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Influence of number of records on reliability of myotonometric measurements of muscle stiffness at rest and contraction

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Purpose: The aim of this study was to determine an effect of myotonometric records' number on stiffness measurements' reliability in muscles at rest and contraction. *Methods*: Muscle stiffness was measured using Myoton-3 device. Twenty records were taken for: (i) biceps (BB) and triceps brachii (TB) at rest and for BB at 10% of maximal voluntary contraction (MVC) in healthy elderlies (HE) and in Parkinson's disease patients (PD); and (ii) brachioradialis (BR) at rest and at 25, 50 and 80% MVC in healthy young (HY) subjects. Also, in HY group, the 3-records mode was used for BR's measurements at maximal contraction. Each measurement taken with 20-records was classed into five records groups: the whole 20- and the first 15-, 10-, 5- and 3-records. Test-retest reliability for these records groups was analyzed. *Results*: In HE and PD group measurements' reliability was excellent for all groups of records (20–3 records). In HY group, for the five groups of records taken at rest and submaximal levels of contraction (25, 50 and 80% MVC) the measurements reliability: (i) was mostly excellent or rarely average; and (ii) only in one per three 50% MVC conditions was unacceptable, i.e., for the 3-records group. The reliability of 3-records mode measurements at maximal contraction were unacceptable. *Conclusions*: Reliable myotonometric stiffness measurements in muscles at rest and during submaximal contractions can be achieved with less than 20 records (15, 10, 5 records) and even for the most of measurements with 3 records in HY and HE as well as in the PD patients. Myotonometric stiffness measurements with 3-records mode during maximal contraction were not reliable.

Key words: *muscle stiffness, myotonometry, measurements reliability*

1. Introduction

Myotonometry is a an objective, reliable, noninvasive and easy to use technique with potentially wide applications for investigating muscle properties in areas such as scientific research, medicine, sport, and rehabilitation [1]–[4], [7], [9], [10], [18]–[21], [24], [26]. This method is an ideal alternative to other technically complicated non-invasive methods [8] and to invasive biomechanical methods for an assessment of mechanical properties of soft tissues in humans [6], [12] and animals [5]. The muscle stiffness measured by myotonometer is determined as a ratio of low mechanical shock force (transmitted via testing end to skin surface overlying tested muscle and not inducing muscle reflex activity) to depth of the tissue deformation [N/m]. In any case of scientific measurements, from data quality point of view, it is important to know reliability of measurement. The previous studies have indicated moderate to very high or excellent reliability of myotonometirc stiffness measurements for various muscles, either for repeated single record [20] or three [7], [13], five [10], ten [1], [3], [19] and twenty [4], [9], [10], [18], [24] records mode both for relaxed and contracted muscles (at different force levels), in young and old healthy or diseased subjects. According to Safrit [22], higher number of measurement trials in physical education and exercise science is associated with greater reliability. Therefore, in the

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myotonometric stiffness measurements the multiscan 20–records mode was used to perform a reliable measurements [4], [9], [10], [18], [24]. However, this mode takes from 30 seconds to 1 minute to run, which might thus limit its usefulness for assessing muscle properties in some specific experimental conditions. It is especially a case in short lasting protocols (when a single measurement trial lasts from few to several tens of seconds). For example when measurements are taken during muscle contraction, particularly at high force levels, muscle force deviation related to motor control mechanisms [15] might influence the myotonometricic stiffness measurements reliability. Also, other factors might influence the myotonometricic stiffness measurements when using various testing protocols with various number of records. In healthy subjects, the stiffness measurements might be affected by: voluntary muscle tremor, tixotropic properties of muscle fibers, short time changes of stiffness depending on muscle state, the length of tested muscles (as some data might be obtained at different joint angles) that affects afferent input from muscle receptors to motoneurons and antagonistic muscle activity. On the other hand, in old population the muscle stiffness measurements might be affected by muscle atrophy. In clinical conditions, for example in patients with Parkinson's disease, the muscle tendon unit rigidity and resting tremor would be important factors affecting measurements reliability.

In our previos studies [18], [10] we performed 20-records mode measurements of myotonometricic stiffness in: (i) Parkinson's disease patients and healthy elderly subjects in biceps and triceps brachii muscles at rest and at low level of muscle contraction; and (ii) in healthy young subjects in brachioradialis muscle at rest and at muscle contraction in the range from low to maximal level of muscle contractions and in different elbow joint angles. At submaximal level of force we encountered difficulties to perform the 20-records mode measurements in stable muscle state conditions. For the same reason we were able to perform only measurements with 3-records mode at maximal contractions. Based on this experiences, we raised the question regarding a minimal necessary number of records that must be taken (instead of 20-records mode) in these different experimental conditions to obtain reliable myotonometricic stiffness measurements. Given that myotonometry offers an objective measuring method of mechanical properties of skeletal muscles, this metodological information would have its practical relevance in areas such as scientific research (studying mechanism of muscular plasticity), sports (monitoring of training effects), medicine and rehabilitation (diagnosis and treatment efficacy). To answer the question, in the present study, we used the data set of the 20-records mode of myotonometricic stiffness measurements from the two abovementioned experiments and analyzed the test-retest reliability for the groups of measurements with decreasing number of records starting from taking into analysis the whole 20, throughout the first 15, 10, 5 and 3 records. By this simulation approach we wanted to determine the effect of the number of records, captured during myotonometric measurements, on the reliability of these measurements under various testing conditions, including resting conditions and during muscle contraction. Our hypothesis was that an excellent reliability scores would be achieved mostly for stable resting conditions and at low levels of muscle contraction independent of the number of records taken, but for the measurements at a higher levels of muscle contraction the excellent reliability would be less affordable and the myotonometric measurements would be characterized by lower reliability scores.

2. Materials and methods

2.1. Experimental protocol and measurement device

Based on our simulation approach (described in the point 2.4), we determined the effect of number of records on the reliability of myotonometric stiffness measurements, using the data collected during two separate experiments conducted under different experimental conditions (the experiments are described in the point 2.2 and 2.3). The experiments were approved by the local ethics committee and complied with the Declaration of Helsinki. All subjects participating in both experiments gave their written consent prior to participation in the study. Myotonometric measurements of stiffness were conducted using a Myoton-3 device (Müomeetria AS, Estonia) in accordance with the methodological recommendations of Bizzini and Mannion [4], with the testing end of the myotonometer placed on and perpendicular to the surface of the skin overlying the tested muscle and simultaneously positioned parallel with the gravity vector. From the myotonometricic outcomes we used only the stiffness measure, since it is the parameter most often used in studies using myotonometry. We also think that, since this study is a mixture of data from different protocols, extending the material with the data for the all

myotonometricic parameters would make the results and discussion sections less affordable because of its huge complexity.

2.2. Experiment 1 (E1)

Subjects

Eight women diagnosed with mild to moderate Parkinson's disease (PD, mean age 77 ± 3 years, body mass 60 ± 9 kg, height 160 ± 5 cm) and 10 age-matched healthy elderly women (HE, mean age 77 ± 4 years, body mass 70 ± 11 kg, height 160 ± 5 cm) participated in the study. The PD patients were tested during their medication-on phase that was defined as a period of beneficial effects of anti-parkinsonian medication, which was mainly L-dopa (monopharmacotherapy), or L-dopa with piribedil or ropinirol (polypharmacotherapy) in some patients. Hereby, the tested subjects are the same ones as the those enrolled in our previous study [18], therefore detailed subjects' anthropometric and clinical characteristics can be found in [18].

Measurement procedure

Myotonometric measurements were taken from the short head of the biceps brachii (BB) and long head of the triceps brachii (TB) muscle with the subject lying on a comfortable wide massage table (BB in supine position and TB in prone position), with their upper extremities along their trunk and their forearm between pronation and supination. In the HE subjects the dominant hand was tested, while in PD patients it was the affected or more affected one. Both muscles were tested at rest (as described above) and BB was also tested whilst holding a 2-kg load attached to the wrist, equivalent to about 10% of the subject's maximal voluntary contraction (MVC). The MVC procedure and results have been presented in [17]. During measurements under resting conditions, the researcher instructed the subject to relax their muscle and then placed the testing end of the myotonometer on the skin surface overlying the muscle and performed 20 consecutive records (multiscan 20–records mode) with 1-second time delay between the successive records. During measurements taken whilst holding the 2-kg load, a researcher instructed the subject to flex their forearm at the elbow joint to an angle of 15° between the forearm and the surface of the massage table and hold the load for 1 minute, and also the 20-records mode measurements were taken when the position in the elbow joint was stable.

2.3. Experiment 2 (E2)

Subjects

Thirty healthy young (HY) male subjects participated in the study (mean age 22 ± 2 years, body mass 75 ± 7 kg, height 180 ± 6 cm). The tested subjects consist partly of the group (n = 17) of the healthy young subjects enrolled in our previous study [18] and 13 additionally enrolled ones. An injury in the right arm within 6 months prior to the start of the study was an exclusion criterion. Myotonometric measurements were taken from the brachioradialis muscle (BR) of the right extremity, which was also dominant for all subjects.

Measurement procedure

Myotonometric measurements were performed over the muscle belly of the right BR in subjects sitting on the chair of our custom-made BIODYNA dynamometer, as described in [10], [11], [16]. The tested upper extremity was abducted to 90° at the shoulder joint. The forearm was in neutral position (between the pronation and supination), aligned with the dynamometer arm and held by a mount attached to the instrument arm at the wrist-joint level. During measurements, the arm of the dynamometer was locked in the required elbow-joint position. In a separate session, elbow flexors maximal voluntary contraction (MVC) was measured during isometric contraction of each subject at elbow-joint angles of 60°, 90°, and 150°, as described in [10], [11], [16]. Myotonometric measurements were performed at the three elbow joint angles (in order as described above) during muscle rest and at either 25 or 50% of MVC. Additional measurements were performed at 80 and 100% of MVC at an elbow joint angle of 90°.

During measurements under resting conditions, the researcher instructed the subject to relax their muscle, and then placed the testing end of the myotonometer on the skin surface overlying the muscle and performed 20 consecutive records (with 1-second time delay between the successive records).

For the measurements at submaximal levels of muscle contraction, an acoustic signal was a cue for a subject to develop respectively 25, 50 or 80% of MVC, based on the force feedback displayed on a computer screen. The desired force level had to be hold stable for 50 seconds, while the researcher placed the testing end of the myotonometer on the surface of the BR and carried out measurements using the 20-records mode. Subjects rested for 2 minutes between consecutive submaximal contraction trials.

The measurements at 100% of MVC were performed similarly, but without the visual force-feedback (to ensure real voluntary maximal contraction), and with holding the force for 6 seconds, while the researcher took measurements with three consecutive records (multiscan 3-records mode with 1-second time delay between the successive records). The measurement duration at 100% of MVC and the equivalent number of records were adjusted according to the subject's ability to maintain the MVC as stable as possible. Subjects rested for 3 minutes between consecutive 100% of MVC trials.

2.4. Description of our simulation approach

We did not perform separate measurements using 20-, 15-, 10-, 5- and 3-records modes for all of these above described conditions but only used the data previously recorded by us in the E1[18] and E2 [10], using the 20-records modes for the rest and submaximal contraction conditions. From these data set of the 20-records modes measurements, we chose five groups of records, i.e., the whole 20 records and the first 15, 10, 5 and 3 records from each measurement to the statistical analysis of the test-retest reliability. By this approach we simulated the reliability analysis of performances of measurements with 20-, 15-, 10-, 5- and 3-records modes, respectively. Additionally, we added the 3–records mode measurements taken at 100% of MVC to the analysis of reliability. Using all these data set with different protocols of myotonometricic measurements we were able to check whether the myotonometricic stiffness measurements can be taken with acceptable reliability with various number of taken records (20, 15, 10, 5 and 3 records) in various subjects groups not only at rest, but also when muscle is contracted in the range form 10% MVC, throughout the 25, 50, 80 and 100% of MVC.

2.5. Statistical analysis

An absolute agreement intra-class correlation coefficient (ICC) was applied to determine the test-retest reliability of the myotonometric measurements for: (i) each of the five groups of records taken in multiscan 20-records mode (at rest and 10, 25, 50 and 80% of MVC) and (ii) for the records group taken in multiscan 3-records mode (at 100% MVC). The reliability was rated according to Sleivert and Wenger [23]: unacceptable – ICC < 0.60; average – ICC 0.60–0.79; excellent – ICC 0.80–1.00. Additionally, the mean and standard deviation values of muscle stiffness (S-MYO [N/m]) were calculated for all the above-described groups of records and differences among these groups (at rest and submaximal contraction conditions) were analyzed using an analysis of variance. A value of $\alpha \leq 0.05$ was considered significant for all analyses.

3. Results

3.1. Reliability in Experiment 1

In the elderly subjects (HE and PD), the stiffness measurements' reliability was excellent for all groups of records (20, 15, 10, 5 and 3 records), in the both tested experimental conditions (rest and 10% of MVC) and for the both tested muscles (BB and TB) (Table 1).

Table 1. Test-retest reliability (ICC, intra-class correlation coefficient) of myotonometric measurements of stiffness for the five groups of records (20, 15, 10, 5, 3) taken at rest and submaximal levels of force in the Experiment 1

Experimental conditions		ICC of Stiffness for the five groups of records				
		20	15	10	5	3
E1	BB R PD	0.995	0.958	0.964	0.950	0.956
	BB 10% PD	0.997	0.996	0.993	0.993	0.988
	TB R PD	0.991	0.989	0.991	0.974	0.984
	BB R HE	0.937	0.993	0.989	0.981	0.966
	BB 10% HE	0.996	0.995	0.991	0.979	0.965
	TB R HE	0.981	0.968	0.937	0.919	0.889

Groups of records: all 20 records and first 15, 10, five, and three records (20, 15, 10, 5, 3; respectively); Experiment 1 (E1); muscles: BB = biceps brachii, TB = triceps brachii; levels of force: R = rest, 10% percent of MVC; tested subjects: PD = Parkinson's disease patients, HE = healthy elderly subjects.

3.2. Reliability in Experiment 2

In the HY group, for BR stiffness measurements in the 20, 15 and 10-records groups the reliability was excellent in all tested muscle conditions (resting state and 25, 50 and 80% of MVC) and in all elbow joint angles (60, 90 and 150°) (Table 2).

For the 5-records groups, the reliability was also excellent in almost all the above-described meas-

Table 2. Test-retest reliability (ICC, intra-class correlation coefficient) of myotonometric measurements of stiffness for the five groups of records (20, 15, 10, 5, 3) taken at submaximal and maximal levels of force in the experiment 2

Experimental conditions		ICC of Stiffness for the five groups of records				
		20	15	10	5	3
E2	BR R 60° HY	0.974	0.964	0.940	0.912	0.884
	BR R 90° HY	0.980	0.976	0.965	0.937	0.888
	BR R 150° HY	0.952	0.938	0.914	0.809	0.628
	BR 25% 60° HY	0.991	0.989	0.986	0.981	0.971
	BR 25% 90° HY	0.992	0.991	0.989	0.975	0.958
	BR 25% 150° HY	0.987	0.985	0.984	0.975	0.958
	BR 50% 60° HY	0.966	0.968	0.947	0.854	0.669
	BR 50% 90° HY	0.949	0.930	0.852	0.727	0.347
	BR 50% 150° HY	0.980	0.978	0.972	0.941	0.921
	BR 80% 90° HY	0.895	0.879	0.840	0.801	0.659
	BR 100% 90° HY	–	–	–	–	0.156

Groups of records: all 20 records and first 15, 10, five, and three records (20, 15, 10, 5, 3; respectively); Experiment 2 (E2); muscles: BB = biceps brachii, TB = triceps brachii, BR = brachioradialis; levels of force: R = rest, 25%, 50%, 80% and 100% = percent of MVC; elbow joint angles: 60°, 90°, 150°; tested subjects: HY = healthy young subjects.

urement conditions (when considering the elbow joint angles and muscle state conditions) and only one measurement (taken under 50% of MVC at 90° elbow-joint angle) characterized of average reliability (Table 2).

For the 3-records groups, the BR stiffness measurements reliability was mostly excellent (resting state at 60° and 90° elbow joint angle; all three elbow joint angles at 25% of MVC; and at 150° elbow joint angle during 50% of MVC) or average (150° elbow joint angle at rest; at 60° of elbow-joint angle during 50% of MVC and at 90° of elbow joint angle during 80% of MVC), with one exception of an unacceptable reliability for the measurements taken under 50% of MVC at 90° elbow joint (Table 2).

The reliability of 3-records mode measurements at maximal contraction were unacceptable (Table 2).

3.3. Comparison of mean values between groups of records in Experiment 1 and 2

The mean stiffness values did not differ significantly ($P > 0.05$) among the five groups of records (20, 15, 10, 5, and 3 records) for all muscles under all

Table 3. Myotonometric measurements of stiffness for the five groups of records (20, 15, 10, 5, 3) taken at submaximal levels of force (mean and standard deviation) in the healthy elderly subjects (HE, $n = 10$) and in PD patients (PD, $n = 8$) in the Experiment 1

Experimental conditions		Descriptive statistics – stiffness [N/m] for the five groups of records					ANOVA for groups of records	
		20	15	10	5	3	*F* value	*P* value
E1	BB R PD	203 ± 22	203 ± 23	202 ± 24	201 ± 26	199 ± 25	0.064	0.992
	BB 10% PD	294 ± 38	292 ± 38	290 ± 38	287 ± 40	285 ± 38	0.033	0.998
	TB R PD	226 ± 24	225 ± 23	223 ± 21	218 ± 22	214 ± 24	0.020	0.999
	BB R HE	192 ± 8	191 ± 10	191 ± 12	190 ± 13	190 ± 16	0.047	0.996
	BB 10% HE	285 ± 39	286 ± 39	285 ± 38	283 ± 39	285 ± 40	0.032	0.998
	TB R HE	215 ± 28	215 ± 29	214 ± 34	212 ± 36	211 ± 38	0.506	0.732

Groups of records: all 20 records and first 15, 10, five, and three records (20, 15, 10, 5, 3; respectively); Experiment 1 (E1); muscles: BB = biceps brachii, TB = triceps brachii, levels of force: R = rest, 10% percent of MVC; tested subjects: PD = Parkinson's disease patients, HE = healthy elderly subjects. There was no significant difference ($P > 0.05$) between groups of records for values of stiffness under any experimental conditions.

Table 4. Myotonometric measurements of stiffness for the five groups of records (20, 15, 10, 5, 3) taken at submaximal and maximal levels of force (mean and standard deviation) in the healthy young subjects (HY, $n = 10$) in the Experiment 2

Experimental conditions		Descriptive statistics – stiffness [N/m] for the five groups of records					ANOVA for groups of records	
		20	15	10	5	3	*F* value	*P* value
E2	BR R 60° HY	293 ± 34	291 ± 35	288 ± 32	282 ± 33	275 ± 33	0.667	0.617
	BR R 90° HY	291 ± 35	289 ± 35	289 ± 35	291 ± 37	290 ± 37	0.007	1.000
	BR R 150° HY	326 ± 33	324 ± 35	320 ± 37	318 ± 39	312 ± 39	0.324	0.861
	BR 25% 60° HY	388 ± 69	382 ± 68	378 ± 71	366 ± 69	360 ± 71	0.399	0.809
	BR 25% 90° HY	386 ± 58	381 ± 58	377 ± 58	372 ± 55	369 ± 55	0.214	0.930
	BR 25% 150° HY	486 ± 83	474 ± 83	459 ± 84	442 ± 86	438 ± 85	0.886	0.477
	BR 50% 60° HY	446 ± 56	443 ± 60	443 ± 62	438 ± 55	429 ± 48	0.208	0.933
	BR 50% 90° HY	490 ± 58	484 ± 56	487 ± 49	477 ± 51	472 ± 44	0.276	0.892
	BR 50% 150° HY	546 ± 63	542 ± 67	534 ± 66	519 ± 66	518 ± 72	0.517	0.723
	BR 80% 90° HY	614 ± 71	616 ± 69	608 ± 71	594 ± 88	576 ± 82	0.331	0.856
	BR 100% 90° HY	–	–	–	–	590 ± 153	n/a	n/a

Groups of records: all 20 records and first 15, 10, five, and three records (20, 15, 10, 5, 3; respectively); Experiment 2 (E2); muscles: BB = biceps brachii, TB = triceps brachii, BR = brachioradialis; levels of force: R = rest, 10%, 25%, 50%, 80% and 100% = percent of MVC; elbow joint angles: 60°, 90°, 150°; tested subjects: HY = healthy young subjects. There was no significant difference ($P > 0.05$) between groups of records for values of stiffness under any experimental conditions.

experimental conditions in the E1 and E2 (Tables 3 and 4).

4. Discussion

Safrit [22] reported that higher number of measurement trials in physical education and exercise science enables an achievement of greater measurement reliability. Therefore, the multiscan 20–records mode has been recommended and used in previously reported myotonometricic measurements [4], [9], [10], [18], [24]. However, the reliability of this mode might be limited under certain conditions, such as during short-lasting submaximal muscle contraction and particularly at maximal voluntary contraction, or immediately after fatigue. By our current simulation approach, we have analyzed a reliability of the myotonometricic measurements of stiffness with using the multiscan 20-, 15-, 10-, 5- and 3-records modes in experiments conducted with various methodological protocols (diffrent muscles tested in various testing positions and joint angles), in various subjects groups (healthy young subjects, healthy elderly and patients with PD) and not only at rest but also when muscle is contracted in the range from 10% of MVC, throughout the 25, 50, 80 and 100% of MVC. The findings from our study showed that performance of reliable myotonometric measurements of stiffness, at rest and at submaximal muscle contraction, is possible with the multiscan 20-records mode and also with 15-, 10-, 5- or 3-records modes. However, the 3-records mode does not allow to perform a reliable myotonometric stiffness measurements at maximal muscle contraction.

4.1. Reliability of myotonometric measurements of stiffness performed at rest and submaximal levels of force

In the elderly subjects (patients with PD and the healthy age-matched elderly) tested in our Experiment 1, the reliability of the myotonometric measurements of stiffness was excellent at rest as well as at 10% of MVC in both tested muscles (BB and TB), for the five groups of records (20, 15, 10, 5 and 3 records). This excellent reliability was certainly related to the facts that (i) the real resting state measurements of muscle tone were not perturbed by any potential factor that might affect the muscle tone and (ii) that the 10% of MVC was sustained stably by tested subjects. The physiological tremor or tixotropic properties influence, that might occur as an effect muscle fatigue or muscle contraction (respectively), was not present, since the subjects were tested in a relaxed state or at very low level of muscle contraction that did not cause any muscle fatigue. Also, there was no movement of arm and forearm in the elbow joint, and, therefore, it might not change the length of these muscles and this

way it could not act on the receptors and consequently on the muscle activity and its tone. Also, in the PD patients, there was no influence of resting tremor or rigidity, since the patients were tested during their "medication-on phase". Thus, at these two conditions (the real muscle relaxation and very low level of muscle contraction) the muscle stiffness measurements were not perturbed by any factor and therefore it was possible to collect the repeatable 20 myotonometric records that were similar in stiffness values in the consecutive groups of records (3, 5, 10, 15 and 20 records). The above is proven by the ANOVA comparisons of stiffness values among the five groups of records. Thus, irrespectively of the number of taken records, i.e., 20-, 15-, 10-, 5- and even with the 3-records mode, the myotonometric measurements of stiffness would be highly reliable.

In the HY subjects (tested in our Experiment 2), the myotonometric measurements taken at rest were also highly reliable by achieving the excellent scores for almost all of the BR stiffness measurements at the three elbow joint angles (60°, 90° and 150°) and for the five groups of records (3, 5, 10, 15 and 20 records). The only one average reliability score was found for the 3-records mode measurement of BR stiffness in a position of 150° of the elbow joint angle. The reasons of such high reliability of resting BR stiffness measurements are similar as for the above commented measurements in the elderly subjects, i.e., the real muscle relaxation without any influence of perturbing factors, such as fatigue, tixotropic properties and muscle length changes. Thus, irrespectively of the number of records taken, the resting measurements of myometric stiffness in the HY subjects BR muscle would characterize of high or acceptable reliability.

The myotonometric measurements of BR stiffness taken in the HY subjects at submaximal levels of force (25, 50 and 80% of MVC) with 20-, 15-, 10- and 5-records modes characterized mostly of excellent reliability, and only once of average reliability (at 50% of MVC in the 90° elbow joint angle for the 5-records mode). Our results let us state that, irrespectively of the number of records taken, the myotonometric measurements of stiffness at the submaximal levels of force (25, 50 and 80% of MVC) would be characterized as high or acceptable reliability.

For the 3-records mode, the BR stiffness measurements reliability: (i) was mostly excellent, (ii) in two cases characterized of average reliability (at 50% of MVC in the 60° elbow joint angle and at 80% of MVC in the 90° elbow joint angle) and (iii) in one case the reliability was unacceptable (at 50% of MVC in the 90° elbow joint angle). This unacceptable reliability at 50% of MVC might be related to force fluctuations at the onset of sustained isometric contraction (stabilizing phase) at the 90° elbow joint angle, that might affect muscle tone and, consequently, myotonometric records of stiffness. Thus, measurements involving just three records may be used at higher force levels, but researchers should pay particular attention to force fluctuations during myotonometric measurements under these conditions.

Trials conducted at higher force levels (50 and 80% of MVC) using the multiscan 20-records mode required the researcher to start the measurement procedure as soon as possible to avoid any influence of muscle fatigue, given that the procedure takes between 30 seconds and 1 minute to complete. This sustained muscle contraction might result in force/muscle tone deviations caused by fatigue-related physiological tremor [15] that might affect the myotonometric records and especially these from the second half of the 20 records. Also, for the same reason, the initial few records, taken during the stabilizing phase of isometric contraction might be affected by small force/muscle tone deviations. It should be noted that the mean myotonometric stiffness values did not differ significantly among the five groups of records (20, 15, 10, 5, and 3 records) under any conditions at submaximal levels of force. However, the mean values of the first three records tended to be lower than the means of the third, fourth and fifth records groups, what might be related to the force fluctuations on the onset of measurement. Thus performance of shorter measurements modes (3–10 records) instead of the multiscan 20-records mode, with paying an attention on these initial force fluctuations, might enable a performance of highly repeatable measurements with simultaneous shortening of single measurement time.

4.2. Reliability of myotonometric measurements of stiffness performed at maximal level of force

Notably however, we found that the reliability of muscle-stiffness measurements at 100% of MVC were unacceptable using mode of 3 records. This might be because of muscle stiffness changes along with force fluctuations, with the tested subjects unable to control fatigue-related physiological tremor at 100% of MVC [15], resulting in increased variability between consecutive records.

4.3. Methodological recommendations from measurements' reliability point of view

Based on our findings, the most important factor is the testing condition (muscle relaxed state or muscle contraction). The choice of testing group with its specificity, for example the healthy subjects or patients with various movement disorders (tested on-drugs or after withdrawal of drugs), should be taken into consideration as well by the application of specific myotonometric measurements' modes. Also, the limitation of time to complete the testing session should be taken into account. This is especially the case when the long-lasting testing session might cause an intensification of factors affecting myotonometricc measurements of stiffness (e.g., parkinsonian rigidity or tremor in patients with PD or fatigue in healthy subjects). Thus, when a researcher performs the myotonometric measurements in such time-limited sessions (and especially in multimodal projects where myotonometry is one among many other methods used during the session), we recommend to choose the option of lower number of records. In this case, the reliable myotonometric measurements might be performed even with 3 to 5 records at resting condition, but for the measurements at submaximal levels of force at least 5 records should be taken to be sure about the measurements reliability. However, for the myotonometric measurements of stiffness at maximal level of force we do not recommend the moultiscan 3-records mode with the 1-second time delay between the successive records, since we found this measurement mode as an unacceptable measurement procedure. Probably a good alternative approach in this case might be to shorten the time between the successive three records, for example to 0.5 s. Another possible solution might be the one proposed by Pruyn et al. [20], who performed repeated single records.

5. Conclusions

In the healthy elderly subjects and in individuals with PD, the myotonometric measurements' reliability at rest and low level of muscle contraction was excellent for all groups of records (20, 15, 10, 5 and 3 records). In the healthy young subjects, for the five groups of records taken at rest and most of measurements at submaximal levels of force (25, 50 and 80% of MVC), the reliability was excellent or rarely average, and only in one per three measurements at 50% MVC the reliability was unacceptable (for the 3-records group).

To sum up, the reliable myotonometric stiffness measurements in muscles at rest and during submaximal contractions can be achieved with less than 20 records (15, 10, 5 records) and even for most measurements with 3 records in healthy young and elderly subjects as well as in the PD patients. However, the myotonometric stiffness measurements with 3-records mode during maximal contraction were not reliable in healthy young subjects.

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