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**A REDUCTION OF THE SADDLE VERTICAL FORCE TRIGGERS THE SIT-STAND
TRANSITION IN CYCLING**

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Abstract

The purpose of the study was to establish the link between the saddle vertical force and its determinants in order to establish the strategies that could trigger the sit-stand transition. We hypothesized that the minimum saddle vertical force would be a critical parameter influencing the sit-stand transition during cycling. Twenty-five non-cyclists were asked to pedal at six different power outputs from 20% ($1.6 \pm 0.3 \text{ W.kg}^{-1}$) to 120% ($9.6 \pm 1.6 \text{ W.kg}^{-1}$) of their spontaneous sit-stand transition power obtained at 90RPM. Five 6-components sensors (saddle tube, pedals and handlebars) and a full-body kinematic reconstruction were used to provide the saddle vertical force and other force components (trunk inertial force, hips and shoulders reaction forces, and trunk weight) linked to the saddle vertical force. Minimum saddle vertical force linearly decreased with power output by 87% from a static position on the bicycle ($5.30 \pm 0.50 \text{ N.kg}^{-1}$) to power output=120% of the sit-stand transition power ($0.68 \pm 0.49 \text{ N.kg}^{-1}$). This decrease was mainly explained by the increase in pedal forces from $2.84 \pm 0.58 \text{ N.kg}^{-1}$ to $6.57 \pm 1.02 \text{ N.kg}^{-1}$ from 20 to 120% of the power output corresponding to the sit-stand transition, causing an increase in hip vertical forces from -0.17 N.kg^{-1} to 3.29 N.kg^{-1} . The emergence of strategies aiming at counteracting the elevation of the trunk (handlebars and pedals pulling) coincided with the spontaneous sit-stand transition power. The present data suggest that the large decrease in minimum saddle vertical force observed at high pedal reaction forces might trigger the sit-stand transition in cycling.

Key Words: INVERSE DYNAMICS, PEDALING, SEAT, STAND

1. Introduction

Seated (SEAT) and Standing (STAND) are the two common positions chosen during bicycle locomotion. Several studies comparing the two positions have shown that spontaneous pedaling cadences are slower in STAND than in SEAT position (Harnish et al., 2007; Lucía et al., 2001), and that the STAND position is associated with the highest power outputs (McLester et al., 2004; Millet et al., 2002; Reiser et al., 2002). Furthermore, the fact that cyclists tend to spontaneously switch from SEAT to STAND when high force applied to the pedals are needed (i.e. during fast accelerations or steep climb ascensions) suggests that the change in position favors a maximization of the pedal reaction forces (Hansen and Waldeland, 2008). However, the parameters leading to select one position over the other one in order to produce a given combination of pedal reaction force and power output need to be clarified.

Many attempts have been made to understand the mechanisms underlying these positions, particularly to determine the superiority of the STAND position to produce higher power outputs and pedal reaction forces. From a joint torque perspective, a study using the moment cost function defined by Gonzalez and Hull (1989) presented a slight reduction of this cost function above the sit-stand transition power (Poirier et al., 2007), whereas lower limbs net joint torques have been described by others as increasing in STAND position for both the ankle plantarflexion and the knee extension (Caldwell et al., 1999; Li and Caldwell, 1998). From a metabolic energy consumption perspective, the SEAT position has been shown to be more efficient to produce lower power outputs (Ryschon and Stray-Gundersen, 1991; Tanaka et al., 1996), and equally efficient as the STAND one to produce high power outputs (Harnish et al., 2007; Millet et al., 2002; Tanaka et al., 1996). Regarding studies using electromyography, the literature suggests that differences in the temporal profiles and in the level of activation of the muscles could be expected between SEAT and STAND (Li and Caldwell, 1998; Hug et al., 2011). For example, Duc et al. (2008) reported a slight decrease

69 for the *semimembranosus* activation from SEAT to STAND, whereas Li and Caldwell (1998)
70 reported increased activations of the *gluteus maximus*, *tibialis anterior* and *rectus femoris* muscles
71 in STAND position. These differences may influence the coordination patterns in both positions (De
72 Marchis et al., 2013). Nonetheless, the muscle synergies activated in the two positions may remain
73 similar (Hug et al., 2011) and the literature does not provide evidences of an advantage of one
74 position against the other at this level.

75 Since there is no obvious reason to prefer the STAND rather than the SEAT position to
76 produce one given power output, we propose in this study to reverse the questioning and to wonder
77 why the SEAT position is no longer optimal, instead of why the STAND position becomes optimal
78 beyond a given level of crank power. To test our hypotheses, we first propose a criterion that could
79 clearly distinguish the two positions: the SEAT position is characterized by a contact between the
80 cyclist and the saddle (i.e. a vertical force is applied by the cyclist on the saddle) whereas the
81 STAND position is characterized by the absence of this vertical force. In this definition, the force
82 applied by the cyclist on the saddle (and reciprocally) is of central interest, and the sit-stand
83 transition is defined by the disappearance of this force. To the best of our knowledge, only three
84 studies measured saddle forces in cycling. The first one presented saddle force at three pedaling
85 cadences and described a double period pattern with maximum magnitudes decreasing as cadence
86 decreases (Bolourchi and Hull, 1985). However, the second study, did not found this double period
87 pattern (Stone and Hull, 1995) while the third one observed both of these patterns (Wilson and
88 Bush, 2007). To better understand this phenomenon, we propose to investigate the saddle force
89 patterns. According to Newton's second law, this force is the result of a simple mechanical
90 interaction between the cyclist's body weight and the other forces applied on his bicycle.
91 Consequently, a downward vertical force applied on the pedal would result by reaction in an upward
92 force on the hip, accelerating the trunk in an upward direction, and decreasing the force applied on
93 the saddle by the cyclist. Therefore, we propose to measure vertical forces applied on the saddle, in

complement with the other forces acting on the trunk of the cyclist (i.e. hips and shoulders reaction forces, trunk weight, and acceleration of the trunk's center-of-mass) at different pedal reaction forces. The aims of this study are to validate a full-body inverse dynamics model of cycling and to test the hypothesis that saddle vertical force would decrease and reach values close to zero with increasing pedal forces, making the SEAT position irrelevant given its definition and leading the cyclist to spontaneously adopt the STAND position.

2. Methods

2.1. Participants

Twenty five male sport science students (23.2 ± 3.6 y, height 1.77 ± 0.06 m, body mass 71.5 ± 9.1 kg) volunteered for this investigation. The participants were non-cyclists and belonged to category 4-5 according to Ansley and Cangle (2009) classification. Each participant was informed of the experimental procedure and signed an informed consent form prior to the study. The study was conducted in accordance with the declaration of Helsinki and was approved by the University of Toulouse ethical committee. Participants were asked to avoid high-intensity or exhaustive exercise at least 72 hours before the laboratory trials.

2.2. Experimental Protocol

The cycling tests were performed using an electromagnetically braked cycle ergometer Excalibur (LODE, Groningen, Netherlands). To limit bike positioning effects, standardized settings were adopted. Briefly, pedal cleats were positioned under the first metatarsal bone (Viker and Richardson, 2013), the saddle height was set at a 150° knee angle during maximum leg extension,

119 the seat tube angle was set to 73°, the crank length was 0.17 m in length and the handlebar was flat.
120 The latter was positioned to standardize drop (the vertical distance between the top of the saddle
121 and the handlebar mediolateral axis) and reach (the horizontal distance between the back of the
122 saddle and the handlebar mediolateral axis) lengths according to torso and arm lengths (de Vey
123 Mestdagh, 1998). The mediolateral positioning of the two hands on the handlebar was left up to the
124 participant (handlebar width: 0.7 m).

125 After bike positioning, participants were first weighed on the cycle ergometer in order to
126 measure a static level of saddle vertical force (representing 0% of the sit-stand transition power).
127 This weighing was made with the shoes fixed on the pedals, the hands on the handlebars, and the
128 cranks in horizontal position. Then, after a five-minute warm-up at 100W, they performed a cycling
129 test to determine their spontaneous sit-stand transition power (Figure 1). In this test, phases of 20 s
130 with a starting power output of 200 W incremented by 25 W at each step rest were alternated with
131 rest phases of 40 s at a power output of 50 W. The sit-stand transition power was considered as the
132 power output at which participants rose from the saddle during at least 10 s. A visual feedback of
133 the pedaling cadence was provided to the participants who were instructed to maintain it at
134 90 ± 5 RPM.

135

136 *PLEASE INSERT FIGURE 1*

137

138 Then, after a five-minute rest period, participants performed six randomized trials at power
139 output corresponding to 20, 40, 60, 80, 100 or 120% of their sit-stand transition power and were
140 asked to remain seated throughout these sequences. Each pedaling trial began with a minimum
141 stabilization time of 10 s at the target power output at 90RPM, followed by 10s of data recording.
142 Three minutes of passive rest were given between each of these six trials.

143

2.3. Data acquisition

The 3D force and moment components applied to the handlebar, saddle tube and pedals were recorded from three tubular sensors (SENSIX, Poitiers, France), and by two instrumented pedals (I-Crankset-1, SENSIX, Poitiers, France) at 1 kHz (Figure 2). According to the manufacturer, these dynamometers had a maximum 1% error on each direction (combining linearity and hysteresis errors), and a maximum 1.5% error on the 6 components combination.

PLEASE INSERT FIGURE 2

Kinematics data were collected from 56 passive markers recorded by twelve infrared cameras (VICON, Oxford, United-Kingdom) at 200 Hz. The kinetics sensors' reference points were defined as shown in Figure 2. The ankle (because of the impossibility to stick one kinematic marker on the *medial malleollus* in reason of the crank proximity), shoulder and hip joint centers were located using the SCoRE method (Ehrig et al., 2006). For this method, a preliminary recording asking the participants to repeat flexion-extension, abduction-adduction and circumduction of the tested joint allowed the localization of their centers-of-rotation (Begon et al., 2007). Body segments masses, center-of-mass positions, and radii of gyration were defined in accordance with De Leva's anthropometric charts (de Leva, 1996). All kinetics and kinematics data were recorded in three-dimensions.

2.4. Data reduction and analysis

Kinetics and kinematics data were synchronized using Nexus 1.7.1 system (VICON, Oxford, United-Kingdom) and filtered using a 4th order, zero phase-shift, low-pass Butterworth with a 8 Hz

cutoff frequency (McDaniel et al., 2014). In order to determine the factors affecting the saddle vertical force, the trunk was represented (comprising the head and the pelvis) as being submitted to external forces applied on the shoulders, hips, and saddle contact. The following equality has been computed by isolating the head and trunk solid according to Newton's second law:

$$F_s = m_t a_t - (W_t + F_h + F_{sh}) \quad (\text{Equation 1})$$

where m_t is the mass of the head and trunk solid according to De Leva's anthropometric chart, a_t is the linear acceleration of the head and trunk center-of-mass, W_t is the sum of the head and trunk weights, F_s is the saddle reaction force obtained from the saddle tube sensor, F_{sh} is the shoulder reaction force calculated by inverse dynamics method from the handlebar sensors, and F_h the hip reaction force calculated by inverse dynamics method from the pedal sensors. To compute F_h and F_{sh} , a classic inverse dynamic process was used (Winter, 1990). In this method, body-segments from upper and lower limbs were considered rigid and interconnected by frictionless joints and their inertial parameters were derived from the scaling equations (de Leva, 1996). Given the aims of the study, only the vertical components in Equation 1 were considered. This model is illustrated in Figure 3. The entire data processing was performed using custom-made codes written in Scilab 5.4.0 (SCILAB, Scilab Enterprises). All the data were normalized to the subject's body mass. During the crank cycle corresponding to the minimum saddle vertical force observed among the 10 s of recording for each power output, vertical forces presented in Equation 1 were extracted. In this crank cycle and at the instant corresponding to the minimum saddle vertical force, vertical force values were retained for further analyses.

PLEASE INSERT FIGURE 3

2.5. Statistics

Before each statistical test, data normality and variance homogeneity were assessed using Shapiro-Wilk's, and Levene's tests, respectively. A one-way repeated measures ANOVA (power output = 20, 40, 60, 80, 100 and 120% of sit-stand transition power) was performed to compare saddle force levels across Power outputs. Post-hoc analyses were performed using Bonferroni's method. To check the accuracy of the experimental model represented by the equality computed in Equation 1, the difference between saddle vertical reaction force and the equivalent sum of forces was quantified for each power output condition using the root-mean-square error (RMSE). In addition, Pearson's coefficients (R) were used to determine the correlation between the two patterns. Partial eta-squared (η^2) was used to quantify the size of the effect of power output on vertical forces. All statistical analyses were performed using STATISTICA (STATSOFT, Maisons-Alfort, France). A p-value of 0.05 was defined as the level of statistical significance.

3. Results

The sit-stand transition power reached during a pedalling phase of 20 s at 90 RPM (i.e. during the first test, see methods) was 568 ± 93 W (8.0 ± 1.4 W.kg⁻¹) and the power outputs corresponding to 20, 40, 60, 80, 100 and 120% of sit-stand transition power were 114 ± 19 W (1.6 ± 0.3 W.kg⁻¹), 227 ± 37 W (3.2 ± 0.5 W.kg⁻¹), 341 ± 56 W (4.8 ± 0.8 W.kg⁻¹), 454 ± 74 W (6.4 ± 1.1 W.kg⁻¹), 568 ± 93 W (8.0 ± 1.4 W.kg⁻¹) and 682 ± 111 W (9.6 ± 1.6 W.kg⁻¹), respectively.

The static vertical force on the saddle (0% of sit-stand transition power) was 5.30 ± 0.50 N.kg⁻¹.

Descriptive statistics about saddle vertical force are shown in Table 1. A significant main effect ($p < 0.001$) of power output was found, showing that the magnitudes of minimum saddle

219 vertical forces decreased with increasing power output. Post-hoc tests indicated that the saddle
220 vertical force decreased significantly between each power output condition.

221

222 *PLEASE INSERT TABLE 1*

223

224 Accuracy of the model was assessed and the results of the saddle vertical force pattern
225 reconstruction using the equality described in Equation 1 are presented in Table 2. An illustration of
226 this reconstruction is presented in Figure 4.

227

228 *PLEASE INSERT TABLE 2*

229 *PLEASE INSERT FIGURE 4*

230

231 Vertical saddle, trunk inertial force, shoulders and hips reaction force patterns are presented
232 in Figure 5.

233

234 *PLEASE INSERT FIGURE 5*

235

236 The variation with power output of each term detailed in Equation 1 at the instantaneous
237 minimum saddle vertical force in the cycle is presented in Figure 6.

238

239 *PLEASE INSERT FIGURE 6*

240

241 **4. Discussion**

242

243 The primary purpose of this investigation was to test the hypothesis that the saddle vertical
244 forces would decrease with increasing power output. Our findings supported our hypothesis with a
245 linear decrease of 87.4% of the saddle vertical reaction force, from $5.30 \pm 0.50 \text{ N.kg}^{-1}$ to 0.68 ± 0.49
246 N.kg^{-1} , between a static position on the bicycle and the minimum instantaneous value obtained
247 while pedaling at 120% of the sit-stand transition power (Table 1). Another purpose of the study
248 was to determine the forces applied on the trunk during cycling at different pedal reaction forces in
249 order to interpret the decrease in saddle vertical force. The model presented in Equation 1 provided
250 an accurate examination of the forces associated with the saddle vertical force (Table 2 and Figure
251 4). These data suggest that the vertical saddle force decreased mainly in response to the increase in
252 hip vertical reaction forces (Figures 5 and 6). Consequently, with increasing pedal reaction forces,
253 the body weight was less and less supported by the saddle. The results indicated that when the
254 saddle force approached 1 N.kg^{-1} , the participants tended to spontaneously transit to the STAND
255 position, suggesting that the saddle force could be a predictor of the sit-stand transition power.

256 A combination of several strategies was observed to limit the decrease in saddle vertical
257 force in response to the increasing demand in pedal force, potentially increasing both the sit-stand
258 transition power and the delay before the occurrence of the sit-stand transition. These strategies are
259 likely to help maintaining the SEAT position when high level of pedal reaction forces are created
260 and may also explain why the saddle vertical force did not reach zero (Figure 6). However, these
261 strategies have been previously reported as particularly metabolically costly (Korff et al., 2007;
262 Edwards et al., 2009; McDaniel et al., 2005). The first strategy observed was to pull on the pedal to
263 create downward reaction forces at the hip level (Figure 5). This pedal pulling may be associated
264 with the advantage of increasing the mechanical effectiveness of pedaling (Korff et al. 2007), and
265 explains the non-linear increase in the sum of pedal vertical forces during with increasing crank
266 power (Figure 6). However, and probably because human's lower limb is far stronger to produce
267 force in extension than in flexion (Anderson et al., 2007), increasing the mechanical effectiveness

by training cyclists to pull more on the pedals has been reported to decrease their metabolic efficiency (Korff et al., 2007; Edwards et al., 2009). Because experts in cycling have been reported to push more on the pedals at equivalent power output (Coyle et al., 1991) it could be expected that they would have to create downward forces by pulling their handlebars and/or pedals and/or accelerating their trunk downward simultaneously to the decrease in vertical saddle force at lower power outputs than the non-cyclists from our study, and more frequently in their daily practice because of the higher power output that they develop. Further investigations are needed to confirm this hypothesis which could lead to improvement in cycling performance. A second strategy observed to limit the reduction of the saddle vertical force was to accelerate the trunk's center-of-mass downward (Figure 5). It is worth noting that the pattern of these accelerations are synchronized with the pattern of saddle vertical force from 100% of the sit-stand transition power: when the saddle force was at its minimum, the trunk's center-of-mass was accelerated downward, and reciprocally, the upward acceleration of the trunk's center-of-mass occurred while the saddle vertical force was at its maximum, the whole occurring twice by pedaling cycle. A third strategy was to create a downward reaction force at the shoulders by pulling on the handlebar, this last strategy was mainly observed above the sit-stand transition power (Figure 6). Both of these strategies involve additional muscular efforts from the upper limbs. As highlighted by McDaniel et al. (2005), the upper limbs' metabolic cost is important in cycling. These authors showed that the use of a modified saddle allowing the stabilization of the trunk and a potential decrease in upper limb muscular efforts decreased the metabolic cost of pedaling for a fixed power output. The reductions were of 1.6, 1.2, and 0.2% at 40, 60, and 80 RPM, respectively and they showed that the best improvement in metabolic cost was obtained at the highest level of pedal forces (for a fixed power output), i.e. in the conditions corresponding to the highest handlebars and pedals pulling and trunk inertial forces observed in our study. The present data are in agreement with the interpretation that with increasing pedal forces, the body weight was less and less supported by the saddle, and

293 that downward forces acting on the trunk were required to maintain the SEAT position above one
294 level of crank power (for a given pedaling cadence of 90 RPM). The fact that costly strategies to
295 counteract the elevation of the trunk emerged at the power at which the participants spontaneously
296 switched to the STAND position suggests that this position could have been chosen in order to
297 avoid these strategies. It is worth mentioning that several other factors may influence the choice of
298 the cycling position in the field such as aerodynamics (Debraux et al., 2011; Millet et al., 2014), or
299 slope gradient (Bertucci et al., 2005; Duc et al., 2008). However, the difficulty to keep force on the
300 saddle during high pedal reaction force production observed in this study is making the SEAT
301 position less attractive in these conditions, giving a mechanical reason to trigger the sit-stand
302 transition. Our study is the first to present saddle force patterns at different levels of pedal reaction
303 force as a justification to trigger the sit-stand transition, and to explain these patterns by a
304 mechanical decomposition of the forces applied on the trunk during cycling. In order to further
305 confirm the present results, experimental designs manipulating the body weight, and/or testing
306 pedaling cadence effects on the magnitude of saddle vertical force and the occurrence of the sit-
307 stand transition are warranted. Additionally, Hansen and Waldeland (2008) implemented repeated
308 cycling bouts to exhaustion with experimented cyclists and reported smaller sit-stand transition
309 power output than the one observed in this study with non-cyclists. This difference illustrates a
310 potential protocol-dependence of the sit-stand transition power, which may therefore also be
311 affected by the duration of the cycling trial. Altogether, further investigations on the sit-stand
312 transition paradigm in cycling may lead to improvements in pedaling efficiency by potentially
313 decreasing the mechanical cost of pedaling in SEAT position at high pedal reaction forces, and by
314 determining the precise pedal reaction force level at which the sit-stand transition is necessary to
315 maximize performance for different cadences, weights and durations conditions.

316 By determining the parameters involved in saddle force patterns, the present study also have
317 implication for clinicians, researchers, and manufacturers trying to understand the etiology of groin

injuries and erectile dysfunction associated with cycling (Bressel et al., 2010; Bressel and Larson, 2003; Carpes et al., 2009; Lowe et al., 2004). Indeed, the inconsistency of the patterns of saddle force observed previously (Bolourchi and Hull, 1985; Stone and Hull, 1995; Wilson and Bush, 2007) can be explained by the different pedaling conditions used in these studies. Due to the sensitiveness of saddle forces (and thus saddle pressures) to pedal reaction forces, cyclists suffering from these pathologies should decrease their pedaling cadence for the same workload, as this is supposed to increase hip upward reaction force in order to decrease the saddle reaction force.

It is important to note some limitations of the present study. The use of a cycling ergometer is a common practice for testing, rehabilitation and training, but it differs with cycling in the field (Bertucci et al., 2012). Likewise, the potential protocol-dependence of the spontaneous sit-stand transition power determination needs further investigations.

Conclusion

The body weight is gradually less supported by the saddle as pedal reaction forces increase, thus decreasing the mechanical advantage of pedaling in the SEAT position. Strategies counteracting the upward vertical pedal forces were observed around the power corresponding to the sit-stand transition, suggesting that the spontaneous choice to rise in the STAND position may be a solution to reduce the need to overcome these constraints. The spontaneous sit-stand transition occurred at minimum saddle vertical force about 1 N.kg^{-1} ; the high linearity of the relationship between saddle vertical force and power output for a given cadence suggesting an ability of prediction of the sit-stand transition.

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346

347 **Conflict of Interest**

348

349 The authors have no financial or personal relationships with other people or organizations that could
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483 **FIGURE 1 – Experimental protocol to determine the sit-1 to-stand transition power (SSTP).**
484 **SSTP was considered as the CPO at which the participants rose from the saddle during at**
485 **least 10 s.**

486
487 **FIGURE 2 – 3D force and moment sensors. A. Pedal. B. Saddle Tube. C. Handlebars.**
488

489 **FIGURE 3 – Theoretical model of the cyclist. For clarity only one side of the body is**
490 **represented. Red arrows represent external forces (saddle, pedals and handlebars), and**
491 **dashed red arrows represent reaction forces applied on the trunk at the hip and shoulder**
492 **levels calculated by inverse dynamics. Only the vertical components of these forces are**
493 **represented. White dots represent kinematic markers. Black dots represent joint centers**
494 **calculated using the SCoRE method.**
495

496 **FIGURE 4 – Illustration of the mean saddle vertical reaction force and mean sum of forces**
497 **applied on the trunk (presented in Equation 1) patterns for all participants (n = 25) for CPO**
498 **= 20% of SSTP.**
499

500 **FIGURE 5 – Vertical reaction force patterns presented along the crank cycle corresponding**
501 **to the minimum saddle vertical reaction force recorded for each CPO. Mean lefts (red line)**
502 **and rights (blue line) are presented \pm one standard deviation. Data normalized by body-mass.**
503 **A. Saddle. B. Mass time acceleration of the trunk's center-of-mass. C. Shoulders. D.**
504 **Hips.**
505

506 **FIGURE 6 – Evolution of the vertical reaction forces across CPOs. Diamonds: saddle vertical**
507 **reaction forces. Squares: product between the mass of the trunk and the acceleration of its**
508 **center of mass. Triangles: sum of the two hip vertical reaction forces. White circles: sum of**
509 **the two shoulder vertical reaction forces. Black dots: weight of the 26 head and trunk. Each**
510 **data point corresponds to the instantaneous vertical force observed while the saddle vertical**
511 **force was minimal. Positive values indicate upward reaction forces (except for the trunk's**
512 **weight, reverted in a purpose of readability).**
513

514 **TABLE 1 – Minimum saddle vertical reaction forces across CPOs. Data are expressed in**
515 **N.kg. as mean \pm standard deviation [range]. *: Main CPO effect. a, b, c, d, e, and f represent**
516 **significant differences compared to 20, 40, 60, 80, 100, and 120% of SSTP conditions,**
517 **respectively (p < 0.001).**
518

519 **TABLE 2 – Accuracy of the mechanical decomposition. Root-Mean-Square-Error (RMSE)**
520 **expressed in N.kg⁻¹, and coefficients of correlation (R) between the pattern of vertical saddle**
521 **force and the pattern of the sum of forces applied on the trunk (terms described in Equation**
522 **1) are presented as MEAN (\pm SD). * represents significant coefficient of correlation**
523 **40 (P < 0.001).**

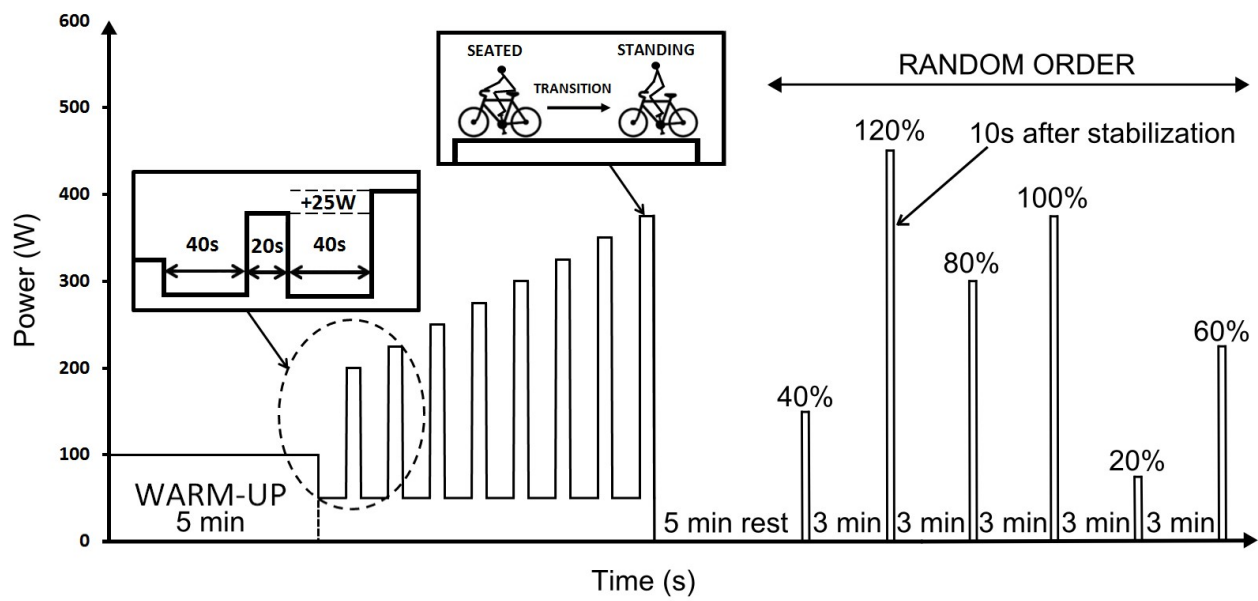


FIGURE 1

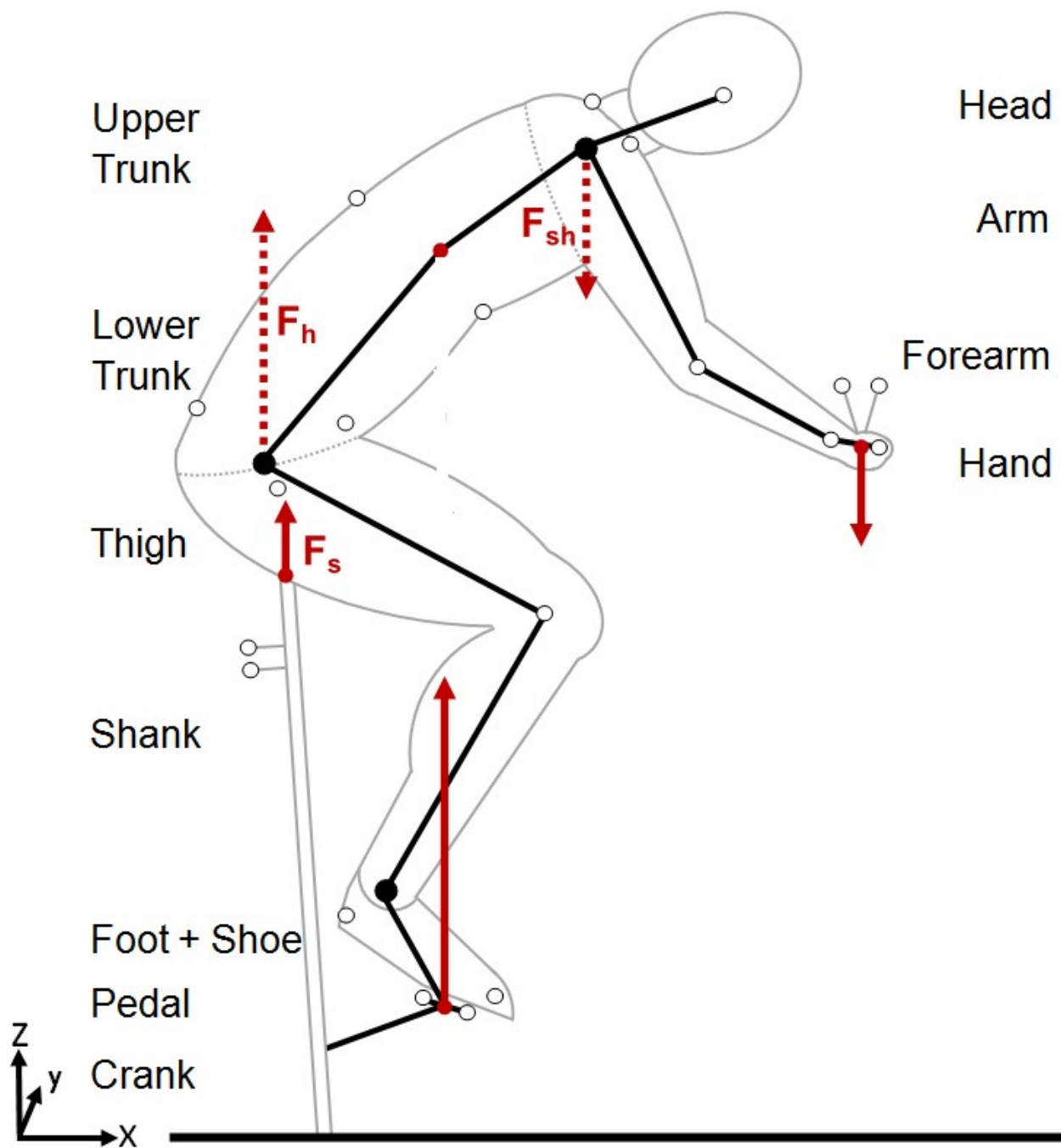


FIGURE 3

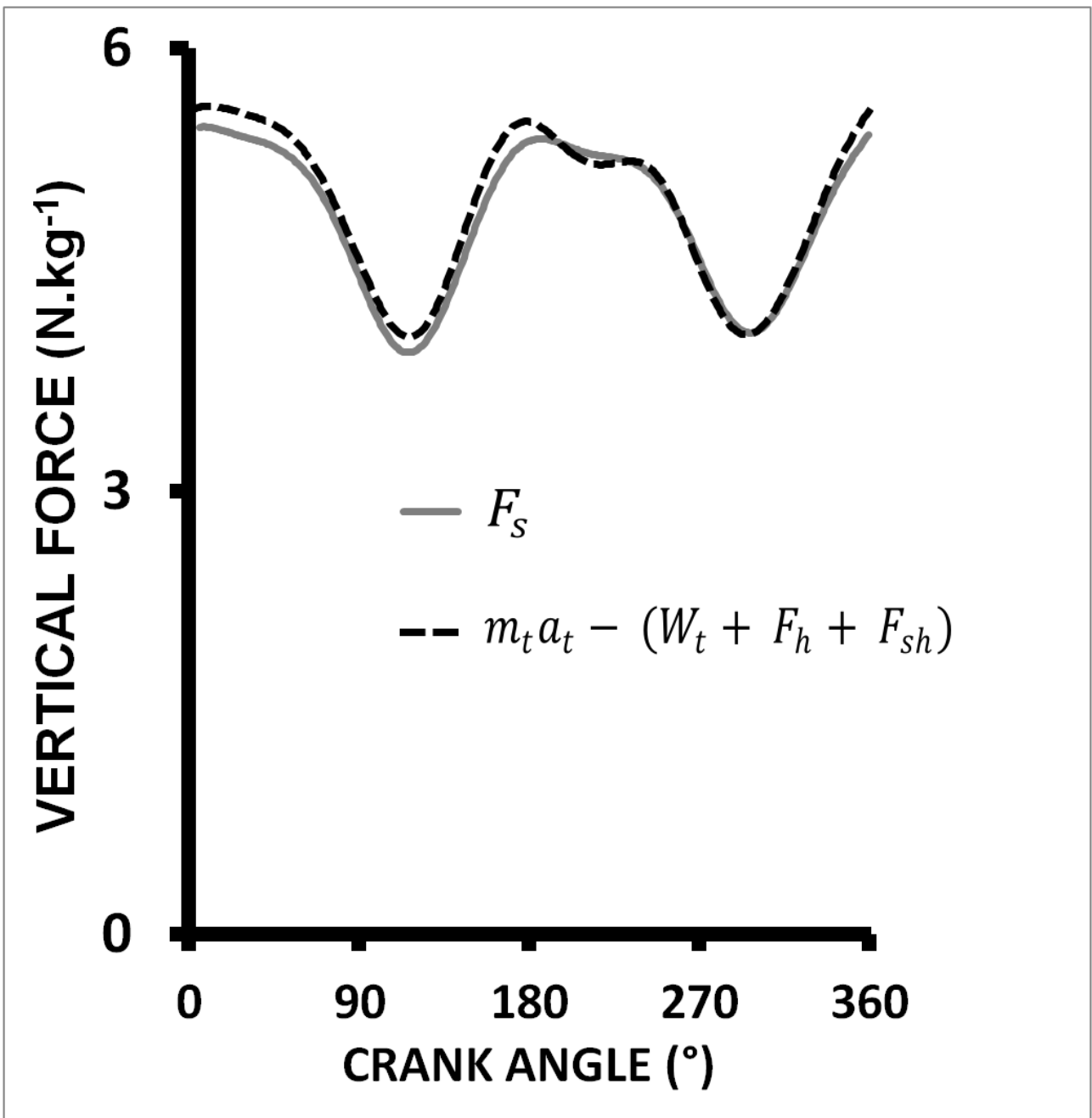


FIGURE 4

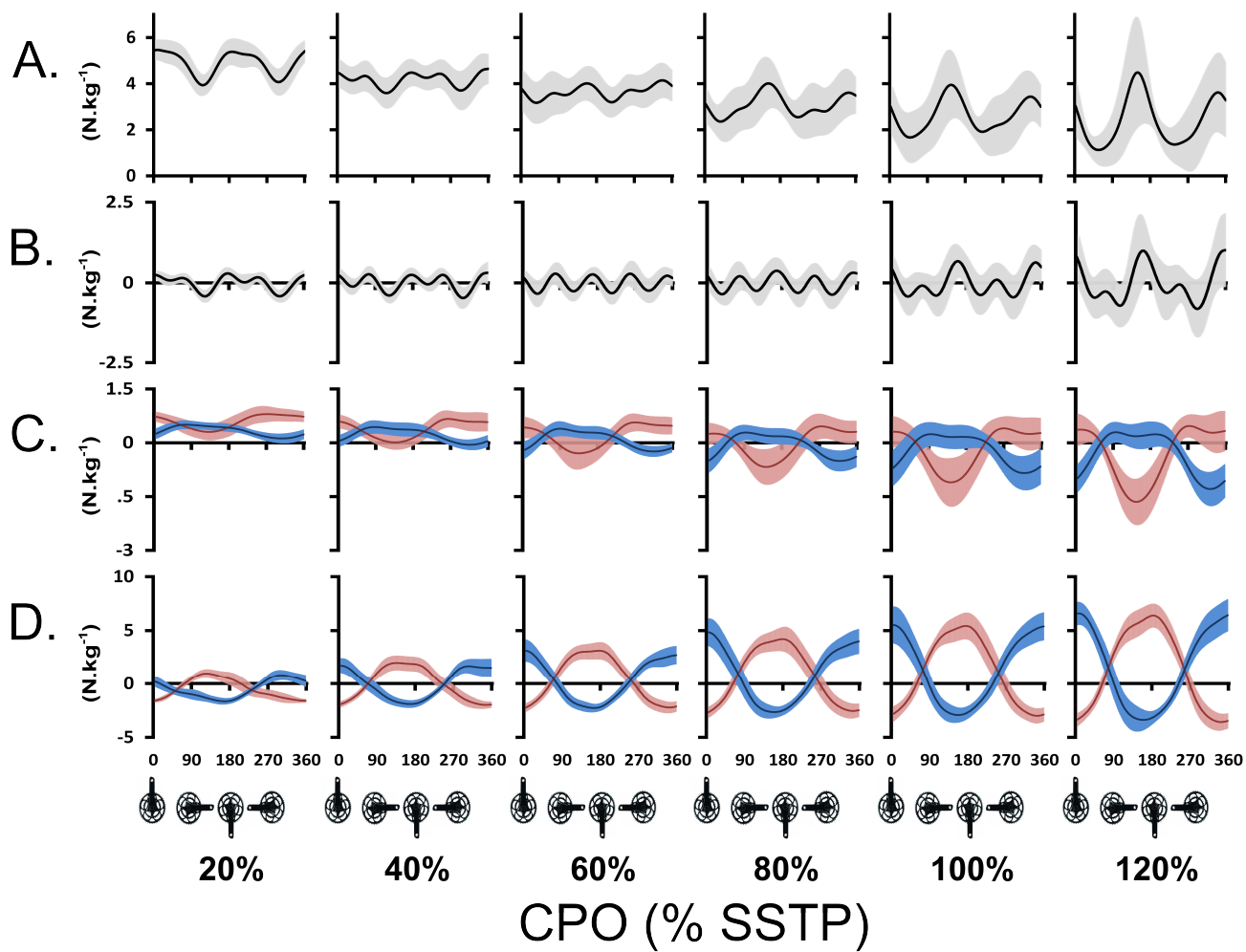


FIGURE 5

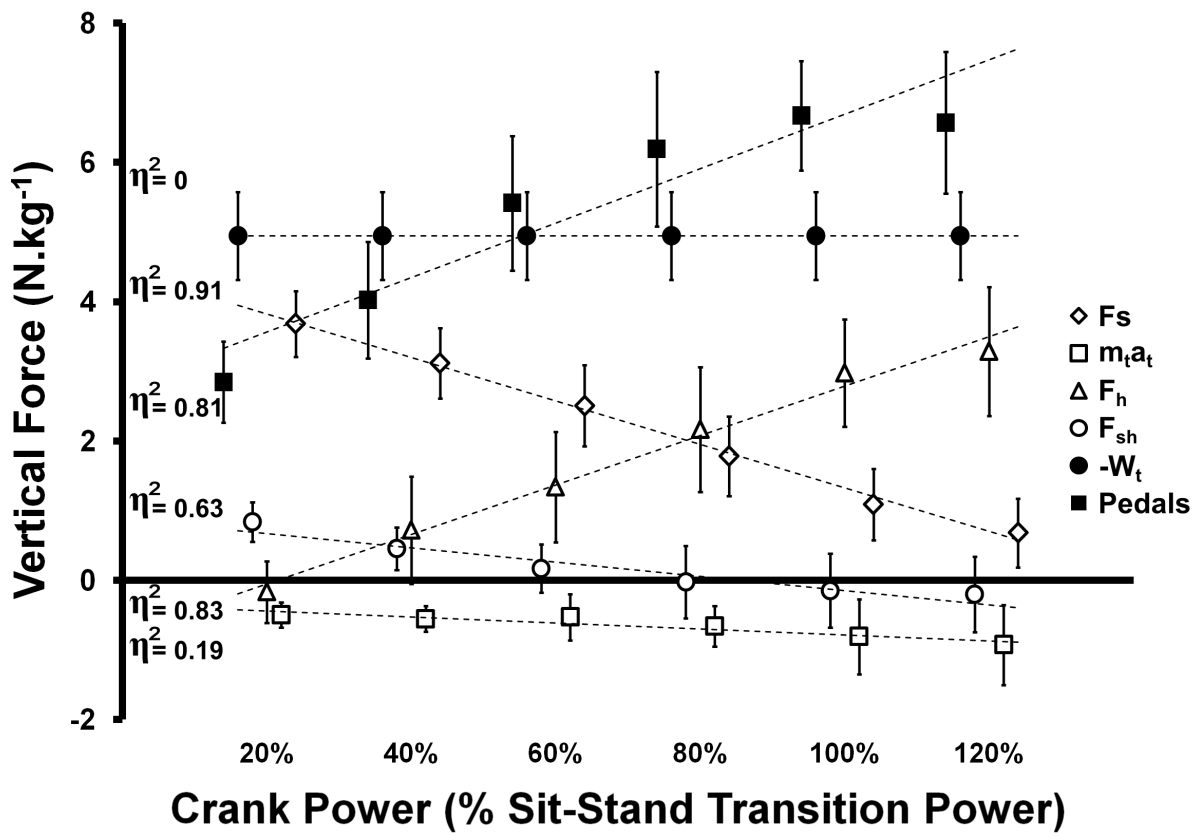


FIGURE 6