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Research article

Characterizing the functional ANOVA model for repeated measures via PCA application to biomechanical data

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Abstract: Gait analysis is a branch of biomechanics where its purpose is the study of mechanical laws relating to the way the body moves from one place to another. In most cases, the data sets for human gait analysis consist of continuous recordings of multiple physical activities, including kinematics and muscle performance. Despite the registered data being functions, the most common practice to detect any anomalies among experimental conditions consists of analyzing the vector of discrete observations or even summary measures of the curves. This fact causes an important information loss since the continuous nature of the data is being ignored. A suitable solution is to apply functional data analysis for analyzing continuous biomechanical data as functions, revealing the true nature of movement and allowing us to model and forecast the data with more precision. In the current paper, a new functional methodology for the analysis of variance with repeated measures was introduced. In particular, since functional data variability can be summarized by their first principal component scores, we proposed to turn the functional model into a multivariate one for the response of the most explicative principal components, and then, considered a semi-parametric approach to overcome the restrictive assumptions required in the classic repeated measures design. The motivation of this research was to contrast the differences in gait patterns of elementary school students when walking to school, depending on the type of bag they use to carry their school materials. The analysis reveals that gait joint movement is influenced by sex and the type of schoolbag, regardless of the load carried.

Keywords: functional data analysis; analysis of variance; principal component analysis; repeated measures; biomechanics; gait analysis
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1. Introduction

In numerous fields of science, we find magnitudes (spectrums, signals, images, etc.) characterized by the evolution of a continuous random variable. These variables have been historically examined through multivariate techniques or time series analysis over a vector of discrete observations at different time points. By way of motivation, data characterizing human motion in biomechanical studies are usually waveform curves that represent joint measures such as flexion angles, velocity, or linear acceleration, among others. A common practice in this area is to analyze discrete summaries of sample curves [1–3]. In other words, the information of each curve is represented by a single scalar measure (mean, maximum, minimum, etc.) of the discrete observations instead of using a magnitude evaluated completely in the whole domain. This approach not only leads to biased results but also causes a significant loss of information such as the continuity or smoothness of curves. Moreover, it is common to have high-dimensional data where the number of variables exceeds the number of sampled individuals, so that traditional statistical methods are not appropriate.

In response to the growing need to develop a comprehensive approach encompassing multivariate statistical methods to model functions (generally curves) depending on time or another continuous argument, functional data analysis (FDA) is gaining momentum in the last three decades, also motivated by the technological progress (see [4–8] to get global and structured knowledge of the basic theory, applications, and computational aspects in FDA). Taking its characteristics and good performance into account, FDA can be used for analyzing biomechanical data in a continuous way, revealing the true nature of the movement [9], detecting the location and magnitude of differences within the observed functions [10], and providing more consistent [11] and discriminative [12] results. A more general systematic review about the importance and benefits of conducting FDA in biomechanical studies can be found in [13, 14].

The current work aims to analyze how the type of schoolbag affects the gait patterns of primary school students when walking to school. The details of the experimental study are provided later. In particular, we are interested in detecting significant differences among the joint rotation angle in each axis direction for several types of schoolbags. Given the underlying theoretical framework in this study, a new functional analysis of variance approach with repeated measures (FANOVA-RM) is performed. This technique tests the equality of mean curves of a functional variable observed on the same sample individuals under various experimental conditions. The majority of the previous works are focused on the independent measures design (see [15] for an exhaustive analysis of the key elements in the FANOVA problem and [16] for a wide review of different tests for the one-way layout). However, the literature for the repeated measures design is sparse. For example, the initial testing strategy for the pairwise case was suggested in [17]. Nevertheless, only the variability between groups was considered in this procedure. In order to heed the variability within groups, two new statistics were proposed in [18] which were subsequently extended in [19] by assuming a basis expansion of sample curves. In a similar way, two different basis expansion estimation approaches for the FANOVA-RM model were proposed and compared in [20]. However, the inherent problem of this proposal was the high multicollinearity caused by the own basis coefficients, as well as the need of having a moderate sample size in order to guarantee the power of the hypothesis tests.

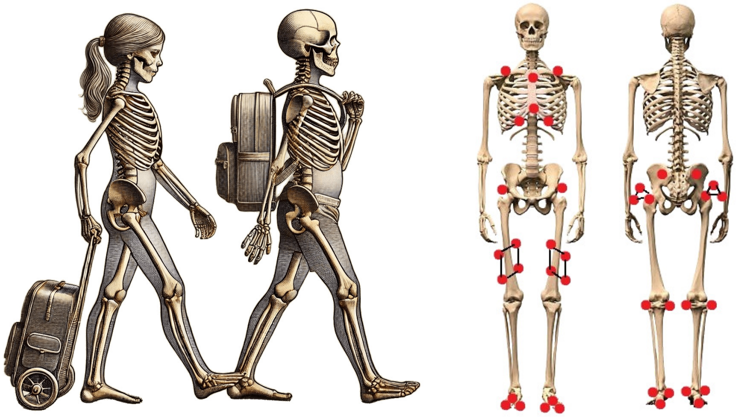
Functional principal component analysis (FPCA) is a powerful tool in FDA that can help to reduce the infinite dimension of functional data and to solve the limitations in the estimation of functional statistical learning models. FPCA has already been considered in biomechanical studies to explain the variability structure of data making use of a small set of uncorrelated principal components both for the univariate scenario [21, 22] and for the case of having more than one functional variable [23, 24]. In broad terms, FPCA is a technique for reducing the dimensionality of such datasets, increasing interpretability but at the same time minimizing information loss [25–28]. Moving from an infinite-dimension space to a finite-dimension one generated by a reduced set of principal components is a common practice that provides good results. Different unsupervised functional classification models have been developed, for example, clustering [29, 30] and random forest [31, 32]. On the other hand, several functional logistic regression approaches for supervised classification of curves were developed in [33, 34]. A regularization approach for function-on-function principal component regression based on the merge of functional data analysis with group Lasso was introduced in [35]. The case of function-on-scalar regression was studied in [36] where a functional mixed regression model was considered with application to positron emission tomography (PET) data. In addition, FPCA has been used by [19, 37] within the FANOVA model with independent measures for the univariate and multivariate functional framework, respectively.

Assuming that curves variability can be summarized by the first principal component scores, in this work we propose that the FANOVA-RM model can be reduced to a multivariate analysis of variance with repeated measures (MANOVA-RM) for the multivariate response vector of the most explicative principal components. There are multiple procedures for estimating the MANOVA-RM model. The multivariate mixed model and the doubly multivariate model were the first approaches proposed in the literature to include the within-subject effect (the schoolbag type in our case) in the analysis [38–40]. These parametric approaches assume multivariate normality and covariance homogeneity, very restrictive assumptions from an applied viewpoint. A more flexible option is a semi-parametric model that does not require any of these conditions. In particular, we adapt the semi-parametric bootstrap approach proposed by [41, 42], in which a Wald-type statistic (WTS) is computed. This procedure allows us to evaluate together the interaction effects among variables within subjects and between subjects with minimal assumptions, and also, the possibility that effects on response variables may depend on group levels of covariates.

In addition to this introduction, the rest of manuscript is organized as follows. The experimental study that motivates this work is detailed in Section 2. The theoretical aspects are in Section 3. The results obtained after applying the methodology are shown in Section 4. Finally, the most important conclusions are summarized in Section 5.

2. Experimental study

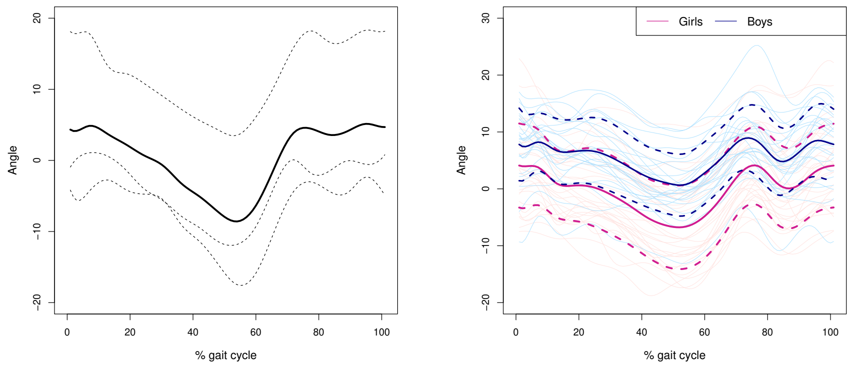
The Sport and Health University Research Institute of the University of Granada (iMUDS, for its acronym in Spanish) conducted an experimental study on 53 children aged between 8 and 11, including 25 boys and 28 girls. Seven experimental conditions were considered: only walking, walking with a trolley, and walking with a backpack (see Figure 1(a)) with different loads in the schoolbags: 10, 15, and 20% of their body weight. The technical details about the instrumentation and procedures employed for the data collection are given in [21]. In summary, a 3D motion capture system (Qualisys AB, Göteborg, Sweden) was used to record the gait cycle by means of twenty-six reflective markers and nine infrared high-speed cameras at a capture rate of 250 Hz. Figure 1(b) reveals the position of these markers on the children's bodies.



a) b)

Figure 1. a) Children walking with a trolley and backpack (image generated with ChatGPT). b) Position of the reflective markers on the children's bodies to register the gait cycle.

To obtain the experimental data, each child walked three times along a 15-meter platform. In each gait cycle, the angle for ankle, hip, knee, pelvis, thorax, and foot progress was registered for each experimental condition described above in each axis direction: X, Y, and Z that represent flexion/extension, adduction/abduction, and internal/external rotation, respectively. Note that only one direction was taken for foot progress. Once the three cycles are monitored, the mean curve is computed and selected as the representative observation of the gait for each child (see Figure 2(a)). Hence, the sample consists of 53 curves observed in 101 equidistant points for each articulation, axis direction, and experimental condition. For example, the sample curves together with the mean curves and pointwise confidence bands distinguishing the subjects by sex for the hip articulation on the Z axis in the scenario of carrying a trolley with a load of 10% body weight are shown in Figure 2(b). In addition to the sex and the stochastic evolution of the rotation angle, other demographic and clinical variables of interest such as the age, back pain, or body mass index were collected as well. However, these variables will not be considered in the present research.



a) b)

Figure 2. a) The three registers (dashed lines) of the gait cycle and the corresponding mean (solid line) for a subject. b) Sample curves together with the mean curves and the confidence bands distinguished by sex for the hip articulation on the Z axis considering a load of 10% body weight in the trolley.

This study aims to find dissimilarities and possible relationships in the rotation angle over each joint and axis controlling the load's weight for each type of schoolbag, differentiating the students by sex. In other words, we want to analyze if the schoolbag prototype has an influence on the gait patterns, as well as to detect if there are differences depending on gender. From a mathematical viewpoint, this problem is reduced to comparing the mean curves among the different experimental conditions for each of the articulations and axes. Historically, different approaches have been considered in the literature to address the classical ANOVA problems (or simpler models such as the two sample test or two sample Hotelling's T2 test) but most of them do not take the whole curves into account. For example, a common practice is to consider as the response variable the vector of the discrete measures without taking into account the continuous nature of the data. Other research even chose scalar variables related to the functional response variable such as velocity, cadence, stride length [2], and stance and swing phases [43]. On the contrary, a spatiotemporal analysis of these data by using a point-to-point ANOVA test, without taking into account the temporal dependence, was developed in [44]. Pointwise analysis approaches ignore the functional nature of the data, which may hinder the ability to find subtle differences between experimental conditions and/or subject populations. This was warned in [45], where the use of mixed effects spline smoothing analysis of variance to analyze differences in cyclic biomechanical data was proposed (see [46] for a better understanding). In this regard, and with the aim of providing new functional tools that provide rigorous results in practice, a new two-way FANOVA-RM aproach is proposed and applied in the present paper. By way of clarification, we have a repeated measures design because each child is exposed to all experimental conditions (seven curves for each student) and two-way analysis because there are two categorical variables as factors: type of schoolbag (W: within-subject factor) and sex (B: between-subject factor).

3. Theoretical framework

FDA provides a conceptual framework for the building, treatment, and study of functional objects both in an individual way and in groups. Currently, the efforts are focused on the paradigm of generalized the methods of the supervised and unsupervised statistical/machine learning to the functional scenario. For example, classical statistical techniques such as regression, classification, and clustering have been extended to functional data in approaches as diverse as the parametric (see, e.g., [47]), semi-parametric (see, e.g., [48]), non-parametric (see, e.g., [49]), or Bayesian (see, e.g., [50]).

Formally, let us consider $\{X_{ijk}(t) : i = 1, \ldots, m; j = 1, \ldots, J; k = 1, \ldots, n_j; t \in T\}$, a random sample of curves related to a functional variable X. That is, $X_{ijk}(t)$ is the observed value of the response variable for the k-th subject in the j-th independent group, measured under the i-th experimental condition at time t in a continuous time interval T. Besides, let us suppose they are realizations of a second-order and continuous quadratic mean stochastic process $X = \{X(t) : t \in T\}$, whose sample paths belong to the Hilbert space $L^2(T)$ of square integrable functions with the usual inner product $< f, g >= \int_T f(t)g(t)dt, \forall f, g \in L^2(T)$.

Functional data are intrinsically of infinite dimension, and therefore their treatment poses serious complications because of the impossibility of observing the continuum. In practice, we have a set of discrete observations at different time points that could be unequally spaced and different among the sample individuals and/or even could be greater than the sample size (high-dimensional data). Unlike the scalar data, functional data need pre-processing in which several assumptions are required for the analysis. One of the main assumptions is that functional data belong to a space of functions with certain analytical and topological characteristics, equivalents to the euclidean spaces in which the classical statistic has been developed. In this sense, it is common to assume that functional data are elements of a space generated by a finite basis [4, 5]. With this approach we are transforming the infinite space into a finite-dimension one generated by a set of scalar variables that represent the curves in a correct and rigorous way. In particular, the information and characteristics of sample curves are summarized by the vector of basis coefficients. However, the inherent problem is the high correlation produced by the basis coefficients, which provides a serious problem of multicollinearity when the functional model is reduced to a multivariate one based on the matrix of basis coefficients. Despite the good predictive ability of this procedure, the multicollinearity makes model interpretation more difficult. The most-studied solutions consist of assuming approaches based on using uncorrelated explicative variables. For this reason, FPCA plays a fundamental role in the estimation of FDA models not only to solve the multicollinearity problem but also to reduce dimensionality, which avoids overfitting.

3.1. Functional PCA

FPCA has the same motivation [51] as its multivariate counterpart and it is based on the Karhunen-Loève expansion, which provides an orthonormal representation of the functional observations in terms of uncorrelated variables with maximum variance.

The l-th functional principal component is a generalized linear combination of the original functional variable with maximum variance, that is,

$$Z_{ijk}^{(l)} = \int_T (X_{ijk}(t) - \mu(t)) f_l(t) dt, \tag{3.1}$$

where $\mu(t)$ is the overall mean function and $f_l(t)$ is the principal component weight function obtained by maximizing the following objective function with the corresponding constraints:

$$\begin{cases} Var[\int_T (X_{ijk}(t) - \mu(t))f(t)dt] \\ \text{subject to} \int_T f(t)f(t)dt = 1 \text{ and } \int_T f_r(t)f(t)dt = 0, r = 1, \ldots, l-1. \end{cases}$$

Then, $f_l(t)$ are the solutions to the eigenequation $\int_T C(t,s)f_l(s)ds = \lambda_l f_l(t)$ with $C(t,s)$ being the sample covariance function and $\lambda_l = Var[Z_l]$ the l-th eigenvalue, respectively. Finally, the process admits the following orthogonal decomposition known as Karhunen-Loève expansion, which can be truncated in terms of the first q principal components providing the best linear approximation of the sample curves in the least squares sense:

$$X_{ijk}(t) = \mu(t) + \sum_{l=1}^{\infty} Z_{ijk}^{(l)} f_l(t) \rightarrow X_{ijk}(t) \approx X_{ijk}^{q}(t) = \mu(t) + \sum_{l=1}^{q} Z_{ijk}^{(l)} f_l(t). \tag{3.2}$$

There are different rules in the literature to determine the optimal number of principal components to be selected [52]. A suitable option to choose q when the functional variable is the response variable in a regression model, as in our case, consists of selecting a cut-off of total variability which is large enough to obtain an accurate prediction.

3.2. FANOVA-RM

Functional analysis of variance is a fundamental problem in statistical inference with functional data, aiming to determine differences in the average curves through a hypothesis test of equality of means as the null hypothesis. This allows us to assess the effect that a given treatment or experimental condition has on the sample.

According to the characteristics of our experimental design, the FANOVA model can be expressed as follows:

$$x_{ijk}(t) = \mu(t) + \alpha_i(t) + \beta_j(t) + \theta_{ij}(t) + \epsilon_{ijk}(t) \ \forall t \in T, \tag{3.3}$$

where $\mu(t)$ is the overall mean function; $\alpha_i(t)$ and $\beta_j(t)$ are the i-th and j-th main effects functions associated with the experimental conditions and the independent groups, respectively; $\theta_{ij}(t)$ is the functional interaction parameter, and $\epsilon_{ijk}(t)$ are independent and identically distributed (i.i.d.) errors. In our real dataset, the number of experimental conditions (schoolbag type) is $m = 3$ and the group effect (sex) has two levels, $J = 2$.

Since the functional parameters are not uniquely defined, certain constraints must be applied. An appropriate sequence of positive weights should be considered to define the constraints in the unbalanced design [15]. The following constraints are assumed in a balanced design:

$$\sum_{i=1}^{m} \alpha_i(t) = \sum_{j=1}^{J} \beta_j(t) = \sum_{i=1}^{m} \theta_{ij}(t) = \sum_{j=1}^{J} \theta_{ij}(t) = \sum_{i=1}^{m} \sum_{j=1}^{J} \theta_{ij}(t) = 0.$$

It is interesting to test if the within and between subject effects have an influence on the response variable, as well as the possible interaction between them (the impact of one factor depends on the level of the other one). For this purpose, the following null hypotheses are respectively proposed against the alternative, in each case, that its negation holds:

$$
\begin{aligned}
&H_0(W) : \alpha_i(t) = 0, \forall t \in T, \quad i = 1, \ldots, m, \\
&H_0(B) : \beta_j(t) = 0, \forall t \in T, \quad j = 1, \ldots, J, \\
&H_0(WB) : \theta_{ij}(t) = 0, \forall t \in T, \quad i = 1, \ldots, m, \quad j = 1, \ldots, J.
\end{aligned} \tag{3.4}
$$

Assuming the principal component expansion given by 3.2, we propose to reduce the FANOVA-RM model to a MANOVA-RM model for the multivariate response defined by the most explicative functional principal components.

3.3. MANOVA-RM for the principal components

Assuming the principal component expansion given by 3.2, we propose to reduce the FANOVA-RM model to a MANOVA-RM model for the multivariate response defined by the vector Z of the first q functional principal components.

As in many statistical studies, our data violate certain assumptions of classical multivariate methods. In particular, parametric MANOVA-RM models require restrictive assumptions such as multivariate normality, multivariate sphericity, or equal covariance matrices among groups that are barely fulfilled in practice. In addition, they cannot be used for small sample sizes. To solve these limitations, we adapt the methodology in [41, 42] in which none of the above constraints are necessary by applying a semi-parametric bootstrap approach.

Formally, the MANOVA-RM model for the response vector of the first q principal components, $Z = (Z^{(l)})_{l=1}^{q}$, can be formulated as the following MANOVA model where Z_{jk} is a column vector of dimension $m \times q$ whose components are the values of the selected principal components for the k-th sample unit in the j-th group under the m-th experimental condition:

$$
\mathbf{Z}_{jk} = \boldsymbol{\mu}_j + \boldsymbol{\epsilon}_{jk}; \;\; j = 1, \ldots, J; k = 1, \ldots, n_j; \text{ and } N = \sum_{j=1}^{J} n_j, \tag{3.5}
$$

with

$$
\begin{aligned}
\mathbf{Z_{jk}} &= ((\mathbf{Z}_{ijk}^{(l)})_{l=1}^{q})_{i=1}^{m}, \\
\boldsymbol{\mu}_j &= ((\boldsymbol{\mu}_{ij}^{(l)})_{l=1}^{q})_{i=1}^{m}, \\
\boldsymbol{\epsilon}_{jk} &= ((\boldsymbol{\epsilon}_{ijk}^{(l)})_{l=1}^{q})_{i=1}^{m},
\end{aligned}
$$

with i and j representing the subscripts of the within and the between subject factors, respectively, and $\boldsymbol{\mu}_{\mathbf{j}}$ is the mean vector for the j-th group.

The error terms $\boldsymbol{\epsilon}_{jk}$ are independent and identically distributed $m \times q$-dimensional random vectors with mean $E(\boldsymbol{\epsilon}_{jk}) = 0$, assuming positive definite covariance matrices $Cov(\boldsymbol{\epsilon}_{jk}) = \sum_j > 0$ and finite fourth moment $E(||\boldsymbol{\epsilon}_{jk}||^4) < \infty$.

The hypothesis defined in 3.5 can be reformulated by means of an adequate contrast hypothesis matrix $\mathbf{T}$ by

$$
H_0 : \mathbf{T}\boldsymbol{\mu} = \mathbf{0}, \tag{3.6}
$$

where the vector $\boldsymbol{\mu} = (\boldsymbol{\mu}'_1, \ldots, \boldsymbol{\mu}'_J)'$ has dimension $J \times m \times q$.

The Wald-type statistic proposed in [41] is defined as

$$
Q_N(\mathbf{T}) = N \cdot \bar{\mathbf{Z}}'_{\cdot}\mathbf{T}(\mathbf{T}\hat{\mathbf{V}}_N\mathbf{T})^{+}\mathbf{T}\bar{\mathbf{Z}}_{\cdot}, \tag{3.7}
$$

where $()^+$ denotes the Moorse-Penrose generalized inverse, $\bar{\mathbf{Z}}_{\cdot} = (\bar{\mathbf{Z}}'_{1\cdot}, \ldots, \bar{\mathbf{Z}}'_{J\cdot})'$, $\bar{\mathbf{Z}}_{j\cdot} = \dfrac{1}{n_j} \displaystyle\sum_{k=1}^{n_j} \mathbf{Z}_{jk}$,

$$\hat{\mathbf{V}}_N = diag\left(\frac{N}{n_j}\hat{\Sigma}_j : 1, \ldots, J\right),$$

$$\hat{\Sigma}_j = \frac{1}{n_j - 1}\sum_{k=1}^{n_j}(\mathbf{Z}_{jk} - \bar{\mathbf{Z}}_{j\cdot})(\mathbf{Z}_{jk} - \bar{\mathbf{Z}}_{j\cdot})'.$$

It can be proved that $Q_N(\mathbf{T})$ has, as $N \to \infty$, an asymptotic central χ^2-distribution with degrees of freedom equal to the rank of $\mathbf{T}$. However, as this approximation is only valid for large sample sizes, a resampling approach was proposed in [41, 53] that supports a wide range of design configurations [42] and can be used for arbitrary semi-parametric designs, even with unequal covariance matrices among groups and small sample sizes. Given the observations, the idea is to generate semi-parametric bootstrap samples as

$$\mathbf{Z}^*_{jk} \overset{i.i.d.}{\sim} N(\mathbf{0}, \hat{\boldsymbol{\Sigma}}_j),\ \ j = 1, \ldots, J; k = 1, \ldots n_j,$$

in order to obtain a more accurate finite sample approximation by recomputing the test statistic 3.7 as

$$Q^*_N(\mathbf{T}) = N \cdot (\bar{\mathbf{Z}}^*_{\cdot})'\mathbf{T}(\mathbf{T}\hat{\mathbf{V}}^*_N\mathbf{T})^+\mathbf{T}\bar{\mathbf{Z}}^*_{\cdot}.$$

The conditional $(1 - \alpha)$-quantiles from its distribution, $c^*_N(\alpha)$, converge to the asymptotic limit quantile $\chi^2_{rank(\mathbf{T};1-\alpha)}$ resulting in the bootstrap test $\phi^*_N = \mathbf{1}\{Q_N(\mathbf{T}) > c^*_N(\alpha)\}$ where $\mathbf{1}$ is a vector of ones with appropriate order.

As we have a between-subject factor and a within-subject factor, the contrast matrix $\mathbf{T}$ used in 3.6 will be appropriately chosen in the hypotheses for each of the different main effects as

$$H_0(W) : \left\{\left(\tfrac{1}{g}\mathbf{J}_g \otimes \mathbf{P}_m\right)\boldsymbol{\mu} = \mathbf{0}\right\},$$

$$H_0(B) : \left\{\left(\mathbf{P}_g \otimes \tfrac{1}{m}\mathbf{J}_m\right)\boldsymbol{\mu} = \mathbf{0}\right\},$$

$$H_0(WB) : \left\{\left(\mathbf{P}_g \otimes \mathbf{P}_m\right)\boldsymbol{\mu} = \mathbf{0}\right\},$$

where $\mathbf{J}_g$ is a $g \times g$ matrix of ones and $\mathbf{P}_g = \mathbf{I}_g - \frac{1}{g}\mathbf{J}_g$ is the so-called centering matrix, with $\mathbf{I}_g$ being the identity matrix. We have the same reasoning for $\mathbf{J}_m$ and $\mathbf{P}_m$.

When we find significant differences in these contrasts, further analysis is needed to study the reason for these differences. To do this, we use post-hoc comparisons that could be affected by potential inflation of the Type I error rate due to the realization of multiple tests. As it was demonstrated through simulation studies in [42], the semi-parametric procedure used in this paper incorporates adjustments to mitigate this effect without the need for additional methods such as the Bonferroni correction, false discovery rate, etc. [54, 55]

4. Results

The objective is to analyze if, conditioning by weight, there are differences in the angle rotation for each joint in each axis taking as factors the schoolbag type (repeated measures treatments) and gender (independent groups). Note that the results are obtained for each axis and joint separately to be compared with other previous investigations in this field. In fact, the literature states that the axes are independent from a biomechanical viewpoint. Therefore, as it was stated above, the two-way FANOVA-RM is reduced to a two-way MANOVA-RM for the multivariate response of the scores vector of the most explicative principal components. In this sense, the number of principal components has been selected to explain at least 95% of the total variability in order to guarantee a good explanation of the gait process. All the results have been obtained through R, a free software environment for statistical computing. More specifically, the *fda* [6] package has been considered to perform the FPCA (assuming, previously, a cubic B-spline basis of dimension 20 for the functional reconstruction of sample curves) and the *MANOVA.RM* [56] package to conduct the two-way MANOVA-RM. These libraries are available in the official repository for R packages.

A summary of the outcomes for the pelvis articulation can be seen in Table 1. The mean curves for this joint are displayed in Figure 3. The results for the rest of the articulations are available in the Appendix.

Table 1. P-values associated with the bootstrapped WTSs for the pelvis articulation.

		PCs	SEX	SCHOOLBAG	SEX:SCHOOLBAG	POST-HOC
X AXIS (flexion/extension)	10% WEIGHT	1	0.043	<0.001	0.748	TRL-BCK 0.003 WALK-BCK 0.000 WALK-TRL 0.013
	15% WEIGHT	1	0.039	<0.001	0.490	TRL-BCK 0.000 WALK-BCK 0.000 WALK-TRL 0.000
	20% WEIGHT	1	0.046	<0.001	0.500	TRL-BCK 0.000 WALK-BCK 0.000 WALK-TRL 0.000
Y AXIS (adduction/abduction)	10% WEIGHT	4	0.003	<0.001	0.124	TRL-BCK 0.000 WALK-BCK 0.000 WALK-TRL 0.887
	15% WEIGHT	4	0.004	<0.001	0.331	TRL-BCK 0.000 WALK-BCK 0.000 WALK-TRL 0.989
	20% WEIGHT	4	0.023	<0.001	0.226	TRL-BCK 0.000 WALK-BCK 0.000 WALK-TRL 0.963
Z AXIS (internal/external)	10% WEIGHT	3	0.645	<0.001	0.708	TRL-BCK 0.000 WALK-BCK 0.000 WALK-TRL 0.806
	15% WEIGHT	3	0.645	<0.001	0.335	TRL-BCK 0.000 WALK-BCK 0.000 WALK-TRL 0.750
	20% WEIGHT	3	0.699	<0.001	0.527	TRL-BCK 0.000 WALK-BCK 0.000 WALK-TRL 1.000

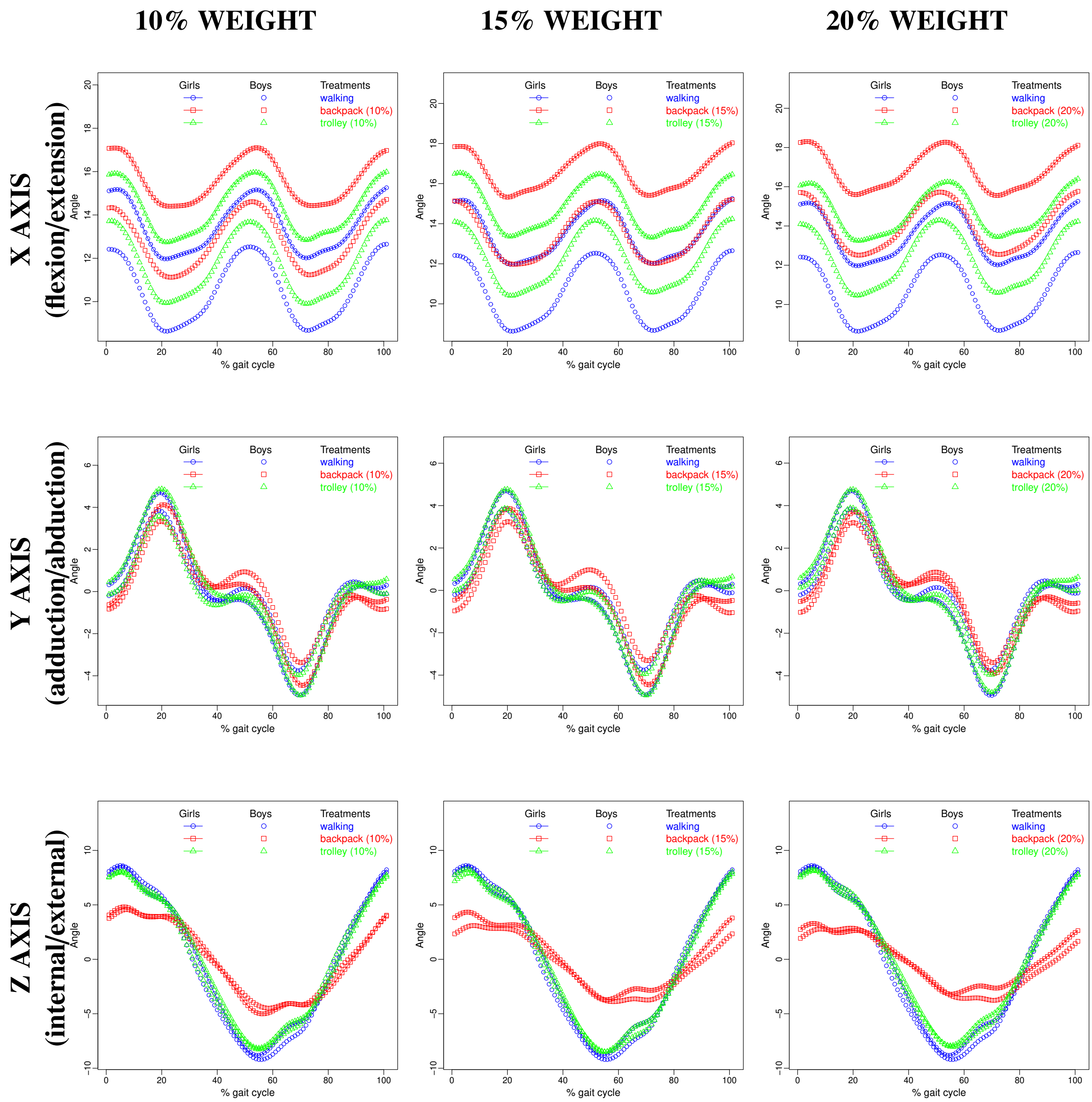


Figure 3. Mean curves of the rotation angle for the pelvis articulation in each experimental condition (treatment).

Focusing on the pelvis articulation, significant differences are found in all axes for both factors, except on the Z axis for sex. Figure 3 reveals how the rotation angle is smaller in boys than in girls on the X axis, whereas the variation range is wider in girls than in boys on the Y axis. Regarding the schoolbag type, the three experimental conditions are different from each other on the X axis. In particular, the rotation angle is arranged in decreasing order as follows: backpack, trolley, and only walking. On the other hand, there are only significant differences between backpack-trolley and backpack-walk on the Y and Z axes. Here, the evolution of the rotation angle in the backpack condition is more constant in comparison with the remainder, especially notable on the Z axis.

Analyzing the results for the rest of the joints, there are no differences by sex in the advancement of the foot. Furthermore, in all joints except for the pelvis, gender has a significant effect on the Z axis where the rotation angle in girls is greater in the ankle and smaller in the hip and knee than in boys. On the Y axis, we find differences in the hip, knee, and pelvis and, on the X axis, in the pelvis and thorax where the rotation angle in boys is lower in the hip and higher in the thorax. In relation to the schoolbag type, we find no significant differences between using a trolley and walking without a schoolbag for the knee, pelvis, and hip on the Y and Z axes, as well as in the thorax only for the Z axis. However, there are differences between using the backpack and the other two experimental treatments. Besides, we also discover dissimilarities for the pelvis and thorax in the three cases on the X axis, whereas only differences are detected in the hip when walking without a schoolbag is involved.

4.1. Discussion

The American Academy of Pediatrics and the Spanish Association of Pediatrics recommend the use of a trolley when the weight of the schoolbag exceeds 10% of the corporal weight. In this vein, [44] suggests that children should avoid loads of more than 10% and 20% of their corporal weight when carrying a backpack and trolley, respectively. In addition, they highlight the importance of using a trolley even in light loads, since the differences in comparison with the control treatment (i.e., walking without anything) are lower with a trolley than with a backpack, independent of the transport load.

Focusing on this research, the results obtained are in line with the findings of other published studies on this topic. As was concluded in [43], we find a significant increase in pelvis flexion/extension in relation to the action of only walking, being greater when carrying a backpack. As with the pelvis, the thorax displays similar results: greater flexion/extension occurs with a backpack than with a trolley; and both are greater in comparison with only walking. The same diagnosis was made over this articulation in [44]. In addition, we do not detect that increasing the schoolbag load has an influence on the differences regarding the control treatment either, which is in agreement with the studies cited above. Only in the knee articulation when subjects are distinguished by sex, it seems that differences are significantly reduced as the load increases. However, due to the sample size, this assertion should be reinforced in future research.

Even so, we have also identified dissimilarities in relation to other research. In particular, no differences were found in the hip, knee, and ankle for any load in [44]. In contrast, we detect that the angle rotation is affected in these articulations, independent of the kind of schoolbag, for all axes; especially in the abduction/adduction axis by carrying a backpack, although for the pair "trolley-walking", the differences vanish.

Most of the research hardly ever classify the subjects according to some morphological, sociological, or anatomical variable to analyze these data; despite the fact that this might arouse a great deal of interest by revealing multiple patterns depending on some characteristic. For example, the effect of a school carriage on obese/overweight and healthy-weight children was analyzed in [57]. In this study, we focus on examining the effect of sex, whose interpretation of the results has already been made above. Therefore, we can conclude that the FDA approach introduced in this paper sheds new light on this topic.

Finally, we highlight that although the proposed methodology has been carried out through an application in biomechanics, it is not limited to this field. The FANOVA-RM framework introduced here is broadly applicable to any scientific discipline where the objective is to analyze the influence of one or more factors on a functional response variable when at least one factor represents an intra-subject condition, i.e., the information from each subject is measured at each level of the intra-subject factor.

5. Conclusions

In this article, a new estimation approach for two-way functional ANOVA with repeated measures has been introduced to analyze how the gait patterns are affected by the type of schoolbag under different weights (within-subject factor) in children of primary education, distinguishing them by sex (between-subject factor). Assuming that functional data can be explained by their first principal component scores, we propose to reduce the functional ANOVA model to a multivariate ANOVA model for the most explicative principal components. The inherent problem is that the repeated measures multivariate model has very restrictive assumptions such as multivariate normality or equal covariance matrices across groups. To avoid these limitations, a solution based on a semi-parametric approach has been considered, whose advantage is providing a rather general and comprehensive theoretical framework to inference for our data.

In addition to its interpretative advantages, the functional methodology introduced in this paper has demonstrated statistical robustness, even with small samples. Previous studies have highlighted the crucial role of sample size in the power of statistical tests, as increased error dispersion tends to make tests less conservative. However, the combination of parametric and non-parametric methodologies based on FPCA has proven to be effective even for small sample sizes, regardless of normality assumptions. Only in extreme scenarios the power of the tests become questionable. Thus, the semi-parametric approach adopted in this study provides a robust alternative to mitigate these limitations while preserving statistical reliability. Likewise, the semi-parametric bootstrap strategy applied in the MANOVA-RM framework has been shown to maintain adequate control of the Type I error rate without the need for additional corrections, even in complex scenarios. This supports the confirmatory nature of the present study and reinforces the reliability of the findings in the biomechanical field.

In broad terms, the results suggest significant effects of the factors of sex and schoolbag prototype and, on the other hand, a negligible interaction with each other. In particular, the post-hoc analysis concludes that the rotation angle formed by the joints when students go to school is less altered by the trolley than by the backpack. These preliminary results reveal important information that might help governments, official organizations, families, and/or teachers to adopt preventive measures that guarantee the health of children.

Author contributions

All authors contributed equally to this work. All authors have read and agreed to the published version of the manuscript.

Use of Generative-AI tools declaration

Figure 1(a) was generated using the free version of ChatGPT (OpenAI) by asking it to give us a single sepia-toned image of a girl skeleton pulling a trolley-style backpack and a boy skeleton with a backpack on his back.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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Appendix

Table 2. P-values associated with the bootstrapped WTSs for the ankle articulation.

		PCs	SEX	SCHOOLBAG	SEX:SCHOOLBAG	POST-HOC
X AXIS (flexion/extension)	10% WEIGHT	5	0.384	<0.001	0.343	TRL-BCK 0.371 WALK-BCK 0.030 WALK-TRL 0.432
	15% WEIGHT	5	0.324	<0.001	0.740	TRL-BCK 0.961 WALK-BCK 0.187 WALK-TRL 0.274
	20% WEIGHT	5	0.281	<0.001	0.866	TRL-BCK 0.561 WALK-BCK 1.000 WALK-TRL 0.548
Y AXIS (adduction/abduction)	10% WEIGHT	5	0.305	0.008	0.570	TRL-BCK 0.795 WALK-BCK 0.101 WALK-TRL 0.368
	15% WEIGHT	5	0.332	0.002	0.188	TRL-BCK 0.699 WALK-BCK 0.122 WALK-TRL 0.419
	20% WEIGHT	5	0.229	<0.001	0.921	TRL-BCK 0.788 WALK-BCK 0.529 WALK-TRL 0.213
Z AXIS (internal/external)	10% WEIGHT	5	0.025	<0.001	0.794	TRL-BCK 0.371 WALK-BCK 0.030 WALK-TRL 0.432
	15% WEIGHT	5	0.011	<0.001	0.832	TRL-BCK 0.961 WALK-BCK 0.187 WALK-TRL 0.274
	20% WEIGHT	5	0.019	<0.001	0.882	TRL-BCK 0.561 WALK-BCK 1.000 WALK-TRL 0.548

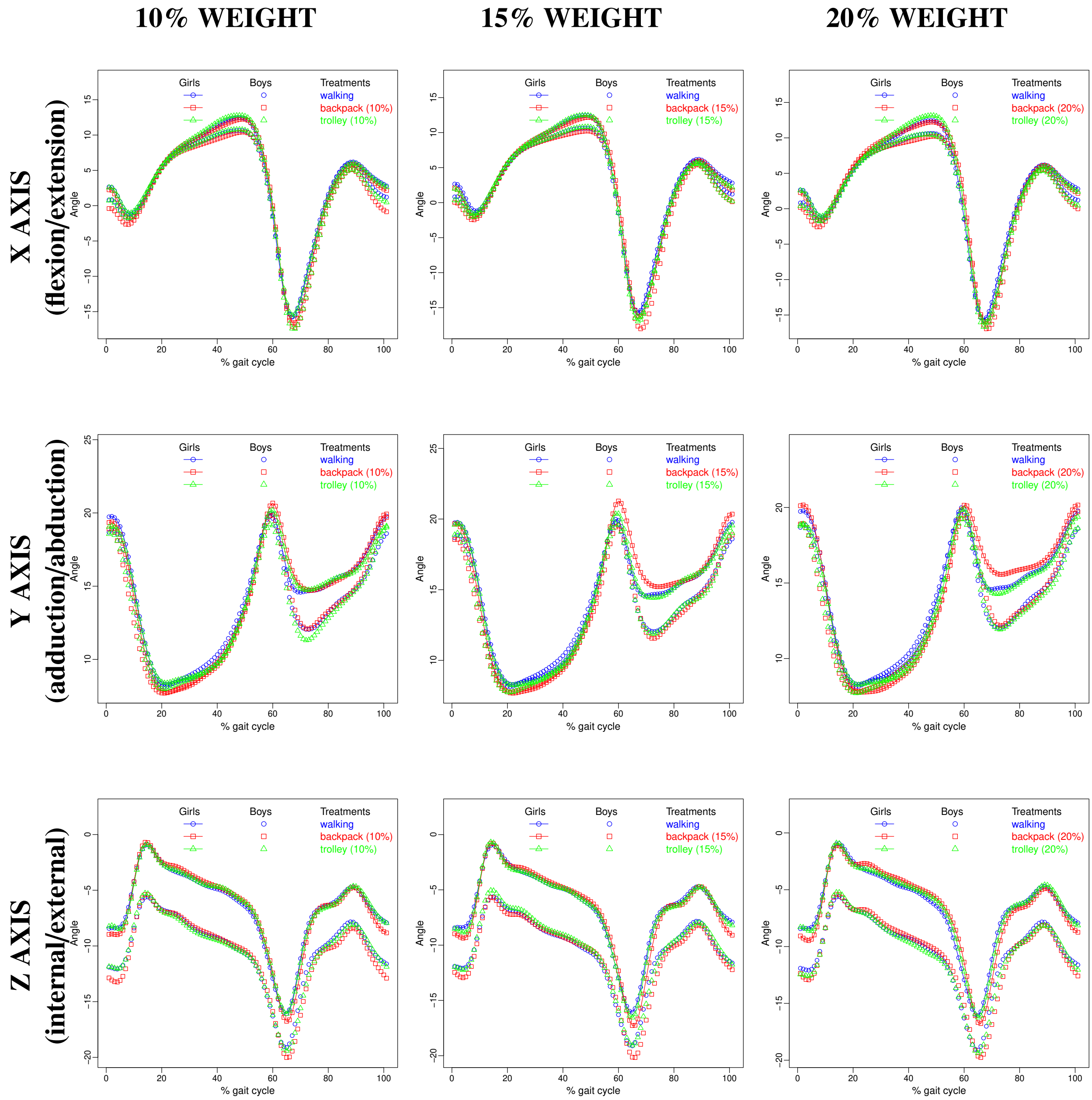

Figure 4. Mean curves of the rotation angle for the ankle articulation in each experimental condition (treatment).

Table 3. P-values associated with the bootstrapped WTSs for the hip articulation.

		PCs	SEX	SCHOOLBAG	SEX:SCHOOLBAG	POST-HOC
X AXIS (flexion/extension)	10% WEIGHT	2	0.169	<0.001	0.567	TRL-BCK 0.092 WALK-BCK 0.000 WALK-TRL 0.033
	15% WEIGHT	2	0.227	<0.001	0.687	TRL-BCK 0.217 WALK-BCK 0.000 WALK-TRL 0.001
	20% WEIGHT	2	0.267	<0.001	0.894	TRL-BCK 0.005 WALK-BCK 0.000 WALK-TRL 0.045
Y AXIS (adduction/abduction)	10% WEIGHT	5	0.015	<0.001	0.265	TRL-BCK 0.000 WALK-BCK 0.000 WALK-TRL 0.418
	15% WEIGHT	5	0.024	<0.001	0.064	TRL-BCK 0.003 WALK-BCK 0.000 WALK-TRL 0.217
	20% WEIGHT	5	0.004	<0.001	0.136	TRL-BCK 0.000 WALK-BCK 0.000 WALK-TRL 0.278
Z AXIS (internal/external)	10% WEIGHT	3	<0.001	<0.001	0.093	TRL-BCK 0.124 WALK-BCK 0.167 WALK-TRL 0.978
	15% WEIGHT	3	0.001	<0.001	0.117	TRL-BCK 0.062 WALK-BCK 0.036 WALK-TRL 0.943
	20% WEIGHT	3	<0.001	<0.001	0.108	TRL-BCK 0.000 WALK-BCK 0.003 WALK-TRL 0.718

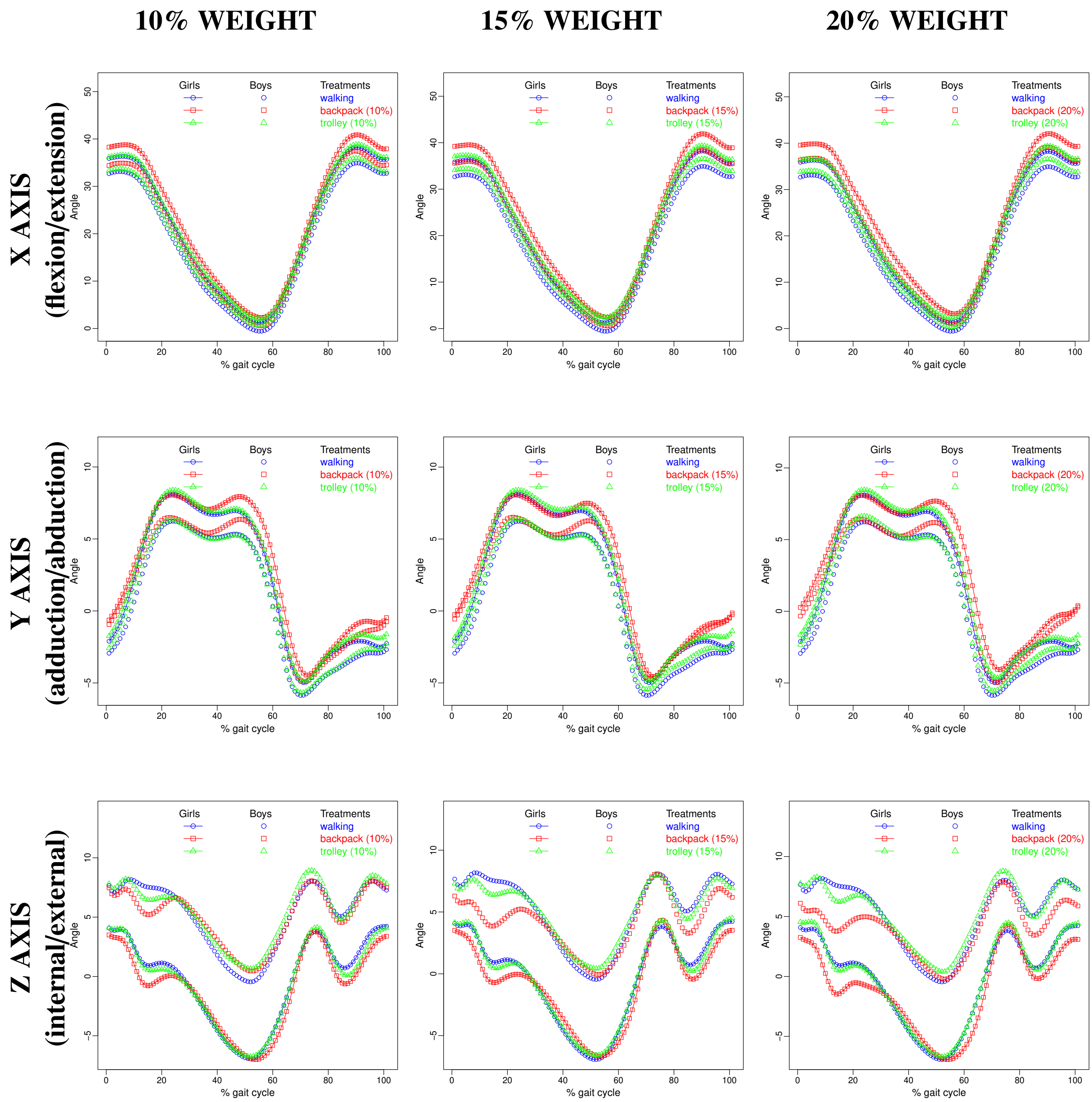

Figure 5. Mean curves of the rotation angle for the hip articulation in each experimental condition (treatment).

Table 4. P-values associated with the bootstrapped WTSs for the knee articulation.

		PCs	SEX	SCHOOLBAG	SEX:SCHOOLBAG	POST-HOC
X AXIS (flexion/extension)	10% WEIGHT	5	0.586	<0.001	0.681	TRL-BCK 0.206 WALK-BCK 0.101 WALK-TRL 0.943
	15% WEIGHT	5	0.551	<0.001	0.898	TRL-BCK 0.495 WALK-BCK 0.245 WALK-TRL 0.858
	20% WEIGHT	5	0.564	<0.001	0.896	TRL-BCK 0.427 WALK-BCK 0.964 WALK-TRL 0.584
Y AXIS (adduction/abduction)	10% WEIGHT	5	0.003	<0.001	0.043	TRL-BCK 0.003 WALK-BCK 0.000 WALK-TRL 0.674
	15% WEIGHT	5	<0.001	<0.001	0.543	TRL-BCK 0.000 WALK-BCK 0.000 WALK-TRL 0.486
	20% WEIGHT	5	0.005	<0.001	0.651	TRL-BCK 0.019 WALK-BCK 0.000 WALK-TRL 0.082
Z AXIS (internal/external)	10% WEIGHT	3	0.028	<0.001	0.894	TRL-BCK 0.000 WALK-BCK 0.000 WALK-TRL 0.488
	15% WEIGHT	3	0.065	<0.001	0.597	TRL-BCK 0.000 WALK-BCK 0.000 WALK-TRL 0.977
	20% WEIGHT	3	0.097	<0.001	0.806	TRL-BCK 0.000 WALK-BCK 0.000 WALK-TRL 0.913

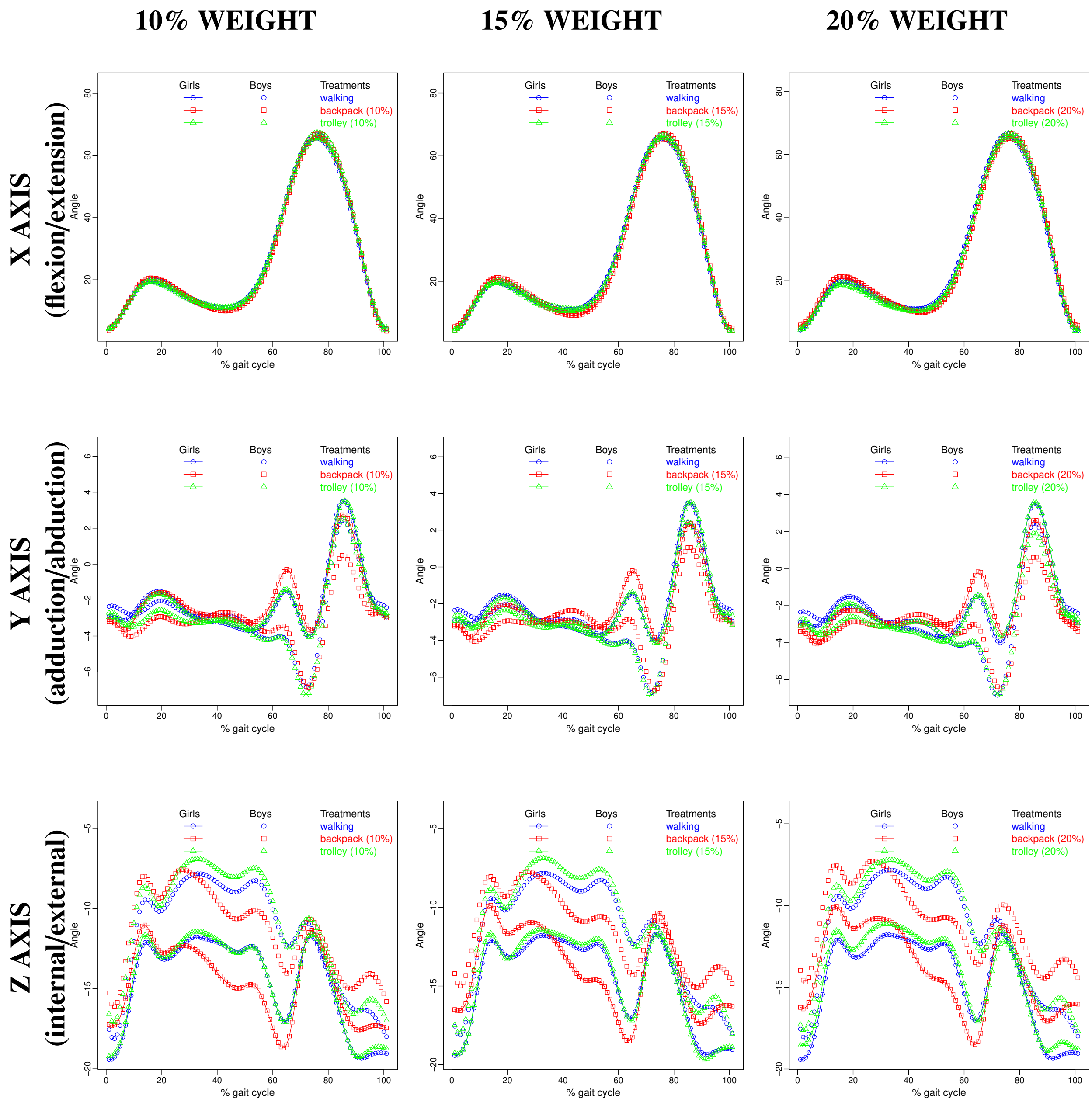

Figure 6. Mean curves of the rotation angle for the knee articulation in each experimental condition (treatment).

Table 5. P-values associated with the bootstrapped WTSs for the thorax articulation.

		PCs	SEX	SCHOOLBAG	SEX:SCHOOLBAG	POST-HOC
X AXIS (flexion/extension)	10% WEIGHT	1	0.009	<0.001	0.903	TRL-BCK 0.000 WALK-BCK 0.000 WALK-TRL 0.000
	15% WEIGHT	1	0.011	<0.001	0.638	TRL-BCK 0.000 WALK-BCK 0.000 WALK-TRL 0.000
	20% WEIGHT	1	0.010	<0.001	0.767	TRL-BCK 0.000 WALK-BCK 0.000 WALK-TRL 0.001
Y AXIS (adduction/abduction)	10% WEIGHT	5	0.715	<0.001	0.237	TRL-BCK 0.735 WALK-BCK 0.319 WALK-TRL 0.776
	15% WEIGHT	5	0.901	<0.001	0.585	TRL-BCK 1.000 WALK-BCK 0.263 WALK-TRL 0.292
	20% WEIGHT	5	0.784	<0.001	0.613	TRL-BCK 0.270 WALK-BCK 0.990 WALK-TRL 0.222
Z AXIS (internal/external)	10% WEIGHT	4	<0.001	<0.001	0.483	TRL-BCK 0.002 WALK-BCK 0.001 WALK-TRL 0.701
	15% WEIGHT	4	<0.001	<0.001	0.467	TRL-BCK 0.000 WALK-BCK 0.000 WALK-TRL 0.695
	20% WEIGHT	4	<0.001	<0.001	0.061	TRL-BCK 0.000 WALK-BCK 0.000 WALK-TRL 0.522

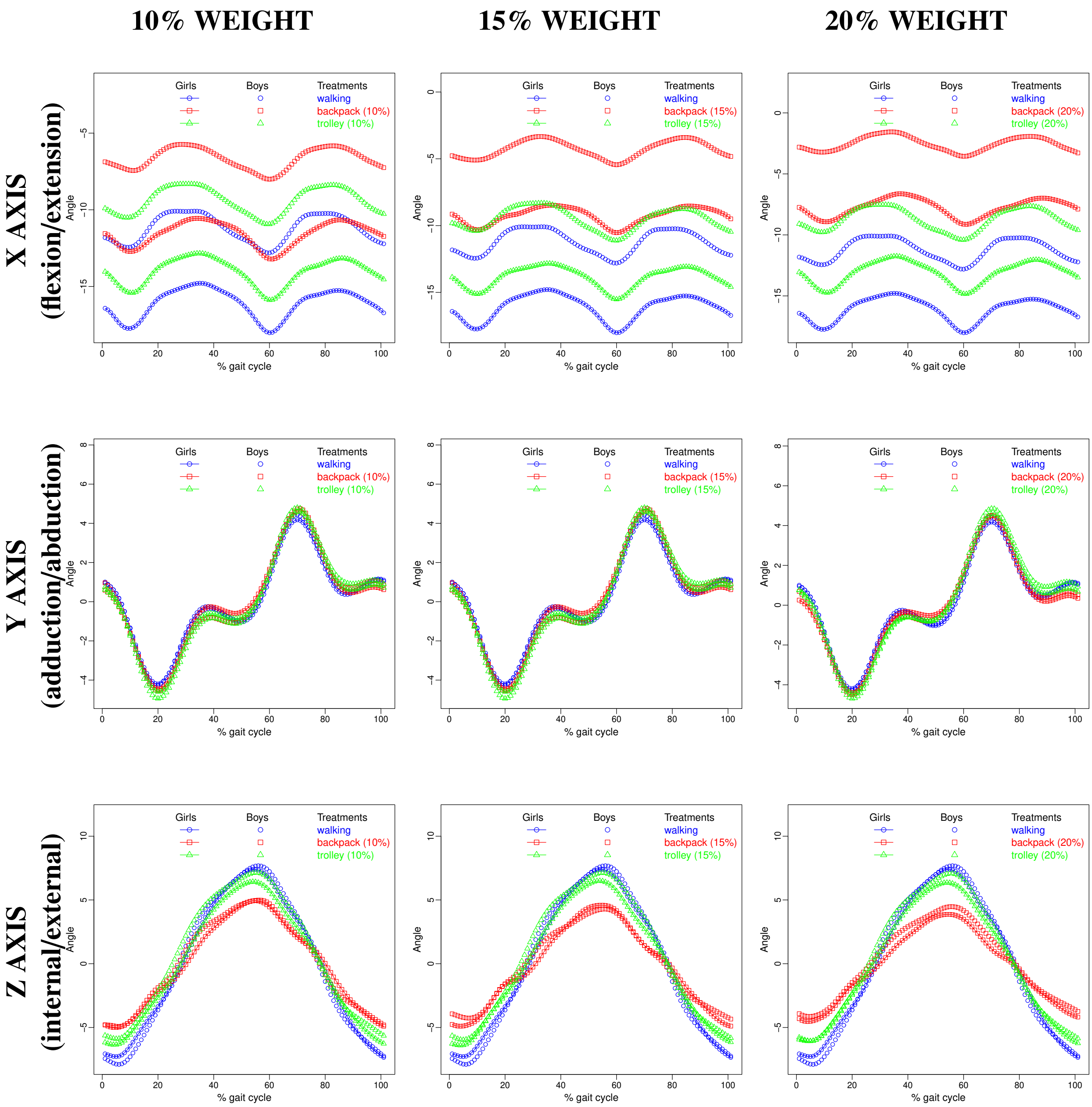

Figure 7. Mean curves of the rotation angle for the thorax articulation in each experimental condition (treatment).

Table 6. P-values associated with the bootstrapped WTSs for the foot progress articulation.

		PCs	SEX	SCHOOLBAG	SEX:SCHOOLBAG	POST-HOC
X AXIS (flexion/extension)	10% WEIGHT	3	0.821	<0.001	0.701	TRL-BCK 0.921 WALK-BCK 0.368 WALK-TRL 0.186
	15% WEIGHT	3	0.836	<0.001	0.960	TRL-BCK 0.279 WALK-BCK 0.039 WALK-TRL 0.701
	20% WEIGHT	3	0.716	<0.001	0.986	TRL-BCK 0.370 WALK-BCK 0.009 WALK-TRL 0.259

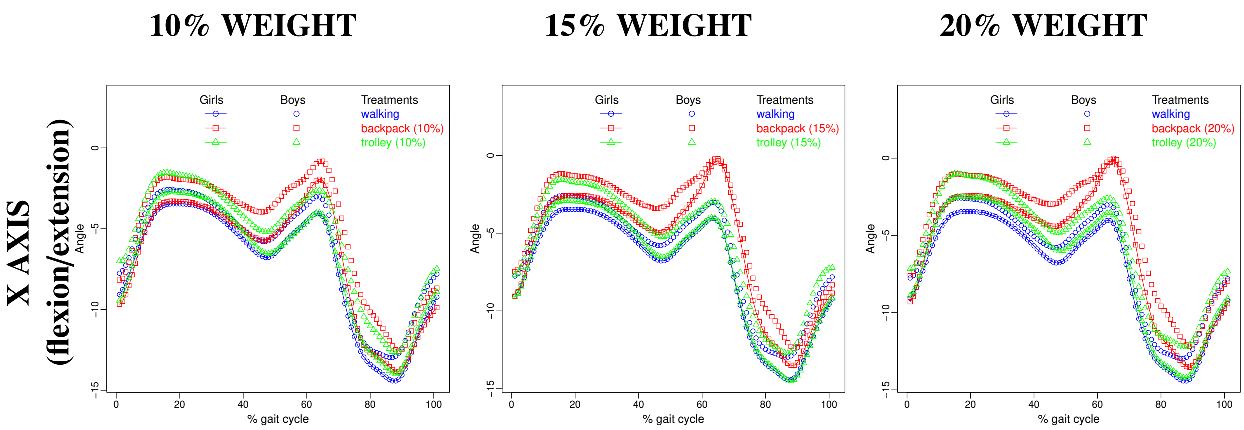

Figure 8. Mean curves of the rotation angle for the foot progress articulation in each experimental condition (treatment).