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Identification of neural and non-neural contributors to joint stiffness in upper motor neuron disease

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CHAPTER 6

Estimating the effects of botulinum toxin A therapy post-stroke: evidence for reduction of background muscle activation

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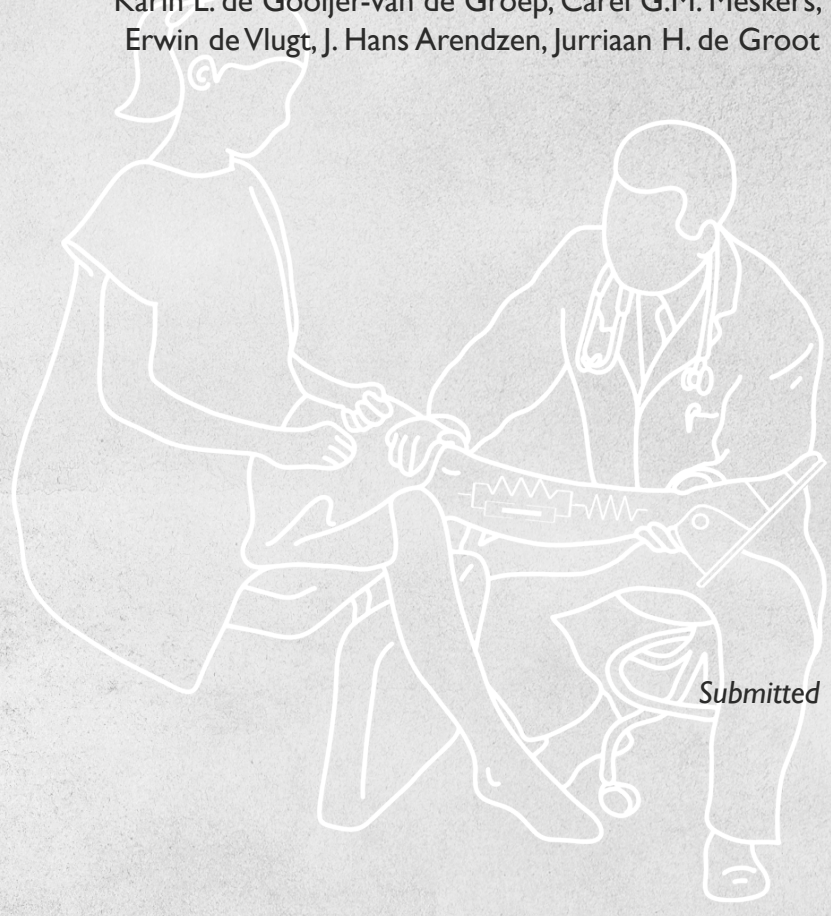

Submitted

Abstract

Clinical application of botulinum toxin (BoNT) will benefit from a better understanding and quantitative assessment of the underlying neural and non-neural properties of spasticity related joint stiffness increase and their response to BoNT treatment.

The effect of BoNT on underlying ankle joint stiffness properties was estimated using an EMG-driven ankle model in 15 chronic stroke patients who underwent instrumented passive ankle rotations before and after BoNT injections in the calf muscles.

Dorsal range of motion (dRoM) increased after BoNT. Baseline values of and changes in non-neural joint stiffness, triceps surae slack length and recorded soleus EMG rest activation were associated with changes in dRoM and neutral rest angle. No associations were found for reflex activity.

Observed and estimated responses are in line with an effect of BoNT on background muscle activity. The instrumented approach may assist to identify those patients who will likely benefit from BoNT therapy.

Introduction

Botulinum neurotoxin-A (BoNT) is recommended for treatment of post-stroke spasticity[1-8]. BoNT blocks the release of acetylcholine from the nerve terminal, thereby uncoupling the excitation-contraction mechanism and thus reducing muscle (hyper)activity[3]. This results in a reduction of muscle tone and joint stiffness and a subsequent decrease in resistance to passive joint manipulation as clinically assessed by the (modified) Ashworth Score (mAS)[9]. Clinical application of BoNT for spasticity treatment post-stroke may benefit from a better understanding and quantitative assessment of the underlying properties of spasticity related joint stiffness increase and their response to BoNT treatment. Is spasticity defined by a velocity dependent resistance to passive stretch according to the concept of Lance[10] or is it better explained by an increased muscle activation at rest, i.e. background muscle activation, according to the Burne concept[11]?

Evaluation of BoNT treatment is hampered by e.g. differences in dosing regimens, injection sites, heterogeneity in patients, concurrent treatment, outcomes selected, and the poor methodological quality of insufficiently powered, placebo-controlled trials[12-15]. Furthermore, assessment of the effects of BoNT by clinical semi-quantitative scaling like the mAS is problematic as the mAS has a questionable reliability and a low responsiveness to change[9;16]. We recognize an urgent need for alternative, high-resolution assessment that enables us to understand the concept of spasticity and its response to BoNT in upper motor neuron diseases like stroke[14].

Neuromuscular modeling in combination with joint manipulation paradigms using high precision robotics is promising in terms of reliable and valid identification of the underlying contributors of joint stiffness[17-23]. An instrumented electromyography (EMG)-driven modeling approach would allow for quantitative estimation of the neural reflexive and non-neural tissue properties to joint stiffness, i.e. muscle shortening and muscle stiffening[18;19;24]. This method should potentially be able to identify (stroke) patient characteristics at baseline prior to BoNT treatment and assess the effects of BoNT post treatment.

The aim of the present study was therefore to estimate the effect of BoNT on underlying neural reflexive and non-neural tissue properties of increased ankle joint stiffness in chronic stroke patients.

Methods

Subjects

Fifteen stroke patients participated in the study. Patients were included following ischemic or hemorrhagic stroke over 6 months at inclusion and were clinically referred for BoNT treatment (Botox, Allergan, Inc., Irvine, CA) in the soleus (SOL), gastrocnemius medialis (GM), gastrocnemius lateralis (GL) and/or tibialis posterior (TP). Patients were recruited from the outpatient clinic of the Leiden University Medical Center between January 2013 and June 2015. The indication for treatment was based on clinical arguments and independent of the present study. Exclusion criteria comprised (other) concomitant neurological and/or orthopedic disorders, treatment within the last 4 months that could interfere with a stable ankle joint stiffness, surgery of leg/foot within last 12 months and inability to participate in the experiment, either physically (i.e. inability to be mounted in the experimental set-up), cognitively (i.e. unable to understand test instructions or to give informed consent). Participants were measured prior to BoNT treatment (baseline T0) and 6-8 weeks (T1) and 12-16 weeks (T2) after BoNT treatment. Written informed consent was obtained from the participants. The study was approved by the medical ethics committee of the Leiden University Medical Center.

Instrumentation

Subjects were seated with their foot fixated onto an electrically powered single axis footplate (Achilles, MOOG FCS Inc., Nieuw-Vennep) with their knee positioned at 70 degrees of flexion (Figure 6.1). The axis of rotation of the ankle and footplate were aligned by visually minimizing knee translation in the sagittal plane during manual rotation of the footplate. For safety reasons and to avoid pain, the maximum tolerated dorsal- and plantar flexion of the ankle were restricted by hard- and software stops of the manipulator and determined by manually moving the ankle through its RoM by a trained operator before starting the measurements. The footplate was aligned visually at 25° plantar flexion with respect to the line connecting the head of the fibula and the lateral malleolus. During robotic movements, EMG, torque and angle were simultaneously recorded.

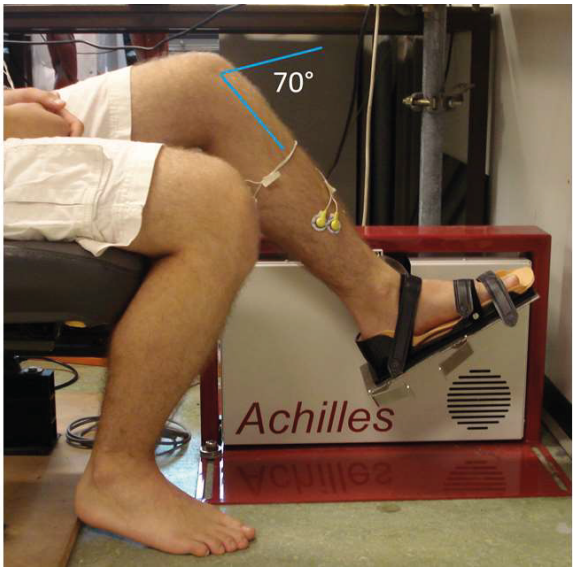

Figure 6.1: *Measurement set-up.*

EMG assessment

Muscle activation of the tibialis anterior (TA) and triceps surae (TS) was recorded using surface EMG (Porti, TMSi B.V. Oldenzaal, The Netherlands) according to the SENIAM guidelines[25] (supplementary table). EMG signals were sampled at 1000 Hz, offline high pass filtered (20Hz, 3th-order Butterworth), rectified and low pass filtered (20 Hz zero overshoot filter). Rest EMG, i.e. the minimal EMG determined for each muscle by applying a moving window of 8 ms, was subtracted from the total EMG because assumed not to contribute to ankle torque. Rest EMG of the TA, SOL, GL and GM were also used as outcome measure. Torque and ankle angle were sampled at 1024 Hz and low pass filtered (20 Hz zero overshoot filter) and resampled to 1000 Hz.

Range of motion and ramp and hold measurements

Maximum dorsal and plantar flexion angles were assessed by gradually increasing flexion torque from 0 to 15 Nm in dorsal flexion direction and subsequently to -7.5 Nm in plantar flexion direction and back to zero torque in dorsal flexion direction. From this measurement the

following outcome measures were determined: 1) dorsal range of motion (dRoM), i.e. the maximum ankle angle at 15 Nm or, in case the patients did not tolerate 15 Nm, at the highest common torque measured over all three measurements (T0,T1,T2) and 2) the neutral angle (NA) which is the ankle angle at zero torque, i.e. the ankle at rest. The zero torque angle is ankle flexion direction dependent (hysteresis) and the NA was defined as the average 0-Nm crossing angle determined for the plantar flexion and dorsiflexion movement.

The instrumented RoM was used as boundary for the subsequent ramp-and-hold (RaH) trials. During the RaH measurements the ankle was rotated at two different angular velocities (15 and 100 deg/sec), starting in maximal plantar flexion and ending in maximum dorsiflexion angle (first ramp). After an approximate 10 seconds hold phase, the ankle was moved back to the maximal plantar flexion angle (second ramp). The subjects were instructed to relax during the RaH trials. Each velocity was repeated, which resulted in four RaH trials and one RoM trial per measurement.

Neuromuscular modeling

A validated neuromuscular model was used to predict ankle torque from measured ankle angle and EMG and subsequently estimating neural reflexive and non-neural tissue properties of ankle joint stiffness[26]. The model encompassed in total 15 parameters, i.e. of both TS and TA: the stiffness coefficient, muscle slack length, optimal muscle length, EMG weighting factors (three for TS and one for TA) and further: mass of the foot and footplate, activation cut-off frequency, relative damping and parameters related to tissue relaxation, i.e. tissue relaxation time constant and tissue relaxation factor. The present study focused on the following properties: 1) The estimated non-neural muscle slack length of TS and TA; 2) The estimated non-neural stiffness coefficient of TS and TA and which is an indicator of muscle stiffness; 3) the estimated non-neural peripheral tissue stiffness, a function of joint angle determined by the combination of slack length and stiffness coefficient of both TA and TS muscles. For comparison purposes, the peripheral tissue stiffness was assessed at an ankle angle of 0 degrees over all subjects and trials, i.e. foot (plate) perpendicular to the lower leg; 4) the estimated neural reflexive torque of TS and TA. The estimated reflexive torque was calculated by the root

mean square of the involuntary active muscle torque during the observed trial. Slack muscle length, stiffness coefficient and peripheral tissue stiffness were determined at an ankle rotation speed of 15 deg/sec; the reflexive torque was determined at an ankle rotation speed of 100 deg/sec. Outcome parameters were averaged over two trials (repetitions), unless trials were excluded due to low variance accounted for (VAF<99%).

Statistical analysis

A linear mixed model was used to compare outcome measures between the different measurement time points. Secondly, a linear regression analysis was performed addressing the association between changes in outcome measures, i.e. estimated neural reflexive and non-neural tissue properties and measured EMG rest activity, as independent variables and respectively changes in measured dRoM and NA as dependent variables. Thirdly, the same procedure was repeated for the baseline values (T0) of the outcome measures, i.e. estimated neural reflexive and non-neural tissue properties and EMG rest activity, as independent variables and changes in measured dRoM and NA as dependent variables to address the predictive value of aforementioned baseline parameters for the outcome after BoNT treatment. Significance level was set at 0.05. Statistical analysis was performed using IBM SPSS statistics 22.

Results

One patient quitted the study after the first measurement due to various reasons leaving 14 stroke patients for further analysis, encompassing a total of 42 measurements. Thus, 42 RoM trials were available, and due to lack of valid RaH data in 2 patients, 144 RaH trials. In 2 patients over 2 trials, the maximum dorsal flexion torque of 15 Nm exceeded the patient's tolerance and thus hardware restricted dRoM was determined at a lower torque level. Of the 144 RaH trials, 3 trials were excluded due to low VAF values (<99%). 9 out of 14 patients were injected in the soleus muscle. Patient characteristics are shown in Table 6.1.

Table 6.1: *Patient characteristics.*

Patient	Age	Gender	Affected side	Years post-stroke	Stroke type	Injected muscles (dose)[+]	Ashworth score	Splint[**]
1	55	M	R	2.6	Ischemic	SOL (100), GM (50), GL (50)	4	1
2	46	M	L	3.0	Ischemic	SOL (50), GM (50)	4	0
3	46	F	R	21.4	Ischemic	SOL (50), TP (100), TA (100)	3	1
4	52	F	L	2.0	Hemorrhagic	GM (25), GL (25), TP (50)	2	1
5	61	M	R	10.4	Ischemic	SOL (100)	3	0
6	41	M	R	16.1	Hemorrhagic	SOL (100)	3	0
7	64	M	R	5.6	Ischemic	SOL (50)	2	1
8	65	M	L	2.5	Hemorrhagic	SOL (100)	2	1
9	68	M	R	3.1	Ischemic	TP (50)	1	1
10	48	F	L	1.6	Ischemic	SOL (100), GM (50), GL (50)	1	1
11	58	M	R	-	-	SOL (100), GM (50), GL (50)	3	1
12	55	F	R	-	Hemorrhagic	GM (100), TP (50)	3	0
13	54	M	L	2.8	Ischemic	GM (50), GL (50)	1	1
14	69	F	L	~4	Hemorrhagic	GM (50), GL (50)	1	0

[+]*SOL: soleus, GM: gastrocnemius medialis, GL: gastrocnemius lateralis, TP: tibialis posterior, TA: tibialis anterior*

*** unspecified, 1=yes; 0=no.*

Measured and estimated parameters before and after BoNT injection

Measured and estimated parameters before and after BoNT injections are presented in Table 6.2. The dRoM improved on average with 4.1 (SD 6.7) degrees from T0 to T1 and the NA shifted from plantar flexion to the (neutral) anatomical ankle angle by 4.6 (SD 7.7) degrees. Significant changes between T0 and either T1 or T2 after BoNT injection were only observed for dRoM and NA (Table 6.2). Estimated neural reflexive and non-neural tissue properties showed no significant change after BoNT injections.

Table 6.2: *Overview of outcome measures before (T0) and after (6-8 weeks: T1 and 12-16 weeks: T2) BoNT injections. P-values for comparison between measurement moments.*

	Measurement moment					
Outcome measure	T0	T1	T2	N	**T0 vs T1 P-value**	**T0 vs T2 P-value**
Dorsal RoM (deg)	5.5 (8.6)	9.5 (5.5)	8.6 (6.7)	14	**.032**	.093
Neutral angle (deg)	-30.1 (10.5)	-25.5 (6.7)	-23.6 (5.4)*	14	.052	**.010***
Peripheral tissue stiffness (Nm/rad)	245 (351)	132 (61)	125 (73)	12	.39	.21
Slack length TS (mm)	22.8 (2.6)	23.2 (2.6)	23.6 (3.8)	12	.60	.38
Slack length TA (mm)	63.6 (11)	60.2 (17)	58.5 (12)	12	.39	.10
Stiffness coefficient TS (1/m)	346 (43)	343 (48)	360 (98)	12	.79	.58
Stiffness coefficient TA (1/m)	186 (54)	192 (62)	185 (42)	12	.57	.96
Reflexive torque TS (Nm)	2.9 (2.1)	3.0 (2.2)	2.7 (2.0)	12	.89	.49
Reflexive torque TA (Nm)	.25 (.20)	.48 (.37)	.31 (.48)	12	.052	.69
Rest EMG SOL (μV)	2.3 (1.6)	2.3 (1.8)	2.3 (1.7)	12	.97	.99
Rest EMG GM (μV)	1.3 (.50)	1.3 (.30)	1.6 (0.92)	12	.50	.21
Rest EMG GL (μV)	1.2 (.24)	1.2 (.37)	1.3 (0.25)	12	.90	.69
Rest EMG TA (μV)	1.5 (.87)	1.5 (.99)	1.5 (.60)	12	.75	.98

**based on 13 stroke patients*

Associations between baseline values and changes in measured and estimated parameters with changes in dRoM and NA

A decrease in estimated peripheral tissue stiffness, an increase in estimated TS slack length and a decrease in measured rest EMG of soleus significantly associated with an increase of measured dRoM and/or a shift in NA towards anatomical ankle angle after BoNT, while no significant changes of reflex torques were observed (Table 6.3). A high estimated peripheral tissue stiffness and a small estimated TS slack length appeared to be predictive for a respectively increase in measured dRoM and NA shift towards anatomical ankle angle. Again, no significant associations between estimated baseline reflex torque and measured dROM and NA were observed (Table 6.4).

Table 6.3: *Association between changes estimated neural reflexive and non-neural tissue properties and measured EMG rest activity and changes in measured dorsal RoM and neutral angle before (T0) and 6-8 weeks after BoNT injections (T1). Unstandardized effects and 95% confidence interval.*

	Dorsal RoM			**Neutral angle**		
	Unstandardized			**Unstandardized**		
	β	**95% CI**	**P-value**	**β**	**95% CI**	**P-value**
Peripheral tissue stiffness (Nm/rad)	-.014	-.023; -.005	**.006**	-.016	-.027; -.005	**.009**
Slack length TS (mm)	1.8	.54; 3.0	**.010**	2.1	.69; 3.5	**.008**
Slack length TA (mm)	-.15	-.60; .31	.49	-.40	-.85; .059	.081
Stiffness coefficient TS (1/m)	-.040	-.19; .11	.55	-.002	-.175; .171	.98
Stiffness coefficient TA (1/m)	-.005	-.12; .11	.93	-.054	-.18; 0.69	.35
Reflexive torque TS (Nm)	.35	-4.1; 4.8	.86	2.9	-1.8; 7.6	.20
Reflexive torque TA (Nm)	.56	-11.5; 12.6	.92	-1.1	-15.0; 12.8	.87
Rest EMG SOL (μV)	-1.5	-3.07;.005	.051	-1.9	-3.6; -.2	**.032**
Rest EMG GM (μV)	-.51	-14.5;13.5	.94	1.2	-15.0; 17.3	.88
Rest EMG GL (μV)	3.7	-11.3;18.8	.59	8.1	-8.6; 24.8	.31
Rest EMG TA (μV)	-10.1	-22.1; 1.9	.090	-10.2	-24.6; 4.1	.14

Table 6.4: *Association between estimated neural and non-neural tisseu properties and measured EMG rest activity at baseline (T0) and changes in measured dorsal RoM and neutral angle between T0 and 6-8 weeks after BoNT injections (T1). Unstandardized effects and 95% confidence interval.*

	Dorsal RoM			**Neutral angle**		
	Unstandardized			**Unstandardized**		
	β	**95% CI**	**P-value**	**β**	**95% CI**	**P-value**
Peripheral tissue stiffness (Nm/rad)	.013	.004; .022	**.010**	.015	.004; .025	**.010**
Slack length TS (mm)	-1.5	-2.9; -0.084	**.040**	-1.6	-3.3; .070	.058
Slack length TA (mm)	.18	-.22; .58	.35	.016	-.47; .50	.94
Stiffness coefficient TS (1/m)	.056	-.040; .15	.23	.071	-.072; .21	.30
Stiffness coefficient TA (1/m)	.008	-.099; .12	.87	-.049	-.17; .070	.38
Reflexive torque TS (Nm)	-.45	-2.6; 1.7	.66	-.94	-3.4; 1.5	.41
Reflexive torque TA (Nm)	-7.7	-30.2; 14.8	.46	2.5	-24.1; 29.1	.84
Rest EMG SOL (μV)	1.6	-.90; 4.1	.18	-2.0	-.90; 4.8	.16
Rest EMG GM (μV)	-2.2	-11.1; 6.8	.60	-1.8	-12.2; 8.6	.71
Rest EMG GL (μV)	-8.4	-26.6; 9.9	.33	-10.3	-31.2; 10.7	.30
Rest EMG TA (μV)	.042	-5.1; 5.2	.99	-.36	-6.3; 6.0	.90

Discussion

Significant improvements in measured dRoM and NA that coincided with an estimated lengthening of the musculo-tendon complex of the TS were observed using an instrumented EMG-driven model approach to evaluate the effect of BoNT injections in the calf muscles in chronic stroke patients on neural reflexive and non-neural tissue properties of ankle joint stiffness. Baseline values and changes of the estimated non-neural tissue parameters peripheral tissue stiffness and the slack length of triceps surae were associated with improvements of dRoM and the NA. The estimated neural and velocity dependent parameter reflexive torque did not associate.

Applicability of the instrumented EMG-driven model approach

With VAF values over 99% and only a few trials being discarded because of low VAF values (2%) the developed model approach seems well applicable to the present clinical case. Previous research showed that the internal model validity was good, test-retest reliability fair to good and that the method has clinical potential[26].

Measured and estimated parameters before and after BoNT injection

The effect of BoNT treatment was reflected by the increase in dRoM and the shift of NA towards the anatomical ankle angle. This is assumed to originate from an increased length of the musculo-tendon complex of the TS muscle during rest conditions and may thus be regarded a main effect of BoNT treatment[27;28]. The other parameters did not show changes as a function of BoNT treatment, which might be due to a large variability within the included cohort and the current lack of means to discriminate potential responders from non-responders in combination with a small sample size. The estimated observations cannot be compared to a gold standard that measures the effect of BoNT treatment because yet lacking. Clinically, we have to do with scales like the mAS with its acknowledged problems of sensitivity and reliability[16]. Significant effects of BoNT on the mAS may only be found in large study samples[29], averaging the large variation in e.g. doses, injection sites and techniques into account[14]. Changes in dRoM and shift of NA to the anatomical ankle angle may be an alternative clinical outcome parameter for

treatment evaluation. Further studies on validity and sensitivity to e.g. variations in dose, are needed in order to fully judge the merits of RoM measurements.

Associations between baseline values and changes in measured and estimated parameters

Changes in measured ankle dRoM and NA were associated with baseline values and changes of the estimated non-neural tissue parameters slack length of TS and peripheral tissue stiffness and estimated EMG rest activity of soleus. Non-neural tissue parameters are assumed to relate to changes in length of musculo-tendon structures thus explaining dRoM and NA more than the neural reflexive torque. Relating the changes in ankle dRoM and NA to the estimated neural reflexive and non-neural tissue properties to joint stiffness and measured rest EMG at baseline provided for the opportunity to predict the effects of BoNT in a heterogeneous group of responders and non-responders to BoNT.

BoNT may decrease background muscle activation: evidence for the "Burne" over the "Lance" concept of spasticity.

Improvement of dRoM and NA following BoNT treatment can potentially be explained by a decrease in background activation of the TS muscle. BoNT results in the relaxation (i.e. lengthening) of the contractile element and thus a shortening and relaxation of the serial elastic length (Figure 6.2). This concept is substantiated by an increase in estimated slack length. Slack length is assumed to reflect the muscle fiber slack length in combination with the muscle fiber pennation angle.

Additional evidence for background activation reduction is provided by the decrease in measured EMG rest activity of soleus, which is associated with the shift of the NA towards anatomical ankle angle. Also the absence of associations of BoNT treatment with reflexive torque suggests the evidence for background muscle activation reduction. The background muscle activation was not accounted for in the model and may therefore also affect the non-neural tissue parameters.

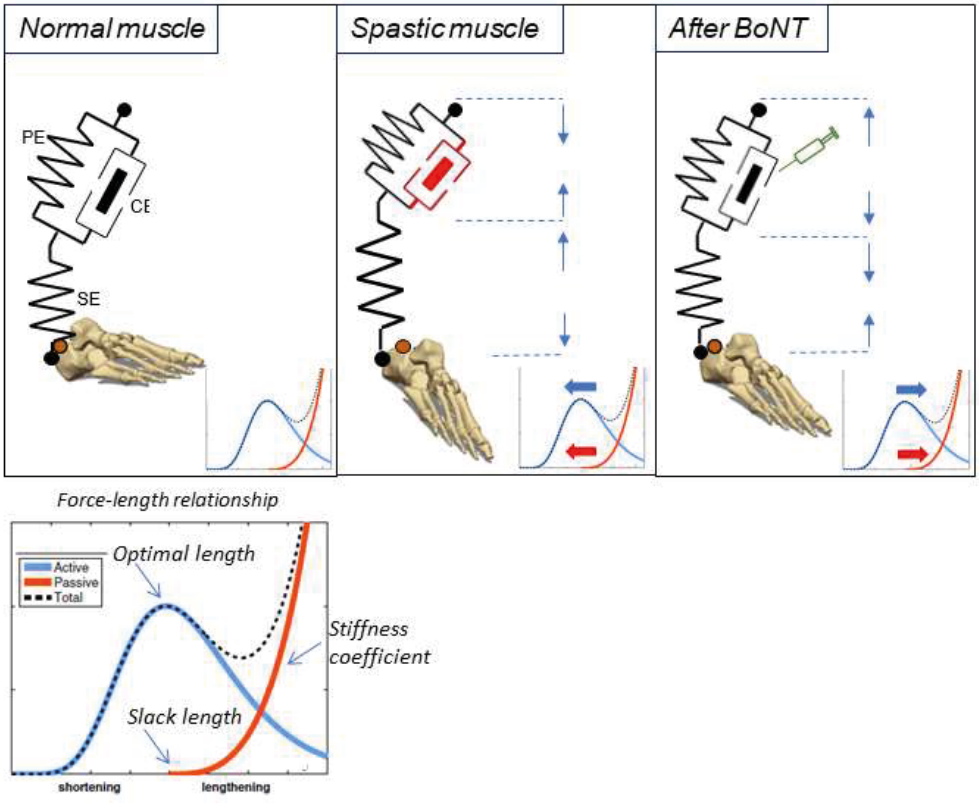

Figure 6.2: *Schematic illustration of a Hill type triceps surae and Achilles tendon (top) with the contractile element (CE), the parallel element (PE) and the serial elastic component (SE) and the muscle force-length relationship (bottom). Left: in the "normal" relaxed condition the foot is in a neutral position, with the SE and PE in 'slack length'. Middle: In the spastic/contractile condition, the CE is activated and the PE is shortened, while the SE length is increased, the foot rotated in equines position. The passive force-length relation and the working point (state) at the active force-length relationships are shifted to the left, meaning that in the neutral position of the ankle, the joint stiffness is increased, resulting in decreased dorsal RoM. Right: BoNT results in the relaxation (i.e. lengthening) of the CE and thus a shortening and relaxation of the SE length. The triceps surae muscle slack length changes/normalizes: the passive force-length relationship and the contractile state of the active force-length relationship are shifted to the right, i.e. towards "normal".*

Note: The shortened triceps surae in the contractile state is a combination of a shortening of the CE in combination with an increase of the muscle fiber pennation angle.

The combination of aforementioned findings is in favor of the "Burne" hypothesis of increased background activation as the underlying pathophysiological mechanism for spasticity[11] and the observed clinical effects of BoNT treatment. The potential mechanism of BoNT treatment may be to reduce background muscle activity so that the contractile muscle tissue relaxes and dorsal RoM increases (Figure 6.2).

All noticed effects are observed under resting task conditions. The higher contractile state of the resting muscle may have an indirect effect on the (increased) sensitivity of muscle spindles and Golgi tendon organs and the spinal reflex thresholds related to the "Lance" hypothesis as neural and non-neural tissue components are in tight interaction[30].

Strength, limitations and clinical implications

This study shows the applicability and merits of high resolution clinimetrics by using a robotic manipulator in combination with an EMG-driven neuromuscular model approach that allows for estimation of underlying properties to joint stiffness, in order to understand the pathophysiological effects of BoNT treatment in stroke patients. Patients were instructed to relaxed and do therefore not require the ability to selectively and voluntary activate muscles, making the method applicable in clinical practice. Addressing effects of dose, injection site and technique and the potential to discriminate responders from non-responders may be important topics for further study considering the costs and the wide spread use of BoNT treatment[14].

There are a number of limitations to the present study. First, the study sample was small, especially considering the expected underlying heterogeneity in patients, history of treatment, dose, selected muscles, injection site etc. Also, the current choice for outcome stratification based on increase of dRoM and NA shift needs further investigation. As background activity cannot be estimated directly yet from the current models, evidence of influence on this component was circumstantial as is the relation of background activity to slack length. Slack length is assumed to reflect both the contractile state of the muscle fibers and the muscle fiber pennation angle. Pennation angles were not in the model nor were measured separately. Therefore the increase of estimated slack length could be indicative for a decrease in pennation angle[27;28]. Ultrasound recordings can strengthen the assumption of changed pennation angles.

Conclusion

We used an instrumented EMG-driven modeling approach to address the effects of BoNT treatment on underlying neural reflexive and non-neural tissue properties of ankle joint stiffness in chronic stroke patients. Significant improvement of measured dorsal range of motion and neutral angle following BoNT treatment were associated with baseline values and changes of estimated peripheral tissue stiffness, estimated triceps surae slack muscle length and measured EMG rest activity of soleus and can potentially be explained by a decrease in background activation of the triceps surae muscle. The approach may be a promising solution in treatment selection, quantifying the effect of treatment and exploring the pathophysiology of spasticity.

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Appendix 6: Supplementary table

***Table 6A*:** *Electrode placement according to seniam guidelines (www.seniam.org)*

Muscle	**Location**
Tibialis anterior	At 1/3 on the line between the tip of the fibula and the tip of the medial malleolus.
Soleus	At 2/3 of the line between the medial condylis of the femur to the medial malleolus.
Gastrocnemius medialis	On the most prominent bulge of the muscle.
Gastrocnemius lateralis	At 1/3 of the line between the head of the fibula and the heel.