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RESEARCH ARTICLE

Control of Movement

Modular reorganization of gait in chronic but not in artificial knee joint constraint

Carlos Cruz-Montecinos,^{1,2,3} Sofía Pérez-Alenda,¹ Mauricio Cerda,^{4,5,6} and Huub Maas²

¹Physiotherapy in Motion Multispeciality Research Group (PTinMOTION), Department of Physiotherapy, University of Valencia, Valencia, Spain; ²Department of Human Movement Sciences, Faculty of Behavioural and Movement Sciences, Vrije Universiteit Amsterdam, Amsterdam Movement Sciences, Amsterdam, The Netherlands; ³Laboratory of Clinical Biomechanics, Department of Physical Therapy, Faculty of Medicine, University of Chile, Santiago, Chile; ⁴Integrative Biology Program, Institute of Biomedical Sciences, Faculty of Medicine, Universidad de Chile, Santiago, Chile; ⁵Center for Medical Informatics and Telemedicine, Faculty of Medicine, Universidad de Chile, Santiago, Chile; and ⁶Biomedical Neuroscience Institute, Santiago, Chile

Abstract

It is currently unknown if modular reorganization does occur if not the central nervous system, but the musculoskeletal system is affected. The aims of this study were to investigate 1) the effects of an artificial knee joint constraint on the modular organization of gait in healthy subjects; and 2) the differences in modular organization between healthy subjects with an artificial knee joint constraint and people with a similar but chronic knee joint constraint. Eleven healthy subjects and eight people with a chronic knee joint constraint walked overground at 1 m/s. The healthy subjects also walked with a constraint limiting knee joint movement to 20°. The total variance accounted (tVAF) for one to four synergies and modular organization were assessed using surface electromyography from 11 leg muscles. The distribution of number of synergies were not significantly different between groups. The tVAF and the motor modules were not significantly affected by the artificial knee constraint. A higher tVAF for one and two synergies, as well as merging of motor modules were observed in the chronic knee constraint group. We conclude that in the short-term a knee constraint does not affect the modular organization of gait, but in the long-term a knee constraint results in modular reorganization. These results indicate that merging of motor modules may also occur when changes in the mechanics of the musculoskeletal system is the primary cause of the motor impairment.

NEW & NOTEWORTHY It is currently unknown if modular reorganization does occur if not the central nervous system, but the musculoskeletal system is affected. This study showed that in the short-term a knee constraint does not affect the modular organization of gait, but in the long-term a knee constraint results in modular reorganization. These results indicate that modular reorganization may also occur when changes in the mechanics of the musculoskeletal system is the primary cause of the motor impairment.

gait analysis; knee joint; lower limb kinematics; motor control; muscle synergy

INTRODUCTION

Several neurological (e.g., stroke and cerebral palsy) and musculoskeletal diseases (e.g., osteoarthritis, rheumatoid arthritis, and hemophilic arthropathy) result in a chronic limitation in joint range of motion (ROM) (1–6). This interferes with many essential activities of daily living such as walking, stair climbing, or standing up (7–9), affecting people's ability to function independently and their quality of life (10, 11).

The success of surgical and pharmaceutical treatments to recover joint kinematics and neuromuscular control during gait is limited (12, 13), especially when the knee joint is affected (14, 15). To improve treatment and rehabilitation strategies, a more detailed analysis of the neural consequences of chronic joint constraints seems warranted.

For neurological and musculoskeletal diseases, the mechanisms causing the chronic limitation in joint ROM are different. In neurological diseases, a disruption of the central

and/or peripheral nervous system is the primary cause of the joint constraint (14, 16–19). In musculoskeletal disorders, the joint constraint is the result mainly of changes in bone or soft tissues (2, 6, 20–22). In case a disorder of the musculoskeletal system is the primary cause, the central nervous system may also be affected. Such secondary adaptations may lead to altered neuromuscular control during gait, for example, an increased coactivation between antagonistic muscles (23–26). Recently, a temporary immobilization of rat hindlimbs (16 h a day for 28 days) during development was found to cause not only a chronic restriction of joint ROM during locomotion, but also a persistent spinal hyperreflexia and changes in topographical organization of the sensorimotor cortex (27, 28).

The central nervous system is presumed to produce limb movements, not by exciting individual muscles but by exciting a group of muscles in patterns called muscle synergies (29–32). A muscle synergy can be defined as a selected group of muscles that are recruited simultaneously with distinct relative levels of activation (31–34). Muscle synergies can be characterized by electromyographic (EMG) activities of muscles represented by combinations of time-independent weights (motor modules) and time-dependent coefficients (motor primitives) (35–39). The motor modules and motor primitives represent together the modular organization of the movement pattern (40).

In healthy individuals, four different muscle synergies have previously been identified to reconstruct unilateral lower extremity muscle activation patterns during locomotion, each related to a specific part of the gait cycle (e.g., acceptance, propulsion, swing, deceleration) (41, 42). *Synergy 1*, acceptance (with a principal contribution of hip extensors/abductors and knee extensors) is exited during early stance. *Synergy 2*, propulsion (with a principal contribution of ankle plantar flexors) is exited during late stance. *Synergy 3*, swing (with a principal contribution of ankle dorsiflexors and biarticular knee extensors) is exited during early swing. *Synergy 4*, deceleration (with a principal contribution of hamstring muscles) is exited during late swing. In people with chronic stroke, merging of modular organization (i.e., modular reorganization) such as acceptance and propulsion synergies or acceptance and deceleration synergies has been reported (14, 41, 43). However, in patients with subacute stroke (20 wk), the motor primitives were similar to those of a healthy group (36). It is currently unknown if such merging of modular organization does also occur when changes in the mechanics of the musculoskeletal system, not the central nervous system, is the primary cause.

In humans, the short-term effects (immediate to a few minutes following application) of artificial joint constraints have been studied using external devices. For example, to mimic the knee flexion contracture gait pattern typical for people with cerebral palsy and people with knee arthropathy and to mimic full knee extension following knee arthrodesis surgery (44–52). A simulated knee flexion contracture results in an increased forefoot weight bearing and flexion posture during stance, increasing the knee extensor torque (46, 48, 51). Both simulated knee flexion contracture and knee arthrodesis change ankle and hip kinematics to facilitate foot clearance of the braced limb (44–46, 48–50). Only one previous study reported short-term changes in EMG in response

to an artificial knee flexion contracture and found an increase in the amplitude and duration of the gluteus maximus, vastus lateralis, and soleus muscles during the stance (46). It is unknown, however, if an artificial joint constraint does affect the modular control of locomotion.

In one study simulating knee arthrodesis, after the immediate adaptations, no further changes in kinematics were found within 24 h (49). Similar results have been reported following the application of an artificial ankle joint constraints, that is, no significant changes in EMG amplitude of plantar flexors or kinetics gait parameters between immediate and postaccommodation (1 h/6 wk) (53, 54). The above suggest that a longer duration (chronic) joint restriction is required for subsequent changes in the central nervous system. Note that an acute joint restriction may not fully mimic the condition of the musculoskeletal system of people with a chronic joint restriction, because the latter may involve additional adaptations such as muscle weakness and changes in muscle length (55–58).

The aim of this study was to assess short-term (immediate to a few minutes following application) and long-term (years) effects of a constraint of the knee ROM on the modular organization of gait. We hypothesize that the modular organization of gait will not be affected in the short-term, but substantially changed in the long-term. For this purpose, we assessed 1) the acute effects of an artificial knee joint constraint on the modular organization in healthy subjects; 2) the differences in modular organization between healthy subjects with an artificial knee joint constraint and people with a similar but chronic knee joint constraint.

METHODS

Participants

This study was approved by the local ethical committee and conducted in agreement with the Declaration of Helsinki. All participants were informed about the purpose and procedures of the project and gave their written informed consent to participate in the study.

For the chronic knee constraints group, people with hemophilic arthropathy (PWA) were recruited (Fig. 1). Hemophilia is an X chromosome-linked bleeding disorder (thus affecting males only) caused by a deficiency of coagulation factors VIII (hemophilia A) and factor IX (hemophilia B) (59). Repetitive intra-articular and/or intermuscular bleeding is a common manifestation of this disease (60), which results in inflamed synovium and irreversible change in cartilage tissue, which may be accompanied by excessive fibrous or scar tissue within a joint and the consequent restriction of ROM (6).

Eight PWA and eleven healthy control subjects (CG) were recruited. For the CG, inclusion criteria were the following: male, over 18 yr of age and under 65 yr, no hemophilia, and body mass index lower than 30. Exclusion criteria were the following: traumatic injuries; signs or symptoms of injury or symptomatic arthritis to the trunk, lower back, and lower limb within the past 3 mo; scoliosis; history of cardiac and/or respiratory pathology; and neurological disease.

For PWA, inclusion criteria were the following: diagnosed with hemophilia A or B, severe or moderate, diagnosed by a medical doctor specialized in hemophilic arthropathy, less than 30° of knee ROM [note that 30° corresponds to 50% of

Figure 1. A: subject with an articulated knee brace (Blunding, Santiago, Chile) used to acutely constrain the knee range of motion to 20°–40°. B: patient with a chronic (10 yr) knee joint constraint.

the knee ROM during gait in healthy individuals (61)], over 18 yr of age and under 65 yr, prophylaxis treatment with deficient factor (i.e., VIII or IX), and body mass index less than 30. Exclusion criteria: history of hip, knee or ankle arthroplasty, equinus foot, inability to walk without an assistive device (e.g., walker, cane), history of muscle or joint bleeding in lower limbs in the last 2 mo, chronic cardiac and/or respiratory pathology and neurological disease.

Clinical Assessments for PWHA

To assess the intensity of pain (scale 0–10 points) during barefoot walking, the Visual Analogue Scale was used. The joint health status in the knee and ankle was assessed with the Hemophilia Joint Health Score 2.1 (HJHS) (62). This scale (range 0–20) consists of eight items per joint, evaluating 1) joint swelling (0–3 points), 2) duration of swelling (0–1 points), 3) muscle atrophy (0–2 points), 4) strength (0–4 points), 5) crepitus on motion (0–2 points), 6) flexion loss (0–3 points), 7) extension loss (0–3 points), and 8) pain (0–2 points). For the passive knee ROM assessment, a 360° universal plastic goniometer with a 30-cm movable arm and scale of 1° increment (Baseline, Chattanooga Group Inc.) was used for all measurements. The protocol was based on a recommendation of the measurement of passive ROM in patients with knee restrictions (63).

Surface Electromyography Protocol

Surface electromyography (EMG) was collected from gluteus maximus (GMAX), gluteus medius (GMED), biceps femoris

(BF), semitendinosus (ST), rectus femoris (RF), vastus medialis (VM), vastus lateralis (VL), tibialis anterior (TA), soleus (SOL), lateral gastrocnemius (LG), and medial gastrocnemius (MG). In PWHA, the limb with the lowest knee ROM was selected considering the inclusion criteria. In CG, the dominant limb was assessed. Leg dominance was assessed by asking the subjects which leg they would use to kick a ball (23). After shaving and cleaning the skin with alcohol, surface electrodes (Ag–AgCl, Kendall H124SG) were placed (interelectrode spacing 2 cm) on the earlier described muscles according to SENIAM guidelines (64). The EMG signals were measured using a wireless EMG system (MyoSystem DTS, Noraxon USA Inc., Scottsdale, CA) with a sampling rate of 1,500 Hz. Heel strike was detected by a synchronized wireless pressure sensor (Noraxon USA Inc., Scottsdale, CA,) placed underneath the heel of the foot.

Kinematics Protocol

The sagittal kinematics of the hip, knee, and ankle joints were assessed using on inertial measurement units (IMUs). Four IMU sensors (Xsens, Enschede, the Netherlands) were positioned between posterior superior iliac spines, the lateral face of the thigh within the proximal third, lateral face of the shank within the distal third close to the lateral malleolus, and the midfoot. The sensors placed on sacrum, thigh, and shank were fixed with the fixation system provided by the company (Xsens, Enschede, the Netherlands). The sensor placed on midfoot was fixed by adhesive elastic taping (Leukotape K, BSN Medical, Hamburg, Germany) with sufficient tension to avoid movement artifacts. In addition, the magnetometer data for each IMUs were collected to adjust the sagittal joint position in the upright position (see *Kinematics of the lower limb* section). Data were collected at a sampling frequency of 75 Hz. The IMUs and EMG/pressure sensor were synchronized with a trigger pulse.

Experimental Procedures

Surface electromyography (EMG), joint kinematics and temporal gait parameters were obtained during 30 m of bare-foot walking overground twice, at the preferred velocity for PWHA and for CG at a slower velocity (1m/s). For both EMG and kinematics, ten cycles corresponding to the middle 10 m of the 30-m corridor were used for data analysis (20 cycles in total) (23, 65) (Fig. 2).

For the CG, the slow velocity was practiced three times for 10 m. Then, CG was invited to walk 30 m at 1 m/s twice used an articulated knee brace (Blunding, Santiago, Chile) fixed at 20° of flexion to 40° of flexion (Fig. 1). Each participant practiced with the external device three times for 10 m.

Data Analysis

For EMG and kinematic analysis, MATLAB v. 2019a was used (Statistics and Machine Learning Toolbox, MathWorks, Inc., Natick, MA).

Muscle activity patterns and timing of EMG activity.

To assess the EMG amplitude for each muscle, first a bandpass filter (20–500 Hz, Butterworth, fifth-order) was applied. Then the EMG signals were rectified using the absolute values of Hilbert transform using the MATLAB

Figure 2. Surface electromyography recordings of one participant in each group including 10 cycles selected of the middle 10 m of one test of 30-m walking. BF, biceps femoris; GMAX, gluteus maximus; GMED, gluteus medius; LG, lateral gastrocnemius; MG, medial gastrocnemius; RF, rectus femoris; SOL, soleus; ST, semitendinosus; TA, tibialis anterior; VM, vastus medialis; VL, vastus lateralis.

function “Hilbert” (66), and then smoothed with a low-pass filter at 10 Hz (23, 67, 68). The EMG signals during each step cycle (from heel strike to heel strike) were time normalized to 200 points, and the amplitude for each muscle was normalized to the maximum activation of each muscle recorded for each participant at each condition (41, 69, 70).

Computation of muscle synergies.

The non-negative matrix factorization (NMF) was used to extract muscle synergies from the EMG data combined into an $m \times t$ matrix (220×200), where m represents the number of muscles (11 and 20 cycles), and t is the time base (200 points). The gait cycles were concatenated in columns on the assumption of time-varying doses (the temporal synergy model) (71, 72). The NMF results in the muscle weightings for each synergy (W) and the matrix encoding the activation pattern of each synergy (C) (32). Note that the product of W and C should approximate the original EMG data. The NMF algorithm was iterated 20 times for each number of synergies (73) (from 1 to 4 in this study), and the iteration with the lowest reconstruction error was selected. To obtain the 11-element modules, averaging across 20 cycles was applied. The difference between the reconstruction of EMG data (EMGr) and the original EMG (EMGo) data was calculated using total EMG variance accounted for (tVAF). The tVAF was calculated as the ratio of the sum of the squared error values to the sum of the squared EMGo (41), as represented in Eq. 1:

$$tVAF = 1 - \frac{\sum_{i=1}^m \sum_{j=1}^t (EMGo(i,j) - EMGr(i,j))^2}{\sum_{i=1}^m \sum_{j=1}^t (EMGo(i,j))^2}. \quad (1)$$

Complexity of motor control.

To assess the complexity of motor control during gait, the following parameters were calculated: tVAF by one to four muscle synergies, and the lowest number of muscles synergies for tVAF > 90% (first criterion) while adding an additional synergy did not increase tVAF by > 5% (second criterion) (41). For all groups, application of the second criterion resulted in an increase of the number of synergies to the same extent (i.e., >90% of the subjects). A higher tVAF indicate an increase of coactivation between antagonistic and synergistic muscles (23, 70, 74).

Motor Modules and Motor Primitives

To allow for comparison of motor primitives and motor module between groups, we further investigated the four-synergy solution for all individuals. This number was motivated by the fact that most previous studies identified four muscle synergies during walking using the same muscles used in the present study (41, 42).

Because extraction of muscle synergies with NMF (4 in our case) does not extract the synergies in the same order for each individual, the following methodology was applied to allocate the motor modules and motor primitives to each of the four synergies. First, we identified the best match between the synergies and the average of the four synergies

extracted in the control group using the threshold of $tVAF > 90\%$ (Fig. A1). In an iterative approach, we compared the synergies in two sets by computing the cosine similarity between the best matched pairs of motor modules (75). Second, to ensure the correct allocation of each motor module to their respective synergy, the matched synergies were carefully checked by a visual inspection focused on the muscle weightings in the motor modules and the temporal peaks of the motor primitives. Finally, each motor primitive was normalized by the peak activity and each motor module was normalized to the maximal muscle weighting.

To assess the principal muscle weighting in each module, 95% of the confidence interval was used (76). To assess the similarities of motor modules and motor primitives between groups, the cosine similarity was computed. Where a similarity of 1 represents perfect similarity, and a value above 0.8 was defined as similar (75, 77).

Assessment of synergy merging.

To assess if synergies were merged, we modeled the motor modules of the artificial and chronic constraint groups as a linear combination of motor modules from the control group. For the linear combinations of motor modules and their relative contribution to the merged module, the nonnegative least squares implemented with the “lsqnonneg” option in MATLAB was used (78). To assess if a synergy of the artificial or chronic constraint groups may be reconstructed from a pair of merged synergies of the control group, two criteria were adopted. First, the coefficient of the reconstruction (i.e., contribution coefficient) of both pairs of motor modules for control group had to be above 0.3 (79). Second, a similarity between the reconstructed and the single motor module had to be above 0.8 (75, 77). In addition, the frequency of appearance of merged synergies according to the above criteria was calculated.

Kinematics of the lower limb.

The magnetometer data were used to determine the initial position of the hip, knee, and ankle joint in the sagittal plane. To assess the position of each joint, each subject was invited to stand in a neutral position with both feet parallel for 10 s. Then, the flexion-extension angle for the hip, knee, and ankle was estimated using the IMU quaternion orientation. Low-pass Butterworth filters were applied to the IMU joint angle data, with a cutoff frequency of 10 Hz. The segmentation of kinematic signals into strides were determined through gait events signifying heel strike and toe-off from the angular velocity in z-axis obtained from the IMU sensor of the shank (80) and then time normalized to 75 points. To assess the walking velocity in the 30-m walking test, the start and end of walking was assessed using the acceleration signal of the IMU sensor of the shank. For the final analysis, the mean of the same 20 cycles selected for the EMG analysis were used to represent the kinematics and temporal parameters of gait.

Statistics

Sample size calculation.

The a priori power analysis conducted in G*Power (3.1.9.2 v.) software (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) showed that eight subjects per group were sufficient to obtain a statistical power of 0.80 with an $\alpha = 0.05$ and large effect size (Cohen's $d = 1.65$) based on the difference in

walk-DMC index between CG and PWHA with severe joint damage reported previously (23).

Statistical analysis.

Two independent assessments were made for all variables: 1) comparison between CG no constraint (control) and artificial knee joint constraint (artificial) and 2) between artificial joint constraint and PWHA with chronic knee constraint (chronic constraint). The normality of data was evaluated through the Shapiro–Wilk test. For all comparisons, the α level was set at 0.05. The unpaired or paired two-tailed t test, and two-tailed Wilcoxon rank-sum test (unpaired) or Wilcoxon signed-rank (paired) were used to compare the between-subject (artificial vs. chronic) and within-subject differences (control vs. artificial), depending on the normality of data. To compare the distribution of the number of synergies, and the frequency of merged synergies between groups the Chi squared test (χ^2) was applied (23, 41). To test for differences in the frequency of merged synergies between the artificial and chronic constraint groups, a post hoc test with Bonferroni correction was applied. The data are expressed as mean and standard deviation unless stated otherwise.

To evaluate differences in the tVAF as a function of number of synergies, a two-way ANOVA (within subject factor: number of synergies; between subject factor: group) was used. Greenhouse–Geisser correction was used if the assumption of sphericity, as checked by Mauchly's test, was violated. If a significant interaction was found between factors, post hoc tests with Bonferroni correction were applied.

To test for the differences between groups in the contribution of individual muscles to each motor module, the Bonferroni correction of the P values for the total number of comparisons (i.e., 44) was applied. To compare the waveforms of the motor primitives and kinematics of hip, knee, and ankle joints between groups, n dimensional statistical (non) parametric mapping (SPM) was used (81–84). For that, the open-source MATLAB-based spm1d-package was used to generate the two-tailed t values maps (<http://www.spm1d.org/index.html>). Normal distribution of data was tested for the selection of parametric or nonparametric analysis. The motor primitives and joint kinematics time series were considered significantly different if any values of SPM over the entire gait cycle exceeded the critical threshold ($\alpha = 0.05$). To compare the motor primitives between groups, a Bonferroni correction for the four comparisons (i.e., for each of the four synergies) was applied. To compare the joint angle patterns, a Bonferroni

Table 1. Characteristics of control group and people with chronic knee restriction due to hemophilic arthropathy

Clinical Characteristic	CG (n = 11)	PWHA (n = 8)	P Value
Age, yr	34.6 ± 10	39.1 ± 16	0.448
Height, cm	174 ± 5	170 ± 3	0.069
Body mass index	25.1 ± 2	26.4 ± 4	0.342
Pain during walking, VAS 0–10	0 [0 0]	4.0 [1 8]	<0.001
Chronicity of knee joint restriction, yr	NA	15 [10 20]	NA

Parametric distribution: means ± SD; nonparametric distribution: median [range]. CG, control group; NA, not applicable; PWHA, people with hemophilic arthropathy; VAS, visual analogue scale. Significant differences ($P < 0.05$) are in bold.

Table 2. Clinical characteristics of people with chronic knee restriction due to hemophilic arthropathy

Joint Clinical Assessment	PWHA (n=8)
HJHS knee score, 0–20 pts	12.6 ± 3.1
HJHS ankle score, 0–20 pts	9.9 ± 2.5
Passive knee extension, degrees	–25.0 ± 11.3
Total passive range of motion of knee, degrees	12.5 ± 10
Passive ankle dorsi flexion, degrees	9.0 ± 5.3
Total passive range of motion of ankle, degrees	23.2 ± 12.4

Parametric distribution: means ± SD. HJHS, hemophilia joint health score; PWHA, people with hemophilic arthropathy.

correction for the three comparisons (i.e., for each of the three joints) was applied. In the final step, cluster specific *P* values were calculated over the entire gait cycle.

RESULTS

We did not find differences in age, height, and BMI between the CG and the PWHA (chronic constraint), except for pain (Table 1). In PWHA, the joint health score (HJHS) for knee and ankle joint was not different ($P = 0.160$). The mean of passive ROM was $12.5^\circ \pm 10^\circ$ for the knee and $23.2^\circ \pm 12.4^\circ$ for the ankle joint (Table 2). The motor modules and motor primitives data were not normally distributed.

Effects of Artificial Knee Joint Constraint on Lower Limb Kinematics

Applying the knee constraint, the amount of hip flexion was similar during the stance phase and during the swing

phase. At the knee joint, the knee constraint resulted in a more flexed position during the stance ($P < 0.001$) and more flexion angle during late swing ($P = 0.015$) (Fig. 3). At the ankle joint, the knee constraint resulted in a more dorsiflexed position during the early stance ($P = 0.013$), middle stance ($P < 0.001$), and swing phases ($P = 0.001$) (Fig. 3).

The knee constraint did not affect the hip ROM (normal: $31.9^\circ \pm 6.5^\circ$; artificial: $36.3^\circ \pm 6.5^\circ$; $P = 0.203$) and the ankle ROM (normal: $24.5^\circ \pm 3.6^\circ$; artificial: $21.1^\circ \pm 5.4^\circ$; $P = 0.203$) during gait. A significant difference was observed for the knee ROM (normal: $49.8^\circ \pm 5.3^\circ$; artificial: $24.4^\circ \pm 5.6^\circ$; $P < 0.001$).

Differences Kinematics of the Lower Limb between Artificial and Chronic Constraint

The extent of knee flexion was lower in the PWHA (chronic constraint) than in the CG with the artificial constraint during the swing phase ($P = 0.015$) (Fig. 3). No differences between groups were found for the hip and ankle joints (Fig. 3).

The knee (chronic: $6.3^\circ \pm 3.8^\circ$; artificial: $24.4^\circ \pm 5.6^\circ$; $P < 0.001$) and ankle ROM (chronic: $11.6^\circ \pm 4.3^\circ$; artificial: $21.1^\circ \pm 5.4^\circ$; $P = 0.001$) were lower in the PWHA (chronic constraint) than in the CG with the artificial constraint. No difference between groups was observed in the hip ROM (chronic: $33.3^\circ \pm 8.3^\circ$; artificial: $36.3^\circ \pm 6.5^\circ$; $P = 0.395$).

Effects of Artificial Knee Joint Constraint on Temporal Variables

Applying the knee constraint did not result in changes of step cycle time (normal: $1.2\text{ s} \pm 0.1\text{ s}$; artificial: $1.2 \pm 0.1\text{ s}$; $P =$

Figure 3. Joint kinematics during gait for hip (top), knee (middle), and ankle (bottom). Statistical results of comparisons between normal and artificial knee constraint in the healthy group ($n = 11$) and between an artificial ($n = 11$) and chronic knee constraint ($n = 8$) are shown. Data are plotted as a function of normalized step time (0, heel strike) and expressed as mean with 95% confidence intervals. The time series for joint kinematics were analyzed with Statistical Parametric Mapping. The vertical dotted line indicates the end of the stance phase. * $P < 0.05$. ** $P < 0.001$.

0.204), stance time (normal: $61.4\% \pm 4.1\%$; artificial: $60.0\% \pm 1.4\%$; $P = 0.162$), and gait velocity in 30 meters walking task (normal: 1.0 ± 0.2 m/s; artificial: 1.0 ± 0.2 m/s; $P = 0.655$).

Temporal Variables between Artificial and Chronic Knee Constraint

The stance time was lower in the PWHA (chronic constraint) than in the CG with the artificial constraint (chronic: $54.0\% \pm 4.4\%$; artificial: $60.0\% \pm 1.4\%$; $P = 0.007$). No differences between groups were observed in the step cycle time (chronic: 1.1 ± 0.1 s; artificial: 1.2 ± 0.1 s; $P = 0.076$) and in gait velocity in 30 m walking task (artificial: 1.0 ± 0.2 m/s; chronic: 0.9 ± 0.1 m/s; $P = 0.425$).

Effects of the Artificial Knee Joint Constraint on Complexity of Neuromuscular Control

The distribution of muscle synergies extracted for normal walking [median 4 (min 2 max 4)] and walking with the artificial constraint [median 4 (min 3 max 4)] did not differ ($P = 0.264$) (Fig. 4 and Figs. A1 and A2). Also, no differences between groups were found for the quality of reconstruction based on the number of synergies extracted (control: tVAF $95.2\% \pm 2.4\%$; artificial: tVAF $95.8\% \pm 1.6\%$; $P = 0.572$). The two-way ANOVA indicated a significant main effect of number of synergies ($P < 0.001$), but no significant group effect ($P = 0.558$) and no significant interaction between factors ($P = 0.137$) on the tVAF (Fig. 4).

Differences in Complexity of Neuromuscular Control between Short-Term Artificial and Chronic Constraint

The distribution of muscle synergies extracted for the chronic constraint group [median 3 (min 2 max 4)] was not statistically different from that of the artificial knee constraint group ($P = 0.062$) (Figs. 4, 5, and A2). Also the quality of reconstruction based on the number of synergies extracted was not statistically different between groups (chronic: tVAF $95.6\% \pm 2.0\%$; artificial: tVAF $95.8\% \pm 1.6\%$; $P = 0.838$). The two-way ANOVA indicated a significant main effect of number of synergies ($P < 0.001$) and significant main effect of factor group ($P = 0.001$), as well a significant interaction between factors ($P < 0.001$) on the tVAF. Post hoc analysis showed a significant difference between groups when including one synergy ($P < 0.001$) and two synergies ($P = 0.002$) (Fig. 4).

Effects of Artificial Knee Joint Constraint on Modular Organization

Applying the knee constraint did not result in statistically different individual muscle contributions (i.e., motor modules) (Figs. 6 and 7). The similarity of motor modules between groups was higher than 0.8 for all synergies (Table 3). Different patterns of the motor primitives between groups were found for synergy 1, 2 and 3. For synergy 1 (acceptance), the motor primitives of the artificial constraint group show a higher activity during the late swing phase ($\sim 80\%$ of the step cycle; $P < 0.001$) (Fig. 7). For synergy 2 (propulsion), the motor primitives of the artificial constraint group show a lower activity during the late stance phase ($\sim 40\%$ of the step cycle; $P < 0.001$) (Fig. 7). For synergy 3 (swing), the motor primitives of the artificial constraint group show a lower

Figure 4. A: the total variance accounted for (tVAF) by 1–4 muscle synergies. Statistical results of comparisons between no and an artificial knee constraint in the control group ($n=11$) and between an artificial constraint in the control group ($n=11$) and the chronic knee constraint group ($n=8$) are indicated. * $P < 0.05$; ** $P < 0.001$. Individual data, means, and 95% confidence intervals are shown. B: the distribution of the number of muscles synergies for each group.

activity during the late swing phase ($\sim 80\%$ of the step cycle; $P < 0.001$) (Fig. 7). The similarity of motor primitives between groups was lower than 0.8 for synergies 3 and 4 (Table 3). No merging of synergies was found in response to the artificial knee constraint (Table A1).

Modular Organization between Artificial and Chronic Constraint

Comparing the artificial and chronic knee constraint groups did not result in statistical differences in individual muscle contributions (i.e., motor modules) (Figs. 6 and 7). The similarity of motor modules between groups was lower than 0.8 for synergies 1, 2 and 4 (Table 3). Different patterns of the motor primitives between groups were found for synergy 1 and synergy 2 (Figs. 6 and 7). For synergy 1 (acceptance), the motor primitive of the chronic group shows a higher activity during the late stance phase ($\sim 40\%$ of the step cycle; $P < 0.001$) (Fig. 7). For synergy 2 (propulsion), the motor primitive of the chronic group shows a higher activity during the late swing phase ($\sim 80\%$ of step cycle; $P < 0.001$) (Fig. 7). The similarity of motor primitives between groups was lower than 0.8 for synergies 1 and 4 (Table 3).

Figure 5. The average and individual data for motor modules and motor primitives of chronic constraint based on the tVAF > 90% criterion. The motor primitives are plotted as a function of normalized step time (0, heel strike). The vertical dotted lines indicate the end of the stance phase. a.u., Arbitrary units; BF, biceps femoris; GMAX, gluteus maximus; GMED, gluteus medius; LG, lateral gastrocnemius; MG, medial gastrocnemius; RF, rectus femoris; SOL, soleus; ST, semitendinosus; TA, tibialis anterior; tVAF, total variance accounted for; VM, vastus medialis; VL, vastus lateralis.

A reorganization of motor modules was observed in the chronic constraint group. *Synergy 1* appeared to be composed of *synergy 1* and *synergy 2* of the control group, and also *synergy 2* appeared to be composed of *synergy 1* and *synergy 2* of the control group (Fig. 8 and Table A1). The frequency of appearance of merged synergies for *synergy 1* and the frequency of appearance of merged synergies for *synergy 2* were statistically different between groups (chronic 63% vs. artificial 0%, $P = 0.003$; chronic 50% vs. artificial 0%, $P = 0.020$, respectively) (Fig. 8 and Table A1). The aforementioned results indicate that a chronic constraint resulted in a modular reorganization characterized by merging of acceptance with propulsion synergies.

DISCUSSION

The aims of the present study were to assess short-term (immediate to a few minutes following application) and long-term (years) effects of a constraint of the knee ROM on the modular organization of gait. The main results of our study are as follows: 1) similar distribution of synergies were found between groups. 2) The tVAF and modular organization of gait was not affected by the artificial knee constraint (short-term), but the timing of motor primitives of the acceptance, propulsion and swing synergies was affected. 3) For the chronic constraint, a higher tVAF for one and two synergies, and significant differences in the motor primitives for acceptance and propulsion synergies were observed. Furthermore, evidence of merged synergies in the chronic constrained

group was found. These results support our hypothesis that the modular organization of gait will not be affected in the short-term, but changes following a long-term constraint of the knee joint ROM. To the authors' current knowledge, this study is the first to report the effects of artificial and chronic knee joint constraints on the modular organization of gait.

Constraining the knee joint in the control group did not result in changes of modular organization of gait, but in changes of the timing of motor primitives of the acceptance, propulsion and swing synergies. Our results are in agreement with previous results describing adaptations of the central nervous system and EMG patterns of individual muscles in response to short-term (minutes) application of an artificial knee constraint, which increased the amplitude and duration of the knee extensors and plantar flexors during the stance phase of gait (46). Whether this involved also a reorganization of modular control was not investigated. The absence of an effect of the artificial knee constraint on the organization of motor modules may be explained by the limited time of exposure (immediate to minutes), which may not be sufficient to recalibrate the sensorimotor system to a novel walking situation (85, 86). During split-belt walking, it has been reported that the central nervous system requires a longer exposure (~15 min, 900 strides) to change neuromuscular control (85). On the other hand, the activity of plantar flexors immediately following the application of an artificial ankle joint constraint did not differ from that after 1–6 h (53, 54). This suggests that adaptations of the central nervous

Figure 6. The average and individual data for motor modules and motor primitives for each group. In the motor modules, the bars with a thick black line indicate the principal muscle contributions. The motor primitives are plotted as a function of normalized step time (0, heel strike). The vertical dotted lines indicate the end of the stance phase. a.u., Arbitrary units; BF, biceps femoris; GMAX, gluteus maximus; GMED, gluteus medius; LG, lateral gastrocnemius; MG, medial gastrocnemius; RF, rectus femoris; SOL, soleus; ST, semitendinosus; TA, tibialis anterior; VM, vastus medialis; VL, vastus lateralis.

system to changes in the external conditions (i.e., belt speed on treadmill) differ from those applied directly to the body (i.e., joint constraint).

Despite the fact that the artificial knee joint constraint resulted in similar ankle, knee, and hip joint kinematics (Fig. 3), modular reorganization (merging of muscle synergies) was found only in the people with the chronic constraint. Up to now, such merging of synergies has been reported only in people with neurological diseases (i.e., chronic stroke, cerebral palsy) (14, 41, 43, 65, 78). Merging between acceptance and propulsion synergies may be explained by different factors. First, merging of these synergies and, thereby, increasing the extent of coactivation, may serve to improve the stance limb stability and decrease pain sensation during stance (23, 24, 26, 87, 88). Several studies on PWhA have reported a reduced postural stability during bipedal, unipedal, and transition tasks (89–91). In addition, the chronic group experienced pain during walking (Table 1). Joint and muscle pain is also known to affect the neural control of individual muscles (92), and how much a muscle is involved in a motor module (93). Second, the long exposure to the joint constraint may also result in secondary changes in the central nervous system (e.g., muscle hyperreflexia, altered somatosensory feedback, and intersegmental facilitative pathways at spinal level) (27, 28, 94), contributing to abnormal timing of leg extensors during the stance phase (94). Third, the repetitive intra- and intermuscular bleeding and, hence, scar tissue formation is a common manifestation of hemophilia (95). This may change the extent of mechanical connectivity between neighboring, synergistic muscles and, thereby, their

intermuscular coordination during locomotion (96). Future studies are needed to investigate if this also results in modular reorganization.

In the chronic constraint group, we also observed a change in the timing of the motor primitives, specifically, a prolonged activity in the stance phase (increase activity ~40% of the step cycle) of the acceptance synergy. Such prolonged activity has also been reported in children and adults people with cerebral palsy and people with chronic stroke (41, 75, 97). Although joint kinetics was not assessed, it is likely that also in the present study a similar increased mechanical demand for the knee extensors was present. The longer activity of the acceptance synergy may serve to compensate for the reduced ability to absorb the impact of foot placement due by the reduced knee extension (50), and increase the stability of the stance limb during propulsion (98–100).

The finding that modular reorganization occurred only after long-term exposure to a joint constraint may be explained by secondary changes in musculoskeletal and the central nervous systems. It has been shown that neuromuscular adaptations in response to a joint constraint do not necessarily occur acutely (101). The long-term exposure to a joint constraint causes secondary changes in the musculoskeletal system (e.g., joint capsule, muscle and connective properties) (6, 20, 102, 103), and secondary changes in the central nervous system (27, 28).

Finally, the differences observed between acute and chronic knee constraint may not only be attributed to the knee joint restriction. PWhA are commonly affected by arthropathy at both the knee and ankle joints (104). Also, in

Figure 7. Comparison of motor modules and motor primitives between groups. The order of synergies displayed in each row (synergy 1, acceptance; synergy 2, propulsion; synergy 3, swing; and synergy 4, deceleration) are the same as shown in Fig. 6. The motor modules are shown as median and interquartile range. Motor primitives are plotted as a function of normalized step time (0, heel strike) and expressed as means with 95% confidence intervals. The time series for motor primitives were analyzed with Statistical Parametric Mapping. The vertical dotted line indicates the end of the stance phase. a.u., Arbitrary units; BF, biceps femoris; GMAX, gluteus maximus; GMED, gluteus medius; LG, lateral gastrocnemius; MG, medial gastrocnemius; RF, rectus femoris; SOL, soleus; ST, semitendinosus; TA, tibialis anterior; VM, vastus medialis; VL, vastus lateralis. ** $P < 0.001$.

severe knee osteoarthritis, not only the kinematics of the knee during gait are affected, but also that of the hip and ankle joints (105). In our data, PWAH showed similar joint damage at the knee and ankle joints (Table 2). However, the extent of joint movement during gait was lower at the knee level than ankle (6.3° vs. 11.6° , respectively), in proportion to the normal ROM reported during gait at slow speed (1.0 m/s) (24, 61).

Limitations

This study has some limitations. First, joint kinetics were not assessed. Therefore, it is unclear if and how the mechanical demands were affected by the acute knee joint constraint and if these were different between the CG with the acute constraint

and people with chronic knee constraint. Second, it was not possible to recruit people with chronic constraint in only the knee joint. Therefore, it is not possible to attribute the altered neuromuscular control in people with chronic knee constraint exclusively to musculoskeletal changes in the knee joint. Third, the time of artificial knee constraint exposure used in our study may not have been sufficient for adaptations in the central nervous system. Future studies are needed to reveal if more prolonged exposure to an artificial joint constraint changes neuromuscular control of gait. Finally, because we only included men in our study, extrapolation to women should be done with caution. Because the arthropathy predominantly affects males, effects of a chronic knee constraint in women cannot be investigated in people with hemophilia.

Table 3. Similarity of motor modules and motor primitives between groups

Synergy	Motor Modules		Motor Primitives	
	Control vs. Artificial	Artificial vs. Chronic	Control vs. Artificial	Artificial vs. Chronic
(S1) Acceptance	0.96 [0.92 0.99]	0.79 [0.66 0.91]	0.92 [0.87 0.97]	0.74 [0.54 0.95]
(S2) Propulsion	0.92 [0.81 0.99]	0.76 [0.59 0.93]	0.86 [0.76 0.96]	0.82 [0.73 0.89]
(S3) Swing	0.81 [0.69 0.94]	0.88 [0.79 0.97]	0.75 [0.61 0.90]	0.81 [0.70 0.92]
(S4) Deceleration	0.86 [0.77 0.94]	0.71 [0.59 0.84]	0.77 [0.64 0.90]	0.50 [0.33 0.67]

Means and 95% confidence interval of cosine similarity. Similarities < 0.8 are in bold. S, synergy.

Figure 8. A reorganization of motor modules was observed in the chronic constraint group. **A:** synergy 1 of chronic constraint group (gray) appeared to be composed of synergy 1 (acceptance) and synergy 2 (propulsion) of the control group (black). **B:** synergy 2 of chronic constraint group (gray) appeared to be composed of synergy 1 and synergy 2 of the control group (black). For both synergies, the two criteria to determine if one synergy may be reconstructed from a pair of merged synergies were achieved. First, the coefficient of the reconstruction (CR) of the two pairs of motor modules was above of 0.3. Second, a similarity (SIM) between the reconstructed and the original motor module was above 0.8. For both synergies, the frequency of appearance was significantly different between chronic and artificial groups (Table A1). BF, biceps femoris; GMAX, gluteus maximus; GMED, gluteus medius; LG, lateral gastrocnemius; M, motor module; MG, medial gastrocnemius; RF, rectus femoris; SOL, soleus; ST, semitendinosus; TA, tibialis anterior; VM, vastus medialis; VL, vastus lateralis.

Conclusions

We conclude that in the short-term a knee constraint does not affect the modular organization of gait, but in the long-term a knee constraint results in modular reorganization. These results indicate that merging of muscle synergies may also occur when changes in the mechanics of the musculoskeletal system is the primary cause of the motor impairment. The present results offer a new perspective on the adaptations of the central nervous system to joint restriction. These new insights may contribute to improving those medical and physical rehabilitation treatments that aim to regain the impaired walking, especially in those diseases where the motor impairment is accompanied with chronic joint constraints.

APPENDIX

The motor modules and motor primitives of the control group (with and without artificial constraint) based on the tVAF > 90 % criterion is shown in Figs. A1 and A2. For those participants with three and four synergies, a similar structure of motor modules was observed. The results of the merged synergies analysis are shown in Table A1. Only in the chronic knee joint constraint, a reorganization of synergy 1 (acceptance) and synergy 2 (propulsion) were observed. The motor modules of synergy 1 appeared to be composed of the motor modules of synergy 1 and synergy 2, also the motor modules of synergy 2 appeared to be composed of the motor modules of synergy 2 and synergy 1.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

C.C.-M., S.P.-A., M.C., and H.M. conceived and designed research; C.C.-M., S.P.-A., and M.C. performed experiments; C.C.-M. and H.M. analyzed data; C.C.-M., S.P.-A., and H.M. interpreted results of experiments; C.C.-M. and H.M. prepared figures; C.C.-M., S.P.-A., M.C., and H.M. drafted manuscript; C.C.-M., S.P.-A., M.C., and H.M. edited and revised manuscript; C.C.-M., S.P.-A., M.C., and H.M. approved final version of manuscript.

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Figure A1. The average and individual data for motor modules and motor primitives of control group based on the tVAF >90% criterion. The motor primitives are plotted as a function of normalized step time (0, heel strike). The vertical dotted lines indicate the end of the stance phase. a.u., Arbitrary units; BF, biceps femoris; GMAX, gluteus maximus; GMED, gluteus medius; LG, lateral gastrocnemius; MG, medial gastrocnemius; RF, rectus femoris; SOL, soleus; ST, semitendinosus; TA, tibialis anterior; tVAF, total variance accounted for; VM, vastus medialis; VL, vastus lateralis.

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Figure A2. The average and individual data for motor modules and motor primitives of artificial constraint based on the tVAF > 90% criterion. The motor primitives are plotted as a function of normalized step time (0, heel strike). The vertical dotted lines indicate the end of the stance phase. a.u., Arbitrary units; BF, biceps femoris; GMAX, gluteus maximus; GMED, gluteus medius; LG, lateral gastrocnemius; MG, medial gastrocnemius; RF, rectus femoris; SOL, soleus; ST, semitendinosus; TA, tibialis anterior; tVAF, total variance accounted for; VM, vastus medialis; VL, vastus lateralis.

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Table A1. All results of merged synergies analysis

	M1 + M2			M1 + M3			M1 + M4			M2 + M3			M2 + M4			M3 + M4		
	CR	Sim	%	CR	Sim	%	CR	Sim	%	CR	Sim	%	CR	Sim	%	CR	Sim	%
Chronic																		
M1	0.46	0.91	63*	0.06	0.78	0	0.23	0.82	13	0.50	0.70	13	0.47	0.80	25	0.13	0.67	0
M2	0.45	0.90	50*	0.09	0.67	0	0.27	0.69	13	0.39	0.78	25	0.45	0.85	38	0.13	0.61	0
M3	0.19	0.57	0	0.09	0.89	13	0.17	0.58	13	0.14	0.89	25	0.16	0.61	0	0.12	0.89	0
M4	0.25	0.71	0	0.35	0.74	13	0.22	0.78	13	0.29	0.67	0	0.18	0.78	25	0.30	0.83	0
Artificial																		
M1	0.03	0.96	0	0.10	0.96	0	0.04	0.96	9	0.18	0.55	0	0.08	0.70	0	0.18	0.70	0
M2	0.01	0.97	0	0.11	0.39	0	0.16	0.42	0	0.09	0.94	0	0.11	0.96	0	0.06	0.40	0
M3	0.19	0.48	0	0.07	0.83	0	0.09	0.54	0	0.14	0.88	0	0.16	0.58	0	0.11	0.84	0
M4	0.11	0.55	9	0.22	0.67	0	0.08	0.87	0	0.11	0.63	0	0.07	0.87	0	0.19	0.89	18

To assess whether one synergy may be reconstructed from a pair of merged synergies of the control group, two criteria were adopted: 1) coefficient of the reconstruction (CR) > 0.3 and 2) similarity (SIM) > 0.8. The merged results with two criteria met are printed in bold. If two criteria were met, the frequency of appearance between groups were tested (chronic vs. artificial). *Statistical significance ($P < 0.05$). M, motor module.

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