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Differences in proprioception, muscle force control and comfort between conventional and new-generation knee and ankle orthoses

This is a pre print version of the following article:

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/147104> since 2016-11-12T16:58:30Z

Published version:

DOI:10.1016/j.jelekin.2014.03.009

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Manuscript Number:

Title: Differences in proprioception and muscle force control between conventional and new-generation orthoses for knee and ankle joints

Article Type: Research Paper (max. 5,000 words)

Keywords: Force accuracy; joint position sense; kinesthesia; knee joint; ankle joint.

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Abstract: The aim of this study was to compare muscle force control and proprioception between conventional and new-generation experimental orthoses. Sixteen healthy subjects participated in a single-blind controlled trial in which two different types of orthosis were applied to the dominant knee or ankle, while the following variables were evaluated: muscle force control (accuracy), joint position sense, kinesthesia, static balance as well as subjective outcomes. The use of experimental orthoses resulted in better force accuracy during isometric knee extensions compared to conventional orthoses (mean difference: 25.0%; $P < 0.05$). Moreover, the use of experimental orthoses resulted in better force accuracy during concentric (mean difference: 24.6%) and eccentric (mean difference: 25.2%) ankle plantar flexions and better knee joint kinesthesia in the flexed position (mean difference: 24.0%) compared to conventional orthoses (all $P < 0.05$). Subjective comfort and preference scores were higher with experimental orthoses compared to conventional ones ($P < 0.05$). In conclusion, orthosis type affected static and dynamic muscle force control, kinesthesia, and perceived comfort in healthy subjects. New-generation experimental knee and ankle orthoses may thus be recommended for prophylactic joint bracing during physical activity and to improve the compliance for orthosis use, particularly in patients who require long-term bracing.

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We the undersigned declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

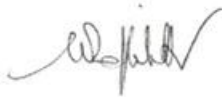
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Signed by the corresponding author (on behalf of all coauthors) as follows:



Dr. Nicola A. Maffioletti
Zurich, 21 October 2013

To: Professor M. Solomonow
Editor-in-Chief, JEK
Professor & Director
Bioengineering Division & Musculoskeletal Disorders Research Laboratory
University of Colorado Health Sciences Center
Mailstop 8343, PO Box 6511, Aurora, CO., 80045, USA

Zurich, 21 October 2013

Dear Prof. Solomonow,

Please find enclosed the research article entitled "Differences in proprioception and muscle force control between conventional and new-generation orthoses for knee and ankle joints" by A. Marchini, S.P. Lauermann, M.A. Minetto, G. Massazza and N.A. Maffiuletti, for a submission to the Journal of Electromyography and Kinesiology.

As the corresponding author, I certify that: (i) the manuscript represents original work; (ii) the manuscript is not under consideration for publication elsewhere; (iii) all authors meet criteria for authorship as they all participated in preparation of the manuscript; and (iv) none of the authors have a conflict of interest to declare.

Should you need any further information, please do not hesitate to contact me.

Sincerely yours,

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1 *Journal of Electromyography and Kinesiology (manuscript submitted)*

2

3 Differences in proprioception and muscle force control between
4 conventional and new-generation orthoses for knee and ankle joints

5

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17

18 *Keywords:*

19 Force accuracy

20 Joint position sense

21 Kinesthesia

22 Knee joint

23 Ankle joint

1 **ABSTRACT**

2 The aim of this study was to compare muscle force control and proprioception between
3 conventional and new-generation experimental orthoses. Sixteen healthy subjects
4 participated in a single-blind controlled trial in which two different types of orthosis were
5 applied to the dominant knee or ankle, while the following variables were evaluated: muscle
6 force control (accuracy), joint position sense, kinesthesia, static balance as well as subjective
7 outcomes. The use of experimental orthoses resulted in better force accuracy during isometric
8 knee extensions compared to conventional orthoses (mean difference: 25.0%; $P < 0.05$).
9 Moreover, the use of experimental orthoses resulted in better force accuracy during
10 concentric (mean difference: 24.6%) and eccentric (mean difference: 25.2%) ankle plantar
11 flexions and better knee joint kinesthesia in the flexed position (mean difference: 24.0%)
12 compared to conventional orthoses (all $P < 0.05$). Subjective comfort and preference scores
13 were higher with experimental orthoses compared to conventional ones ($P < 0.05$). In
14 conclusion, orthosis type affected static and dynamic muscle force control, kinesthesia, and
15 perceived comfort in healthy subjects. New-generation experimental knee and ankle orthoses
16 may thus be recommended for prophylactic joint bracing during physical activity and to
17 improve the compliance for orthosis use, particularly in patients who require long-term
18 bracing.

1 **1. Introduction**

2 Proprioception and muscle force control are important determinants of joint stability.
3 The former can be viewed as the cumulative neural input to the central nervous system from
4 specialized nerve endings called mechanoreceptors, located in joint capsules, ligaments,
5 muscles, tendons, and skin (Grob et al., 2002). Proprioception is generally divided into two
6 aspects: joint position sense, that is restricted to the awareness of the joint position in space
7 (static phenomenon) and kinesthesia, which is defined as the awareness of joint movement
8 (dynamic phenomenon) (Proske and Gandevia, 2012). Besides proprioception, adequate
9 control of submaximal forces is especially important in daily-living activities that are normally
10 executed at a fraction of the available maximal muscle strength (Hortobagyi et al., 2004).
11 Accuracy of force production is one of the most common features of muscle force control that
12 can be easily evaluated during static and dynamic contractions by means of target-tracking
13 tasks (Glatthorn et al., 2010).

14 Lower-limb proprioception and muscle force control can be improved in healthy
15 subjects and athletes through specific training programs (e.g., sensori-motor training)
16 (Guillou et al., 2007; Taube et al., 2008), while orthopedic and neurological patients are
17 generally prescribed with orthoses in addition to their rehabilitation routines with the
18 objective to improve joint stability, mainly for the knee and ankle joints (Kelly et al., 2007;
19 Briem and Ramsey, 2013). However, very little is known on the influence of different orthosis
20 designs on lower-limb proprioception, muscle force control, and even subjective comfort and
21 perceived joint stability.

22 In the present study, knee and ankle joint proprioception (joint position sense and
23 kinesthesia), muscle force control (accuracy), static balance, and subjective outcomes were
24 investigated in a cohort of young healthy subjects wearing conventional vs. new-generation
25 experimental orthoses. The latter integrate a taping system that resembles self-adhesive

1 elastic “kinesiology” tape (such as Kinesio Tape), which is expected to improve knee and ankle
2 functional performance. Knee and ankle orthoses were specifically selected as they represent
3 the braces most commonly prescribed, while healthy subjects were selected as they
4 frequently wear a prophylactic knee/ankle brace to protect the healthy joint from injuries
5 during physical activity. The aim of this randomized, single-blind controlled trial was to
6 investigate whether orthosis design could affect proprioception, muscle force control, balance,
7 and subjective outcomes.

1 **2. Methods**

2 *2.1. Participants*

3 Sixteen healthy subjects (8 men and 8 women; mean age \pm SD: 28 \pm 6 years; body mass
4 index: 22.6 \pm 2.1 kg/m²) volunteered to participate in the study. Subjects were free from
5 neuromuscular or skeletal impairments and were asked to refrain from performing strenuous
6 physical activity during the 24 h prior to the experimental session. Each participant received a
7 detailed explanation of the study and gave written informed consent prior to participation.
8 The study conformed to the ethical principles enunciated in the Declaration of Helsinki and
9 was approved by the local Ethics Committee.

10

11 *2.2. Procedures*

12 Subjects were requested to attend a 90-min orientation session, during which they
13 were fully familiarized with the testing procedures. Two-three days later, they were asked to
14 attend the experimental session (duration: 150 min) in which orthosis type (conventional or
15 experimental), test order (see below) and joints (knee or ankle) were randomized. All the
16 orthoses were applied to the dominant side (kicking leg) in a blinded fashion and the
17 evaluations were performed unilaterally.

18 The following variables were objectively evaluated by means of valid and standardized
19 testing procedures (see below): muscle force control, joint position sense, kinesthesia, and
20 static balance. In addition, the following subjective outcomes were quantified: general
21 comfort, joint stability, and preference. The outcomes obtained with the conventional
22 orthoses NEOMESH (knee) and GAMMA (ankle) were systematically compared to those
23 obtained with the experimental models CKNEE (knee) and CANKLE (ankle), respectively (Fig.
24 1). All the orthoses were manufactured by Tenortho (Tenortho srl, Biassono, Italy).

1 Tests were performed using an isokinetic dynamometer (Biodex, Shirley Corporation,
2 USA) and a stabilometric platform (Win-posturo, Medicauteurs France SAS, Balma, France).
3 Subjects were seated on the dynamometer chair throughout the different tests, with a trunk-
4 thigh angle of 150°. To test the knee, the axis of rotation of the dynamometer was visually
5 aligned to the lateral femoral condyle, and the shin pad was positioned 2-3 cm above the
6 lateral malleolus. To test the ankle, the axis of rotation of the dynamometer was visually
7 aligned to the lateral malleolus and the foot pad was placed over the foot. To correct for the
8 effect of gravity, the mass of either the leg or the foot was measured by the dynamometer at a
9 joint angle of 30° for both the knee flexion and ankle dorsiflexion. During all tests, participants
10 were asked to fold their arms in front of the chest. A standardized warm-up consisting in 5
11 min of light cycling exercise (60-70 W) was systematically completed on a stationary cycle
12 ergometer.

13
14 **Insert Figure 1**
15

16 *2.3. Assessments*

17 *2.3.1. Muscle force control*

18 Subjects were requested to perform torque target-tracking tests (Hortobagyi et al.,
19 2004). They completed submaximal (1 Nm/kg of body weight) knee extensions and ankle
20 plantar flexions in both isometric and dynamic conditions (concentric and eccentric), with
21 three trials per condition for both the conventional and experimental orthosis. Isometric
22 contractions were performed at 45° flexion for the knee joint and at 0° plantar flexion for the
23 ankle joint, and lasted approximately 10 s. Concentric and eccentric contractions were
24 performed at an angular velocity of 10°/s, with a range of motion of 80° for the knee joint
25 (contraction duration: ~8 s) and of 50° for the ankle joint (contraction duration: ~5 s).

1 Passive rest periods of 60 s were interspersed between the different trials. Visual feedback of
2 the actual and target torque traces was provided to the subjects during the test. Force
3 accuracy, defined as the mean absolute percentage error between the actual and the target
4 torque during a 5-s interval, was quantified (mean of 3 trials per condition).

5

6 *2.3.2. Joint position sense*

7 Joint position sense was measured with an active angle-reproduction test (Grob et al.,
8 2002) as the ability to reposition the knee or ankle joints at three arbitrarily predetermined
9 positions: extended (15° knee flexion, 15° ankle dorsiflexion), neutral (45° knee flexion, 0°
10 ankle plantar flexion) and flexed (75° knee flexion, 15° ankle plantar flexion), with three trials
11 per condition for both the conventional and experimental orthosis. Subjects were blindfolded
12 to eliminate visual feedback during the test. Knee and ankle joints were passively moved from
13 the resting position (90° knee flexion and 30° ankle dorsiflexion, respectively) to one of the
14 predetermined positions (and kept constant for 10 s), then the joint was returned to the
15 resting position by the examiner and the subjects attempted to actively reproduce the target
16 joint angle. Passive rest periods of 60 s were interspersed between the different trials. The
17 mean absolute error between the actual and the predetermined target position was quantified
18 (mean of 3 trials per condition).

19

20 *2.3.3. Kinesthesia*

21 Kinesthesia was measured as the detection threshold of a passive extension movement
22 (Grob et al., 2002) performed at a very low angular velocity (1°/s). This angular velocity was
23 selected because it has been shown to maximally stimulate the joint receptors while
24 minimizing the contribution from muscle receptors (Ageberg et al., 2007). During this test the
25 subjects were blindfolded, wore earmuffs, and a vibration device (Novafon Sonossage,

1 Novafon GmbH, Stuttgart, Germany) was fitted over the shin and foot pad to neutralize the
2 slight vibration created by the motor of the dynamometer. The tests were performed starting
3 from three arbitrarily predetermined joint positions: extended (15° knee flexion, 15° ankle
4 dorsiflexion), neutral (45° knee flexion, 0° ankle plantar flexion) and flexed (75° knee flexion,
5 15° ankle plantar flexion), with three trials per condition for both the conventional and
6 experimental orthosis. The isokinetic dynamometer passively extended knee or ankle joints
7 and the participants were asked to push the hold/resume button when they felt any sensation
8 of movement in their joints. When the button was pushed by the subject, the dynamometer
9 stopped and automatically recorded the actual position. Passive rest periods of 60 s were
10 interspersed between the different trials. The mean absolute error between the actual and the
11 predetermined starting positions was quantified (mean of 3 trials per condition).

12

13 *2.3.4. Static balance*

14 Subjects were asked to stand on the stabilometric platform in a single-limb stance,
15 barefoot and blindfolded. The foot was placed on the reference lines of the platform, and
16 participants were asked to stand as calm as possible for 52 s, with four trials per each type of
17 orthosis. Passive rest periods of 120 s were interspersed between the different trials. The
18 following stabilometric parameters were extracted from the polygon-centered version of the
19 detailed report (WinPosture Nv Software, Medicauteurs France SAS, Balma, France): total
20 sway area, sway path length, and mean sway velocity (mean of 4 trials per each type of
21 orthosis).

22

23 *2.3.5. Subjective outcomes*

24 At the end of each test, the sensations of comfort and joint stability perceived by each
25 participant during the different tests were evaluated through a 0-10 scoring scale, where 0

1 indicated the worst score and 10 the best one. In addition, at the end of the experimental
2 session all subjects were requested to express their general preference for one of the two
3 orthoses (conventional or experimental).

4

5 *2.4. Statistical analyses*

6 Normal distribution of data was verified with the Shapiro-Wilk test. All the dependent
7 variables were compared between the two conditions (conventional vs. experimental
8 orthosis) using paired t-tests. Data are expressed as mean \pm SD or 95% confidence interval
9 (95% CI) computed through the modified Wald method (Agresti et al., 2005). The threshold
10 for statistical significance was set to $P < 0.05$. Statistical analyses were performed with
11 SigmaPlot 11.0 software package (Systat Software Inc., Chicago, IL).

1 **3. Results**

2 *3.1. Muscle force control*

3 Fig. 2 shows the mean absolute percentage error measured in the three conditions
4 (isometric, concentric, and eccentric) for the conventional and experimental orthoses and for
5 the knee and ankle joints. A significantly ($P < 0.05$) lower error was detected (i.e., better
6 accuracy) with the experimental orthosis during isometric knee extensions (Fig. 2A; mean
7 difference between experimental and conventional orthoses: 25.0%) and during concentric
8 (Fig. 2B; mean difference: 24.6%) and eccentric (Fig. 2C; mean difference: 25.2%) ankle
9 plantar flexions compared to the conventional orthosis. No significant differences between the
10 two orthoses were observed in the static condition for the ankle joint and in the dynamic
11 conditions for the knee joint ($P > 0.05$).

12
13

Insert Figure 2

14
15 *3.2. Joint position sense*

16 Fig. 3 shows the mean absolute error in the three joint positions (extended, neutral and
17 flexed) for the conventional and experimental orthoses and for the knee and ankle joints. No
18 significant differences were observed between conventional and experimental orthoses ($P >$
19 0.05), for either the knee or the ankle joint.

20
21

Insert Figure 3

22
23 *3.3. Kinesthesia*

24 Fig. 4 shows the mean absolute error in the three joint positions (extended, neutral and
25 flexed) for the conventional and experimental orthoses and for the knee and ankle joints. A

1 significantly ($P < 0.05$) lower error was detected (i.e., better kinesthesia) with the
2 experimental orthosis in the flexed position for the knee joint compared to the conventional
3 orthosis (Fig. 4C; mean difference: 24.0%), while no significant differences between the two
4 orthoses were observed for the ankle joint ($P > 0.05$).

6 **Insert Figure 4**

8 *3.4. Static balance*

9 Fig. 5 shows the mean stabilometric parameters for the conventional and experimental
10 orthoses and for knee and ankle trials. No significant differences were observed between
11 conventional and experimental orthoses ($P > 0.05$), for either the knee or the ankle joint.

13 **Insert Figure 5**

15 *3.5. Subjective outcomes*

16 Fig. 6 shows the mean subjective outcomes for the conventional or experimental
17 orthoses and for the knee and ankle joints. Comfort scores were significantly higher for the
18 experimental orthosis compared to the conventional orthosis ($P < 0.05$), for both knee and
19 ankle joints (Fig. 6A), while no differences in perceived joint stability were observed (Fig. 6B).
20 The percentage of participants who preferred the experimental orthosis was higher than
21 those who preferred the conventional orthosis, for both the knee (81%; 95% CI: 56-94%) and
22 the ankle joint (56%; 95% CI: 33-77%).

24 **Insert Figure 6**

1 **4. Discussion**

2 *4.1. Main findings*

3 This is the first randomized, single-blind, controlled trial investigating the influence of
4 different knee and ankle orthosis designs on muscle force control and on the two main aspects
5 of proprioception (i.e., kinesthesia and joint position sense), which, altogether, are important
6 determinants of joint stability. The main findings of this study are that the use of experimental
7 orthoses resulted in better static and dynamic control of submaximal forces (for both the knee
8 and ankle joints) and kinesthesia (for the knee joint) compared to conventional orthoses.
9 Subjective comfort and preference scores were also higher with the experimental orthoses.
10 On the contrary, no significant differences were observed between the two types of orthosis in
11 knee and ankle joint position sense, static balance, and perceived joint stability.

12

13 *4.2. Orthosis type affects muscle force control and kinesthesia*

14 We found that submaximal force accuracy and kinesthesia were significantly affected
15 by the type of orthosis, with better outcomes for the new-generation experimental model
16 compared to the conventional one. Some possible underlying factors are thought to be inter-
17 orthosis differences in weight (experimental vs. conventional knee orthosis: 100 g vs. 250 g;
18 experimental vs. conventional ankle orthosis: 50 g vs. 150 g) and/or mechanical restraint
19 provided on the joint structures. Although we failed to include a no-brace condition in our
20 present study, it may hypothesized that muscle force control and kinesthesia could
21 progressively deteriorate from a no-brace condition to experimental to conventional orthosis
22 due to differences in joint movement restriction that adversely influence motor output and
23 sensory inputs. In other words, experimental orthoses could have a less negative impact on
24 motor output and sensory inputs in comparison to conventional orthoses due to the lower
25 restriction of joint movement. The influence of orthosis design on muscle force control and

1 kinesthesia could also be related to the taping system integrated into the experimental
2 orthoses considered here (that resembles self-adhesive elastic “kinesiology” tape such as
3 Kinesio Tape), even though it is difficult to prove. While recent studies conducted on healthy
4 subjects showed no effects of quadriceps taping on physical performance, knee extension
5 strength and electromyographic activity (Lins et al., 2013; Wong et al., 2012) as well as no
6 effects of ankle taping on functional balance, jumping performance, multi-joint coordination
7 and proprioception (Ozer et al., 2009), this is the first study investigating the combined effects
8 of joint bracing and taping on force accuracy and proprioceptive acuity of healthy subjects.
9 Further studies are required to examine whether the improvements in muscle force control
10 and dynamic aspects of proprioception induced by the experimental orthoses are related to
11 the joint taping alone or to the combination of taping with joint bracing.

12 13 *4.3. Orthosis type does not affect static balance and joint position sense*

14 It has previously been observed that ankle supports limiting joint motion (i.e., ankle
15 taping and bracing) have detrimental effects on postural control in healthy subjects, while the
16 use of an elastic bandage has no significant effects (Bennel and Goldie, 1994). Consistently,
17 Hadadi et al. (2011) found that postural sway of healthy subjects increased (i.e., postural
18 control was impaired) from a no-brace condition to soft to semi-rigid ankle orthosis.
19 Restriction of ankle movement was offered as a possible explanation of these results: in other
20 words, the higher the joint restraint provided by a taping technique or brace, the worst the
21 postural control. Therefore, one could assume that the use of experimental orthoses, which
22 offer less joint restraint than conventional orthoses, would have resulted in better static
23 balance. However, we observed no differences in static balance, knee and ankle joint position
24 sense, and perceived joint stability between the two types of orthosis. This could be due to the
25 characteristics of the population under study and/or to the study design. It may be

1 hypothesized that normal proprioception and static postural control of healthy subjects can
2 hardly be improved by short-term application of a brace. This is consistent with previous
3 research demonstrating that knee bracing did not influence either static balance (Kaminski
4 and Perrin, 1996) or knee proprioception (Bottoni et al., 2013; Kaminski and Perrin, 1996) in
5 uninjured active subjects. Therefore, proprioception and static postural control could have
6 been hardly affected to a different extent by the application of conventional vs. experimental
7 orthosis in the current investigation.

8 Further studies on populations of patients who usually require a knee orthosis
9 (individuals with functional knee instability, anterior cruciate ligament injury, patellofemoral
10 pain syndrome) or an ankle orthosis (ankle-sprain copers, individuals with functional ankle
11 instability) are needed to document the differences (if any) in proprioception and balance
12 control associated to the use of different braces. For example, in the above-mentioned study
13 by Hadadi et al. (2011) the comparison between soft and semi-rigid ankle orthosis was
14 performed in both healthy subjects and patients with functional ankle instability: decreased
15 postural sway was observed in patients while wearing either of the orthoses in comparison to
16 the no-brace condition, with soft bracing having greater effects.

17

18

19 **5. Conclusions**

20 In conclusion, we found that the use of experimental knee and ankle orthoses in
21 healthy subjects improved force accuracy during submaximal static and dynamic contractions
22 and kinesthesia in comparison to conventional orthoses. These results have important
23 implications because adequate control of submaximal forces is crucial in activities of daily
24 living that are normally executed at a fraction of the available maximal muscle strength
25 (Hortobagyi et al. 2004). Because these improvements were not associated to a worsening of

1 the perceived joint stability, we may thus recommend the use of experimental orthoses in
2 athletes wearing a prophylactic knee/ankle brace to protect the healthy joint(s) from
3 potential injuries. In addition, subjective comfort was higher with experimental orthoses
4 compared to conventional ones, and the proportion of subjects who preferred the
5 experimental orthosis was higher than those who preferred the conventional orthosis. This
6 could imply better compliance for experimental orthosis, particularly in patients who require
7 long-term bracing. The acute benefits of new-generation knee/ankle orthosis on muscle
8 control and kinesthesia observed in this comparative study remain to be confirmed in a
9 longitudinal intervention study, in an attempt to improve joint stability (and thus reduce the
10 risk of injury) in healthy and previously injured subjects.

11

12

13 **6. Conflicts of interest**

14 Knee and ankle orthoses were provided by Tenortho srl (Biassono, Italy). Neither
15 sponsor had any involvement in the design of the study, in the collection, analysis and
16 interpretation of data, in the writing of the manuscript or in the decision to submit the
17 manuscript for publication.

18

19

20 **Acknowledgements**

21 The authors thank A. Tentorio for his support during the experimental phase of the
22 study.

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1 **Figure legends**

2

3 **Fig. 1.** Overview of the conventional (A, B) and experimental (C, D) orthoses used in this study
4 for the knee (A, C) and ankle (B, D) joints. (A) NEOMESH: fabric coated neoprene, airmesh
5 back side. Spiral plastic coated stainless steel stays. Patella hole with stabilizer. (B) GAMMA:
6 coated neoprene foot neck, airmesh foot sock and back side. Side lateral/medial support stays.
7 (C) CKNEE: elastic knee brace with carbon fiber yarn and integrated taping system. (D)
8 CANKLE: elastic ankle brace with carbon fiber yarn and integrated taping system.

9

10 **Fig. 2.** Mean absolute percentage error (and SD bars) for force accuracy during submaximal
11 knee extensions and ankle plantar flexions in isometric (A), concentric (B), and eccentric (C)
12 conditions with conventional and experimental orthoses.

13 Significant difference between the two conditions: $*P < 0.05$.

14 Conv: conventional orthosis; Exp: experimental orthosis.

15

16 **Fig. 3.** Mean absolute error (and SD bars) for joint repositioning at extended (A), neutral (B),
17 and flexed (C) knee and ankle joint positions with conventional and experimental orthoses.

18 Conv: conventional orthosis; Exp: experimental orthosis.

19

20 **Fig. 4.** Mean absolute error (and SD bars) for passive movement detection (kinesthesia) at
21 extended (A), neutral (B), and flexed (C) knee and ankle joint positions with conventional and
22 experimental orthoses. Significant difference between the two conditions: $*P < 0.05$.

23 Conv: conventional orthosis; Exp: experimental orthosis.

24

1 **Fig. 5.** Mean values (and SD bars) of stabilometric parameters for knee and ankle trials with
2 conventional and experimental orthoses: (A) total sway area, (B) sway path length, and (C)
3 mean sway velocity.

4 Conv: conventional orthosis; Exp: experimental orthosis.

5

6 **Fig. 6.** Mean values (and SD bars) of subjective outcomes (0-10 scoring scale) for conventional
7 and experimental orthoses for knee and ankle joints: (A) comfort and (B) perceived stability.

8 Significant difference between the two conditions: $*P < 0.05$.

9 Conv: conventional orthosis; Exp: experimental orthosis.

(A) NEOMESH



(B) GAMMA



(C) CKNEE



(D) CANKLE



Figure 2

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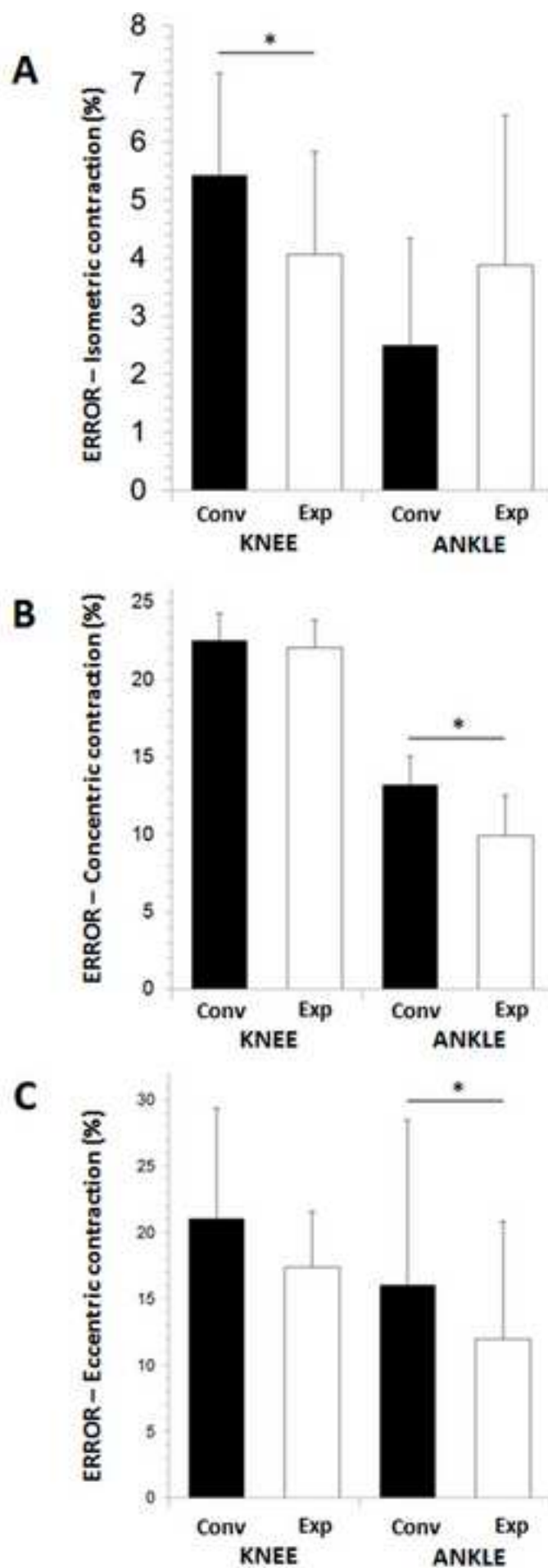


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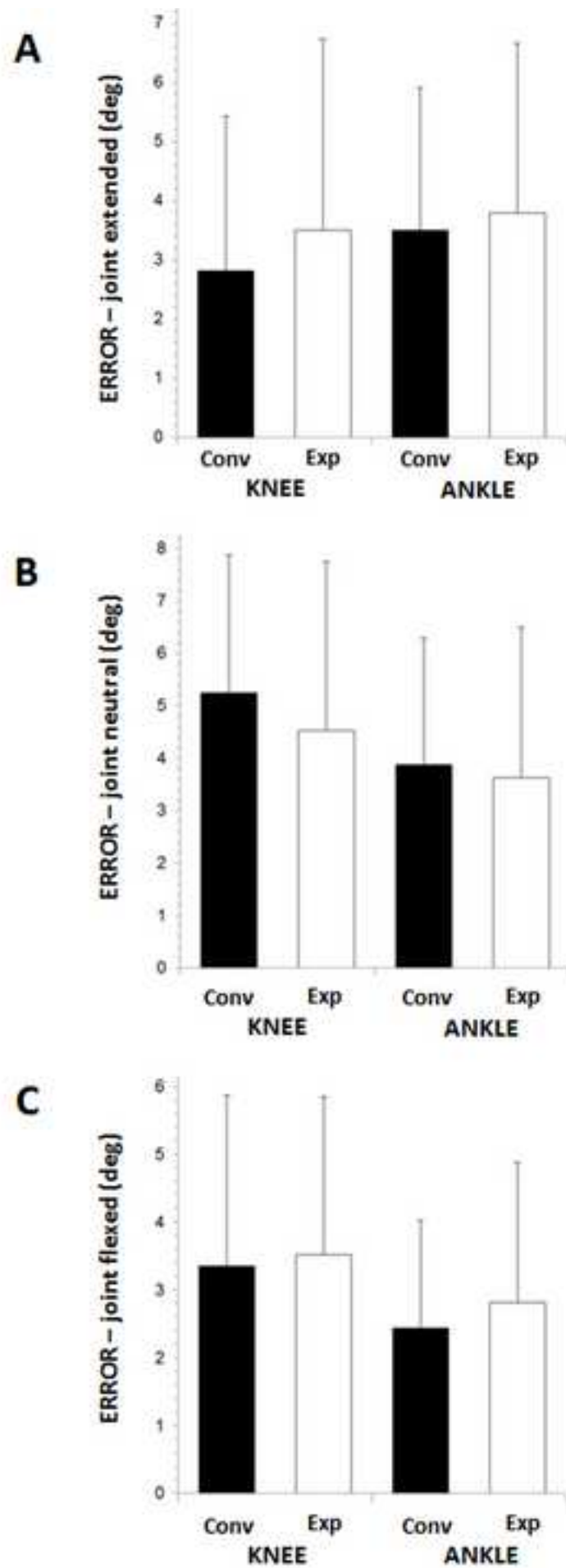


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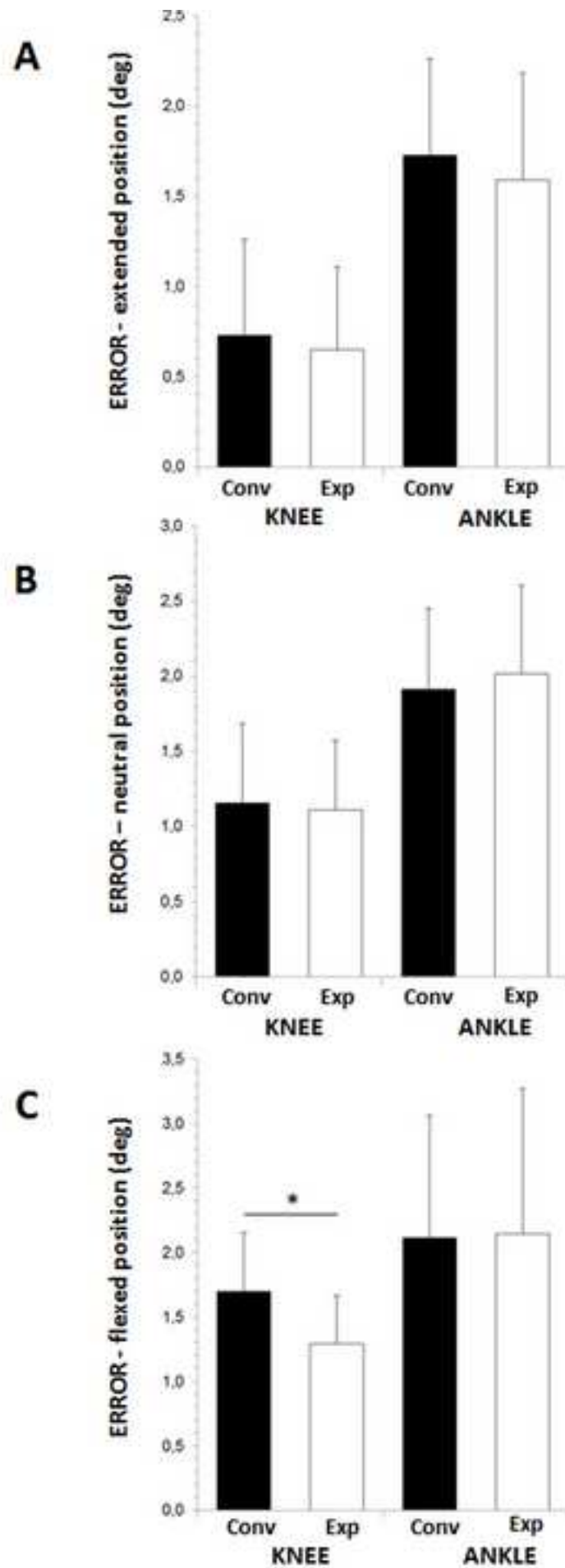


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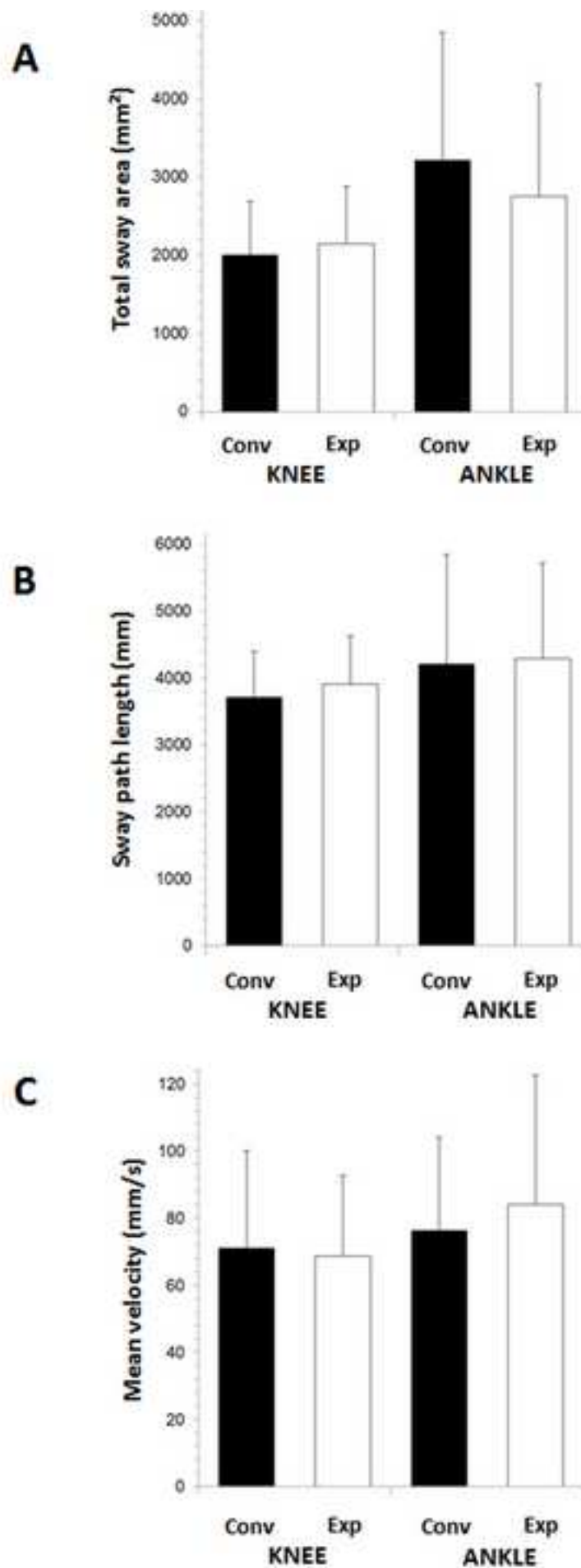


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