ PCa) components of the complex. Human ADI = 0. Lower values indicate greater similarity to human

Table 1

Architectural data and intramuscular collagen content of coccygeus (C), iliococcygeus/iliocaudalis (IC/ICa), pubococcygeus/ pubovaginalis (PC/PVa), and puborectalis/pubocaudalis (PR/PCa) portions of pubovisceralis (PV) for human and rhesus macaque (RM) presented as mean $\pm$ standard error of the mean (SEM)

Muscle	Species	Mass (g)	PCSA (cm ²)	L _n (mm)	L _{mm} (mm)	L _s (μm)	Collagen content (μg/mg tissue)
C	Human	6.7 $\pm$ 0.8	1.5 $\pm$ 0.2	40.3 $\pm$ 2.9	60.6 $\pm$ 2.7	2.64 $\pm$ 0.04	45.1 $\pm$ 3.7
	RM	0.7 $\pm$ 0.1	0.4 $\pm$ 0.03	15.8 $\pm$ 1.0	19.3 $\pm$ 1.1	2.37 $\pm$ 0.06	19.6 $\pm$ 2.1
IC/ICa	Human	4.8 $\pm$ 0.5	0.8 $\pm$ 0.1	55.9 $\pm$ 3.4	77.0 $\pm$ 4.6	2.65 $\pm$ 0.08	42.4 $\pm$ 2.9
	RM	1.7 $\pm$ 0.2	0.5 $\pm$ 0.04	29.4 $\pm$ 1.2	49.0 $\pm$ 1.5	2.18 $\pm$ 0.04	7.9 $\pm$ 1.4
PV	Human						
	PC	2.8 $\pm$ 0.4	0.4 $\pm$ 0.06	65.2 $\pm$ 2.3	89.0 $\pm$ 6.5	2.68 $\pm$ 0.07	50.2 $\pm$ 4.0
	PR	3.6 $\pm$ 0.6	0.5 $\pm$ 0.1	67.1 $\pm$ 5.3	87.3 $\pm$ 5.1	2.62 $\pm$ 0.05	48.9 $\pm$ 1.9
	RM						
C vs IC/ICa	PVa	1.2 $\pm$ 0.1	0.5 $\pm$ 0.05	23.6 $\pm$ 2.0	32.9 $\pm$ 2.0	2.41 $\pm$ 0.04	48.4 $\pm$ 8.5
	PCa	0.7 $\pm$ 0.1	0.3 $\pm$ 0.02	25.9 $\pm$ 1.8	40.8 $\pm$ 1.4	2.32 $\pm$ 0.05	13.3 $\pm$ 2.0
	Human	0.3 *	0.001	0.04	0.08	0.9	0.9
	RM	0.0002	0.06	<0.0001	<0.0001 *	0.01 *	0.1
C vs PV							
	Human	<0.0001 *	<0.0001	0.006	0.02	>0.9	0.7
	RM	0.0003	0.06	0.0016	0.04 *	>0.9 *	<0.0001
	Human	0.02 *	<0.0001	0.04	0.009	0.9	0.8
IC/ICa vs PV	RM	0.9	0.04	0.001	0.0002 *	>0.9 *	0.6
	Human	0.07 *	0.004	0.08	0.5	0.9	0.3
	RM	0.047	0.9	0.1	0.007 *	0.0001 *	<0.0001
IC/ICa vs PR/PCa	Human	>0.9 *	0.03	0.3	0.6	0.9	0.5
	RM	<0.0001	0.0005	0.2	0.4 *	0.05 *	0.7
	Human	0.7 *	0.2	0.9	0.9	0.8	0.9
	RM	0.0009	0.002	0.9	0.9 *	0.6 *	<0.0001

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L_{H} and L_{MH} fiber and muscle length normalized to optimal sarcomere length of each species, $PCSA$ physiological cross-sectional area, L_S operational sarcomere length

* P -values derived from Friedman test, followed by Dunn's pairwise comparisons, within species. All other P -values derived from repeated measures one-way analysis of variance, followed by Tukey's pairwise comparisons, within species

Table 2

Comparison of relative mass, relative physiologic cross-sectional area (PCSA), normalized fiber to muscle length ratio, and operational to optimal sarcomere length ratio of coccygeus (C), iliococcygeus/ iliocaudalis (IC/ICa), and pubococcygeus/pubovaginalis (PC/PVa) and puborectalis/ pubocaudalis (PR/PCa) portions of pubovisceralis (PV), between human and rhesus macaque (RM), presented as mean $\pm$ standard error of the mean

Muscle	Species	Mass fraction (%)	PCSA fraction (%)	L_{in}/L_{mn}	L_s/L_{so}
C	Human	37.6 $\pm$ 1.3	47.3 $\pm$ 1.8	0.69 $\pm$ 0.05	0.99 $\pm$ 0.02
	RM	15.5 $\pm$ 1.6	23.9 $\pm$ 1.6	0.81 $\pm$ 0.03	0.98 $\pm$ 0.02
IC/ICa	Human	26.9 $\pm$ 1.5	25.1 $\pm$ 1.6	0.72 $\pm$ 0.04	0.97 $\pm$ 0.04
	RM	39.2 $\pm$ 2.4	32.1 $\pm$ 1.9	0.60 $\pm$ 0.03	0.90 $\pm$ 0.02
PV	Human				
	PC	15.5 $\pm$ 0.8	12.0 $\pm$ 0.2	0.75 $\pm$ 0.04	0.99 $\pm$ 0.03
	PR	19.9 $\pm$ 1.5	15.4 $\pm$ 1.3	0.78 $\pm$ 0.06	0.97 $\pm$ 0.02
	RM				
	PVa	28.0 $\pm$ 1.9	29.3 $\pm$ 1.7	0.73 $\pm$ 0.04	1.00 $\pm$ 0.02
	PCa	17.6 $\pm$ 1.1	16.4 $\pm$ 1.8	0.63 $\pm$ 0.03	0.96 $\pm$ 0.02
C: human vs RM		<0.0001	<0.0001	0.1	0.9
IC vs ICa		<0.0001	0.04	0.2	0.1
PV: human vs RM					
PC vs PVa		<0.0001	<0.0001	0.9	0.9
PR vs PCa		0.9	0.9	0.05	0.9

L_{fin} , L_{mn} fiber and muscle length normalized to optimal sarcomere length (L_{so}) of each species: Human $L_{so} = 2.7$ μ m; RM $L_{so} = 2.41$ μ m. PCSA physiological cross-sectional area

P values derived from repeated measures two-way analysis of variance (ANOVA), followed by Tukey's pairwise comparisons