Complexity of force output during static exercise in individuals with Down syndrome

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1Department of Kinesiology and Community Health, University of Illinois at Urbana, Champaign, Champaign, Illinois; 2Department of Sports Informatics, University of Seoul, Seoul, Korea; 3Exercise Science Department, Syracuse University, Syracuse, New York; 4Department of Nutrition, Food and Exercise Sciences Florida State University Tallahassee, Florida; and 5Department of Kinesiology and Sports Studies, Wichita State University, Wichita, Kansas

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Heffernan KS, Sosnoff JJ, Ofori E, Jae SY, Baynard T, Collier SR, Goulopoulu S, Figueroa A, Woods JA, Pitetti KH, Fernhall B. Complexity of force output during static exercise in individuals with Down syndrome. J Appl Physiol 106: 1227–1233, 2009. First published January 22, 2009; doi:10.1152/japplphysiol.90555.2008.—Force variability is greater in individuals with Down syndrome (DS) compared with persons without DS and is similar to that seen with normal aging. The purpose of this study was to examine the structure (in both time and frequency domains) of force output variability in persons with DS to determine whether deficits in force control are similar between individuals with DS and older adults. An isometric handgrip task at a constant force (30% of maximal voluntary contraction) was completed by individuals with DS (n = 29, age 26 yr), and healthy young (n = 26, age 27 yr) and older (n = 33, age 70 yr) individuals. Mean, standard deviation (SD), and coefficient of variation (CV) were used to analyze the magnitude of force output variability. Spectral analysis and approximate entropy (ApEn) were used to analyze the structure of force output variability. Mean force output for DS was lower than in young controls (P < 0.05) but no different from old controls. Individuals with DS had greater SD and CV than young and old controls (P < 0.05). The DS group had a significantly greater proportion of spectral power within the 0-to-4-Hz bandwidth than the young and older controls (P < 0.05). The DS group had significantly lower ApEn values than the young controls (P < 0.05), but there were no differences in ApEn between the DS group and the old controls (P > 0.05). In conclusion, young persons with DS demonstrate enhanced temporal structure and greater amplitude of low-frequency oscillations in the force output signal than age-matched non-DS peers. Interestingly, young persons with DS and older persons without DS have similar time-dependent structure of force output variability. This would suggest a possible link between premature aging and less complex force output in persons with DS.

motor control; approximate entropy

DOWN SYNDROME (DS) is the most commonly inherited form of developmental disability in North America (7). It is estimated that 1 out of every 700–1,000 newborns in the United States will be diagnosed with DS, and there are currently over 350,000 persons living with DS in the United States (7). In more than 90% of cases, DS results from an excess of genetic material from chromosome 21 (i.e., trisomy 21). Trisomy 21 results in a constellation of dysfunctions, spanning several physiological systems. Individuals with DS have poor balance, poor coordination, slow reaction time, reduced visual-motor control and sensory acuity, gross and fine motor skill dysfunction, and overall greater movement variability (1, 2, 11, 29, 30, 60). Motor dysfunction in persons with DS prevents successful completion of many activities of daily living, attenuates vocational productivity, and contributes to low physical work capacity (20, 36). This ultimately results in increased dependence on others and assisted living (36). Most striking, lack of mobility has been shown a predictor of mortality in this population (19, 52). As such it is imperative to increase the understanding of the factors underlying neuromotor function in DS.

The origin of motor dysfunction in DS has been attributed to both structural and functional differences in the central nervous system (CNS) (5, 8). Structurally, individuals with DS have reduced volume in the cerebellum, hippocampus, and cerebral gray matter and white matter of the frontal cortex compared with age-matched non-DS peers (5, 6, 38, 53, 55). Cerebellar alteration has been suggested to be the most prominent structural aberration underlying neuromotor impairment in DS (28). The increased motor variability and cerebellar dysfunction that characterizes DS is similar to the effects of advanced age on the neuromuscular system. With healthy aging, all aforementioned regions of the brain undergo structural deterioration (3, 26), and this has been linked to a well-noted decline in motor ability in the aged population (61–63). It is also well accepted that individuals with DS age prematurely (41). Biological age in DS has been estimated to be nearly twofold that of chronological age, and this senescence is global and occurs at multiple organ levels (34). Individuals with DS have somatic degenerative changes in the skin and hair, reduced immune function, increased frequency of neoplasms, amyloid deposition, cata racts, vascular disease, and ultimately shorter life expectancy (34, 41). Studies employing magnetic resonance imaging and computed tomography also note significant premature aging of the brain in DS (41, 53, 54). Thus it is possible that accelerated neural aging in DS may contribute to declines in force control as is seen with healthy aging, but this has not been specifically investigated. Consequently, the primary goal of this investigation is to determine whether force output variability is similar between individuals with DS and older adults.
Traditional means of assessing force output variability in various clinical populations have relied solely on dispersion statistics such as standard deviation (SD) and coefficient of variation (CV). However, these methods ignore the temporal structure of the variability, limiting inferences that can be made regarding underlying control system dynamics (42). Indeed with pathology and aging there may be no change in the amplitude or modal frequency profile of fluctuations but significant differences in the underlying time-dependent structure (57–59). Nonlinear means of assessing force fluctuations yields information on the deterministic and stochastic organization of motor system output, which cannot be inferred from conventional linear methods alone (42). This provides vital insight into the dimensions of feedback control loops governing force output and in turn the underlying state of the neuromotor system (32, 35).

A common nonlinear method of assessing force fluctuations is by examining the complexity of a signal over time. Complexity and variability are not analogous terms (49). Complexity refers to the irregularity of a dynamic process and can be measured quantitatively by assessing the uncertainty of patterns reoccurring within a time event series (31). A force signal dominated by a single attractor, originating from a strong neural oscillator would correspond to a high degree of regularity (i.e., low complexity or high predictability). This rigid mode locking does not allow for appropriate adaptation to changing stimuli. Conversely, a force signal that arises from a network of multiple neural oscillators will be highly irregular (i.e., high complexity or low predictability). Healthy physiological systems demonstrate complex dynamics representing many interacting influences operating over multiple time scales (31). This intrinsic complexity allows for quick and adequate adaptations to internal/external perturbations (35). A loss of complexity with aging may lead to disease and frailty (31), and in the case of DS, it may lead to functional dependence and assisted living.

The purpose of this study was to examine the structure of isometric force output variability, in time and frequency domains, in young individuals with DS, and young and older individuals without DS. We hypothesized that both amplitude and the time- and frequency-dependent structure would be similar in individuals with DS and older individuals without DS.

MATERIALS AND METHODS

Participants. Twenty-nine young participants with DS (mean age 26.3 ± 1.5 yr, 13 men; height 1.55 ± 0.15 m, weight 72.7 ± 2.9 kg), 24 age-matched young control participants without DS (mean age 26.7 ± 1.3 yr, 13 men; height 1.70 ± 0.16 m, weight 71.1 ± 2.4 kg), and 33 older control participants without DS (mean age 70.1 ± 1.0 yr, 10 men; height 1.65 ± 0.19 m, weight: 76.0 ± 2.4 kg) took part in this study. Participants with DS were recruited from community-based service programs for individuals with developmental disabilities. All older subjects were community dwelling and previously cleared for participation in this study following a physical examination and resting/maximal exercise electrocardiograms, conducted on a separate day. All participants were either sedentary or moderately active, with none partaking in a formal exercise training program. Exclusionary criteria were as follows: 1) history of thyroid disease; 2) renal disease; 3) history of diabetes or other metabolic disease that may affect outcome measures; 4) pulmonary/respiratory disorders, including asthma; 5) severe or profound mental retardation; 6) congenital heart disease; 7) any contraindications to exercise or orthopedic injury preventing successful completion of the protocol; 8) arthritis; 9) atlantoaxial instability; 10) cancer; and 11) smoking. All participants had normal or corrected to normal vision.

After thorough explanation of the study protocol to participants, signed informed consent was attained from participants as well as parents or guardians. This study was approved by the University Institutional Review Board.

Familiarization. Subjects with DS were familiarized with all testing protocols before data collection. The number and length of familiarization sessions depended on the response of the individual subject. Subjects were familiarized with muscle force generation procedures as follows. Subjects practiced the handgrip task (i.e., maintaining low intensity output with use of a visual target for a sustained period of time) until they could demonstrate satisfactory performance of the test. Our laboratory has previously shown that, through this familiarization procedure, valid and reliable data can be collected in this population (21, 22). Subjects without DS were familiarized with test procedures on the day of testing before each procedure and demonstrated that they could satisfactorily complete the test tasks before data collection.

Isometric handgrip. Data acquisition commenced once participants established proficiency in completing isometric handgrip (IHG) testing. Participants were seated in a chair facing a 17-inch video monitor (DELL). The monitor had a viewing area of 1,200 horizontal pixels and 1,000 vertical pixels. The monitor was ~50 cm from the volunteer’s eyes and 100 cm from the ground.

Maximal grip strength was determined with the dominant hand using an electronic handgrip dynamometer (model TSD121C, BioPac Systems, Goleta, CA). The force output signal was collected at 1,000 Hz and subsequently down sampled to 100 Hz for signal processing and analysis. The dynamometer had a length of 10.35 cm and a circumference of 10.55 cm. The dynamometer was calibrated before each test using a predetermined standard load. Subjects were tested in the seated position with the unsupported elbow of the dominant arm flexed at 90° and their wrist in a neutral position. Subjects grasped the force transducer with the palmar surface of their hand and wrapped their fingers around it, forming a fist (See Fig. 1). Verbal encouragement was afforded to ensure maximal effort. The highest of three familiarization sessions depended on the response of the individual subject. Protocols before data collection. The number and length of familiarization sessions depended on the response of the individual subject. Subjects were familiarized with muscle force generation procedures as follows. Subjects practiced the handgrip task (i.e., maintaining low intensity output with use of a visual target for a sustained period of time) until they could demonstrate satisfactory performance of the test. Our laboratory has previously shown that, through this familiarization procedure, valid and reliable data can be collected in this population (21, 22). Subjects without DS were familiarized with test procedures on the day of testing before each procedure and demonstrated that they could satisfactorily complete the test tasks before data collection.

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Fig. 1. Subject grasping force transducer.
parsimonious account of the force variability magnitude relationship (9). During contraction, visual feedback was provided via the computer monitor interfaced with the dynamometer. The participant adjusted their force output to match a target line displayed on the midline of the monitor. Participants viewed online feedback of their performance in the form of a continuous line that moved left to right across the screen with time, which corresponded to the force trajectory.

Data analysis. A stable 10-s epoch was used for analysis. All data processing was performed using software written in Labview (National Instruments, Austin, TX). To assess the magnitude of force variability, the SD and CV of the force data were calculated (CV = SD/mean).

To quantify the frequency structure of the force output, power spectral analysis was applied using Welch’s averaged periodogram method (Hanning window). Because most of the power in the resultant spectrum is below 12 Hz, measures derived from the spectrum were limited to the range of 0–12 Hz (47). Because absolute power of the force spectrum increases with the absolute amount of force produced, the proportion of power was calculated to allow comparisons to be made across groups that may be producing different force levels (14). This was accomplished by dividing the power within the desired bandwidth (0–4 Hz, 4–8 Hz, and 8–12 Hz) by the amount of total power within the entire frequency range. The power calculated for the 0- to 4-Hz bandwidth was used as an index of sensorimotor processing (i.e., visuomotor processing) (57). The peak/modal frequency was identified within the 0- to 12-Hz bandwidth, and the proportion of power exhibited at that frequency was determined by dividing the amount of power at the modal frequency by the amount of total power within the entire frequency range (14). The peak/modal frequency reflects the frequency with the greatest overall contribution to the force output signal (14).

ApEn was used to examine the complexity of the time event series and used to quantify the time-dependent structure of the force output (42, 57). ApEn determines the probability of finding specific patterns or matches in a time series. It measures the logarithmic likelihood that runs of patterns that are close for m observations remain close on the next incremental comparisons (37). Highly predictive (i.e., regular) signals such as a sine wave will have lower values, whereas highly irregular signal will have higher values. The embedding dimension m (length of sequences to be compared) may range from 2 to 10 while the filter parameter r (tolerance for accepting matches) may range from 0.10 to 0.50. To ensure a normal distribution of ApEn values, the embedding dimension should be large enough and the filter parameter small enough to ensure a sufficient number of matches. In the present investigation, m was fixed at 2 while the filter parameter r was set at 20% the SD of the time series (37).

Statistical analyses. All data are reported as means ± SE. A priori contrasts were used to compare dependent variables between groups. Homogeneity of variance was assessed with the Levene statistic, and appropriate comparisons were made (equal variance assumed or not assumed) based on results. Significance was set at P < 0.05. Because there was no effect of sex on any of the force control parameters, data collapsed across sexes is reported. All data analysis was carried out using Statistical Package for the Social Sciences (SPSS, v 12.0.1, SPSS, Chicago, IL).

RESULTS

Four elderly individuals and one DS individual were excluded from final analysis as they demonstrated signs of pathological tremor (defined as having modal frequency occur >4 Hz). The DS group was significantly younger than the older control group (P < 0.05). There were no differences in age between the young control group and the DS group (P > 0.05). The DS group was significantly shorter than both the older control group and the young control group (P < 0.05). The DS group was of similar weight to the older control group and the young control group (P > 0.05).

Figure 2 illustrates the force output of a representative young adult without DS and an adult with DS. It is clear that the individual with DS has greater force variability. To further examine these differences, distribution statistics as well as indexes of time- and frequency-dependent structure of force output were calculated.

The DS group produced significantly less mean force than the young control group (Fig. 3; 36.7 ± 2.7 vs. 60.2 ± 6.2 N; P < 0.05). Mean force output was similar between the DS group and the older control group (33.5 ± 2.0 N; P > 0.05). As shown in Fig. 4A, the DS group had significantly greater SD values (7.3 ± 0.8 N) than the young control group (2.6 ± 0.3 N; P < 0.05) and the older control group (1.5 ± 0.1 N; P < 0.05). The DS group had significantly greater CV values (0.210 ± 0.02) than the young control group (0.041 ± 0.002, P < 0.05) and the older control group (0.044 ± 0.007; P < 0.05; Fig. 4B).

As shown in Fig. 5A, the DS group had a significantly greater proportion of spectral power within the 0- to 4-Hz bandwidth (94.7 ± 1.1%) than the young control group (84.5 ± 2.3%; P < 0.05) and the older control group (77.7 ± 2.4%; P < 0.05). The DS group had a significantly lower proportion of spectral power within the 4- to 8-Hz bandwidth (3.5 ± 0.7%) than the young control group (8.6 ± 1.2%, P < 0.05) and the older control group (11.7 ± 1.2%; P < 0.05). The DS group had a significantly lower proportion of spectral power within the 8- to 12-Hz bandwidth (1.8 ± 0.4%) than the young control group (6.9 ± 1.0%; P < 0.05) and the older control group (10.6 ± 1.3%; P < 0.05).

The peak/modal frequency occurred within the 0- to 4-Hz band for all three groups. The DS group and the young control group had peak power at similar frequencies (1.04 ± 0.1 vs. 1.15 ± 0.2 Hz; P > 0.05). The frequency at which peak power
occurred was lower for the DS group than for the older control group (1.29 ± 0.2 Hz; \(P < 0.05\)). The proportion of power at the modal frequency was significantly greater for the DS group (61.2 ± 3.4%) than the young control group (36.0 ± 3.1%, \(P < 0.05\)) and the older control group (28.4 ± 3.2%; \(P < 0.05\)).

The DS group had significantly lower ApEn values (Fig. 5B; 0.38 ± 0.02) than the young control group (0.44 ± 0.01, \(P < 0.05\)). There were no differences in ApEn between the DS group and the older control group (0.40 ± 0.02; \(P > 0.05\)).

**DISCUSSION**

The purpose of this study was to examine the structure (in both time and frequency domains) of force output variability in persons with DS to determine whether deficits in force control are similar between individuals with DS and older adults. The novel findings of the current investigation were 1) amplitude of isometric force output variability in young individuals with DS is greater than in young and older persons without DS, 2) a greater proportion of the spectral power was found in lower frequency bandwidths in DS participants, and 3) the underlying time-dependent structure (ApEn) of the force output is similar in young individuals with DS and older adults.

The most novel finding of the present investigation was that older individuals without DS and younger individuals with DS
have similar time-dependent structure of force output. Time-dependent structure of force variability, as quantified via ApEn, provides indirect insight into the underlying deterministic and stochastic organization of the neurophysiological system. Low values of ApEn correspond to more structured force output, due to fewer attractors/decreased number of control processes, and/or decoupling of control processes (37, 42, 46). In the present study, the more regular (i.e., less complex) force output in persons with DS is similar to that seen with advanced age (57). This would suggest that some of the causal mechanisms that underlie loss of force complexity with aging may also contribute to similar findings in younger persons with DS. Less complex force output in DS may be partly related to premature aging. Thus, in both conditions (aging and DS), there may be shift from multiple equally contributing neural oscillators operating across multiple time scales to fewer, stronger, and more dominating, neural oscillators operating across fewer time scales (48). It should be noted that there were differences between proportion of power in older adults and DS group. The discrepancy between the ApEn results and power analysis suggests that the commonality between DS and older adults change in force control is somewhat limited.

Dispersion statistics (SD and CV) revealed greater variability in force output in persons with DS compared with younger and older persons without DS. Greater amount of variability has been suggested to be mediated by muscular strength (45). Weaker muscles produce more variable force outputs (45). With fewer functioning motor units active during force generation, each motor unit will have a higher firing rate and thus a proportionally greater impact on overall force output (23, 64). Persons with DS typically exhibits 30–40% lower levels of strength compared with their peers with mental retardation and <50% of the expected strength levels of their nondisabled peers (4, 10, 12, 24, 25, 39). These low levels of muscle strength are present in childhood and persist into adulthood (27). Older participants without DS and young participants with DS had similar strengths. Despite equivocal mean force output, persons with DS were still more variable. Lower muscle strength in this population cannot fully explain the more variable force output. Thus the mechanisms that contribute to muscle weakness (i.e., sarcopenia, decreased number of functioning motor units, increases in connective tissue within the muscle) and force variability (i.e., loss of synchronization of motor unit firing, variability in motor unit discharge rate, reduced size of neurons, reduced number of synapses, reduces integrity of gray matter and volume of white matter, and lower neurotransmitter levels) with aging (18) may not be entirely the same as those in DS. More research is warranted to examine the relationship between strength and force variability in this population.

The origin of motor dysfunction in DS has been attributed to both structural and functional differences in the CNS. From a structural vantage, neural deficits are multifocal and may be related to alteration of several key brain regions associated with motor control (8). Several studies have found localized areas of reduced volume, including frontal cortex, limbic areas, hippocampus, and significant left-right cerebral asymmetries in the limbic region and a larger parahippocampal gyrus (6, 38, 53, 55). The cerebella of individuals with DS are smaller and cerebral gray and white matter volume are lower than the general population, and this atrophy is progressive with age (6, 38, 53, 55). Individuals with DS have other white matter anomalies, including narrower corpus callosum, which may affect transfer of semantic information across hemispheres (8).

The cerebellum is crucial in the visual guidance of movement and motor performance (17, 51). In a similar isometric force task as the present study, Vaillancourt and colleagues (56) demonstrated that increased force variability was associated with reduced cerebellar activity (56). Feed-forward processing relies on cerebellar circuitry. The increase in low-frequency proportion of power supports the proposal (15, 16, 56) that deficits in the cerebellum function are at least partially responsible for elevated force variability. The cerebellum constantly receives information from peripheral afferent sensors (i.e., limbs and eyes) and transfers processed information to motor parts of the thalamus and cerebral cortex for commensurate efferent activity. The ability to anticipate the effects of modifications in force and make appropriate adjustments was attenuated in persons with DS. This decrease in anticipation resulted in greater reliance on feedback processing indicated by the elevated low frequency power.

From a functional vantage, motor dysfunction may stem from abnormal sensorimotor integration or cognitive limitations. Results from the present investigation would support a role for impairments in perceptual-motor coupling (visual-motor/sensorimotor processing) in persons with DS (i.e., the amount of time needed to perceive and subsequently correct for an error in motor output). We noted a significantly greater proportion of power located within the 0- to 4-Hz bandwidth in persons with DS. The proportion of power at the modal frequency, which occurred within the 0- to 4-Hz bandwidth, was also significantly greater for DS. Power located within this bandwidth is generally related to movements dominated by online sensory guidance or movements generated in the service of the acquisition of sensory information (44, 47). An association between visual motor processing time and force variability is based on the assumptions of closed-loop theory that individuals act as feedback control systems (46). An increase in processing time results in larger deviations from the intended target (46). A delay in the feedback loop during manual control can interfere with control processes, producing a decrease in performance and increase in error on the task (33). Overall, greater variability in manual control could be due in part to a delay in visual motor processing (33, 46). Indeed, experimental and modeling work has shown that feedback delays lead to an increase in the time and frequency structure of perceptual-motor output (32, 46). Present findings suggest greater closed-loop sensorimotor corrective processing in individuals with DS, contributing to an increase in the temporal and frequency-dependent structure of force output.

Overall findings suggest that there was an increase in the relative contribution of slow time scales and a decrease in the relative contribution of fast time scales to the force output in persons with DS. In younger and older adults without DS, higher frequencies in the motor output would help to maintain a steadier force output with lower amplitude fluctuations (13, 35, 46). The general slowing of the intrinsic dynamics of the motor system in persons with DS would serve to provide constraints on the temporal and frequency properties of movement initiation and execution (14, 50).
The way in which the movements of individuals with DS are performed may be a reflection of an impaired decision-making process and not necessarily a consequence of a primary motor deficit (29). Intellectual disability, which is almost always associated with DS, could affect the decision-making process by delaying the accumulation and translation of information specific to the stimulus and motor response (i.e., Smith predictor) (46, 50). Individuals with DS demonstrate an inability to sustain concentration on the task, consistent with the notion of an impaired central timekeeper (2) and increased feedback processing time. It has also been proposed that an apparently abnormal motor pattern be viewed as a sign that the CNS has rearranged its priorities and designed a new subset of solutions from an infinite memory supplied via the redundancy of the motor system (28, 29). It is also possible that the increased variability demonstrated by the DS group reflects an exploratory strategy and not a decline in function (40).

A limitation of the present investigation was the use of a visually guided isometric force production task to examine motor output variability. Performance on this task is most likely only associated to visually guided grasping tasks, which require precise force production to manipulate objects. The data from this investigation should not be applied when discussing group differences in multidegree of freedom movements such as reaching. Precise mechanisms responsible for loss of complexity and greater variability seen in DS also cannot be ascertained, but only inferred, from the present study design.

In conclusion, young persons with DS demonstrate enhanced structure in the force output, which is reflected in stronger contributions of low-frequency components that have larger amplitudes and more sequential regularity in the time domain. This may be partly attributable to greater reliance on sensorimotor processing. Individuals with DS have a deficit in the ability to control the amplitude of force when processing visual information and generating motor corrections. Young persons with DS have similar time-dependent structure of force output variability compared with older persons without DS. Thus processes related to premature aging in DS may contribute to a less complex force output.

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REFERENCES


