**Using Surface Electromyography To Assess Sex Differences in Neuromuscular Response Characteristics**

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**Abstract:**

Objective: To provide an overview of the continuum of muscular responses that typically occur with joint perturbation. The applications and limitations of surface electromyography (sEMG) in evaluating these responses are also addressed. Research applications assessing sex differences in these neuromuscular response characteristics are discussed along with suggestions for future research.


Data Synthesis: It is widely accepted that efficient neuromuscular control is essential to dynamic joint stability and protection. Many studies have established the significant role of the muscles, and particularly the hamstrings, in providing knee stability. By observing the timing, phasing, and recruitment of reflexive muscular activation after a loading stress to the knee, we can better understand the coordinative mechanisms necessary to protect the joint and prevent ligament injury. A number of research models have employed the use of sEMG to evaluate neuromuscular responses at the knee after joint loading or perturbation. However, very few studies have specifically addressed potential sex differences in these response characteristics.

Conclusions/Recommendations: From the limited research available, it appears that a sex difference may exist in some aspects of neuromuscular responses. However, further research is needed to explore these differences at the knee and their potential role as predisposing factors to the higher incidence of anterior cruciate ligament injuries in females. Future studies should examine sex differences in neuromuscular response characteristics at the knee under functional, weight-bearing conditions while controlling for training and other confounding variables. The limitations of sEMG should be considered when interpreting neuromuscular response studies.
Key Words: dynamic stability, electromechanical delay, reflex, reaction time, anterior cruciate ligament

Article:
The increased incidence of anterior cruciate ligament (ACL) injury in females is a growing concern within the sports medicine community.\textsuperscript{1-10} At the present time, there are no clear explanations for the disparity in injury rates between males and females, although considerable research has attempted to identify potential predisposing factors.\textsuperscript{1,6,11-26} With the sharp increase in both the participation and competitive level of females in sports in recent years, it has been suggested that females have not received adequate training or skill preparation to compete at the level in which they are engaged.\textsuperscript{1,3,14} Therefore, the ability of the neuromuscular system to adequately respond to the substantial joint forces incurred at the knee during sport activity and to provide sufficient joint protection has been suspect. Whether a difference in muscular activation, timing, or recruitment patterns, or a combination of these, exists between males and females may be a significant finding in our attempt to explain the higher incidence of ACL injury in the female athlete. While it appears from the limited research available that certain sex differences may exist in muscular response characteristics,\textsuperscript{14,27,28} more research is needed to establish this relationship as a potential predisposing injury factor.

It is widely accepted that efficient neuromuscular control is essential to dynamic joint stability and protection. The ACL provides as much as 86% of the static resistance to pure anterior tibial translation.\textsuperscript{29} However, forces (both internal and external disturbances) incurred at the joint during sport activity are often beyond the capacity of the passive ligamentous constraints, thus requiring the addition of active muscular forces to maintain joint equilibrium and stability.\textsuperscript{23,30,31} Many studies have established the significant role of muscular activation about the knee, particularly that of the hamstrings, in improving knee stability.\textsuperscript{30-37} Research indicates that timely activation of the hamstring muscles can assist in protecting the ACL from mechanical strain by stabilizing the tibia, thus reducing anterior and rotary tibial translation.\textsuperscript{30,32-34,36,38,39} Therefore, the speed at which muscular activation and subsequent force development can be generated may be an important determinant in providing dynamic joint stability and ligament protection.

It is important to note that no study to date has specifically demonstrated whether, in fact, reflexive muscular activation and joint stiffening can occur quickly enough to protect the joint once an injurious force is applied to the ligament. In fact, research indicates evidence to the contrary.\textsuperscript{40,41} However, researchers have yet to adequately address the contribution of intrinsic muscle stiffness\textsuperscript{42-45} or preparatory alterations in muscular tension at lower applied loads\textsuperscript{46,47} that may prevent excessive joint excursion or provide some measure of immediate joint stiffening until a reflexive response can be generated. Clearly, more research is needed to fully elucidate the capacity of neuromuscular response mechanisms to protect the ACL during joint perturbations.
The evaluation of neuromuscular response characteristics around a particular joint can assist the clinician or researcher in understanding muscular activation and recruitment patterns both during and after a loading stress to the joint. By observing these response characteristics, we can better understand the coordinative mechanisms necessary to protect the joint and prevent injury under sudden loading conditions. Surface electromyography (sEMG) is a valuable tool that may be well suited to provide this assessment if its limitations are realized. Therefore, our purpose is to discuss the research applications, as well as the limitations, of sEMG in the assessment of neuromuscular response characteristics. Our objective is to provide an overview of the continuum of muscular responses that typically occur with joint perturbation and how sEMG can be used to evaluate these responses. Research applications assessing sex differences in neuromuscular response characteristics with sEMG will also be addressed, along with directions for future research.

ASSESSMENT OF NEUROMUSCULAR RESPONSES USING EMG
sEMG has been used extensively in biomechanical applications to describe and quantify a muscle or muscle group's activity or performance about the knee. sEMG can assist the clinician or researcher in determining when a muscle is activated, the timing of that activation in relation to a stimulus or event, and its sequential firing with other muscles. sEMG has also been used extensively in an attempt to quantify or characterize the force output of the muscle, as well as to determine the relative contributions of muscle or muscle groups for a given activity. Muscle fatigue can also be assessed by evaluating the frequency parameters of the myoelectric signal obtained. Our discussion will focus primarily on the evaluation of temporal responses, including the timing and sequential activation of muscles.

In order to fully appreciate the information that can be obtained from evaluating neuromuscular response characteristics with sEMG, an understanding of the various neuromuscular responses that occur and how EMG detects and visualizes these responses is useful.

Neuromuscular Response Mechanisms
The motor unit represents the smallest functional unit of the neuromuscular system and consists of a single motor neuron and all the muscle fibers it innervates. Each muscle fiber comprises a collection of myofibrils that run the length of the muscle fiber and are formed by a series of sarcomeres arranged end to end. The sarcomere represents the smallest contractile unit of the muscle and contains the contractile myofilaments, actin and myosin.

In order for the muscle tissue to function, it requires communication with the central nervous system (CNS) (ie, spinal cord and higher cortical centers) via peripheral nerves. A single peripheral nerve consists of both somatic and autonomic fibers that work in tandem to make appropriate adjustments with the body in response to environmental change. While autonomic nerves control smooth and heart muscle and exocrine glands, somatic nerves provide motor input
from the CNS to skeletal muscle and sensory feedback from muscle and joint structures back to the CNS.  

Alpha motor (efferent) neurons consist of a cell body located in the anterior horn of the spinal cord, a relatively large-diameter axon, and terminal branches that innervate a group of muscle fibers. Upon stimulation of the motor neuron, all the muscle fibers within a particular motor unit fire nearly simultaneously. Depending on the strength of contraction required, smaller motor units are recruited first, followed by the larger motor units to allow for a gradual increase in force.

Sensory (afferent) neurons, on the other hand, transmit information away from muscle, ligament, tendon, and capsular structures to the CNS for the neuromuscular response. This information is provided by specialized receptors, or mechanoreceptors, that function as transducers, converting mechanical energy in the form of physical deformation into electrical energy or action potentials. Collectively, these receptors provide the CNS with information regarding the position, displacement, velocity, and acceleration of the joint. Mechanoreceptors can either be rapidly adapting, to primarily sense rapid changes in deformation, position, or acceleration, or slowly adapting, to sense both static position and position changes of a particular joint structure. These receptors are further classified as low or high threshold, indicating the mechanical sensitivity of these receptors to a stimulus.

Specific mechanoreceptors have been identified based on their morphology and function. Mechanoreceptors found in muscle and tendon include the muscle spindle, sensing changes in length, and the Golgi tendon organ, sensing changes in tension. Mechanoreceptors found within joint structures have been classified by Freeman and Wyke as types I through IV. Type I mechanoreceptors are thought to resemble Ruffini-type endings and are characterized as low-threshold, slow-adapting receptors. Type II receptors are analogous to the Pacinian corpuscle with low-threshold, rapidly adapting characteristics. Golgi tendon-like organs are representative of type III receptors, which are high threshold and very slow adapting. Given their high threshold, these receptors, found exclusively in ligament structures, are thought to provide feedback at the end range of motion once sufficient tension is produced. Type IV receptors, also known as free nerve endings, have been identified in all joint structures except menisci and primarily provide feedback on pain stimuli. In recent years, each of these receptors has been found in the human ACL.

Once a mechanoreceptor exceeds its threshold and is activated, the action potential is rapidly conducted along the large-diameter myelinated afferent neuron to ultimately stimulate the appropriate reflexive muscular response. Generally, reflexive responses have been categorized as either monosynaptic (spinal) or long loop (intermediate) and always occur before
any voluntary activity.\textsuperscript{14,23,24,55,58,72} Given the substantially shorter latencies of these reflexive responses, their importance in providing rapid response to perturbation is evident.

**Monosynaptic reflex.** The simplest reflex arc is a mono- synaptic reflex, which comprises one afferent, stimulated by its sensory receptor, directly synapsing with an alpha motor neuron to cause muscular excitation or inhibition.\textsuperscript{58,61,72} This type of reflex, also termed the M1 response, spinal reflex, or short-loop reflex, originates at the spinal cord level and is generated by a local stimulus that results in a gross, quick movement requiring no cortical input.\textsuperscript{55} An example of this monosynaptic reflex is the tendon tap.\textsuperscript{14,24,72} Because of the simplicity of the spinal reflex, it is also the fastest.\textsuperscript{72} Mono- synaptic reflexes have been reported to occur within 20 to 60 milliseconds after initiation of a stimulus.\textsuperscript{48,55,58,71,73-75}

**Long latency reflex.** The long latency reflex, or late reflex response, represents a delay that exceeds monosynaptic latencies but precedes the earliest voluntary response times.\textsuperscript{55,58,73} Other terms that have been used for this delayed reflex include intermediate response or reflex, polysynaptic reflex, or long loop. While researchers have attempted to explain the origin and function of these reflexes, total agreement is lacking. However, most feel that long latency reflexes represent a spinal reflex consisting of one or more interneuron synapses between the sensory and motor neuron that receive convergent input from higher brain centers and other afferents capable of modifying the reflex response.\textsuperscript{58,61,73-77,23,24} While the length reported for the monosynaptic response latency is fairly consistent at about 20 to 30 milliseconds, the latency reported for the long-loop response appears to be quite varied. Some authors report typical latency times for this response in the 50- to 60-millisecond range,\textsuperscript{58,76} while other authors indicate much longer latency times, on the order of 100 to 150 milliseconds.\textsuperscript{55,73}

**Voluntary muscular control.** All reflexive activity, whether monosynaptic or intermediate, precedes the earliest voluntary response and therefore provides a more rapid response to perturbation or an injurious situation.\textsuperscript{58} Because of the considerable cortical input required for voluntary motor control, these responses occur at a significantly greater delay.\textsuperscript{72} Response times for voluntary movement have been reported to have a minimum delay of 170 milliseconds,\textsuperscript{58} although Chan et al\textsuperscript{73} reported shorter voluntary responses of 117.7 milliseconds in the quadriceps and 157.1 milliseconds in the gastrocnemius following light tendon taps. Substantially longer delays (as much as 400 milliseconds) have been reported in the literature as well.\textsuperscript{14,23,24,55}

**Response variations.** While most experts agree that voluntary responses are too slow to protect the joint from sudden perturbations, reflexive muscular activation may be sufficient to elicit a timely corrective or protective stiffening response in order to prevent further joint deformation.\textsuperscript{78} However, these responses are known to vary considerably depending on the activity state of the muscle,\textsuperscript{74,79-81} movement velocity,\textsuperscript{32} joint angle,\textsuperscript{33,36} weight bearing status and trunk
position,\textsuperscript{54,82,83} and prior training.\textsuperscript{23,24,30} Responses can also be inhibitory. A "silent period," representing a reflexive pause in firing of a contracting muscle, can occur in response to a stimulus such as a shock to skin or peripheral nerve, a tendon tap, a sudden decrease in load against which a muscle is contracting, or a sudden increase in load in an antagonist.\textsuperscript{84} This "silent period" was demonstrated by Marsden\textsuperscript{74} during random trials of sudden unloading of the thumb while moving against a constant force.

In summary, both monosynaptic and long-latency muscular reflexes, whether excitatory or inhibitory, appear to be important in providing a rapid and appropriate response to an imposed perturbation. However, if this feedback is provided by high-threshold Golgi tendon receptors in the ACL, it is likely the initiated muscular responses would not occur quickly enough, given that the delays in force generation would be greater than the time required to reach ligament failure.\textsuperscript{40,41} However, it remains plausible that intrinsic responses and sensory feedback provided by other joint structures at lower threshold may occur before ligament loading and prevent joint deformation from reaching the point of ligament failure. For instance, if the muscle senses tension before the ligament does, there may be time to stiffen the joint through both intrinsic mechanical as well as the stretch reflex mechanisms. Furthermore, Johansson and colleagues\textsuperscript{46,47} present evidence that low-threshold receptors in the cruciate ligaments may influence the sensitivity of the muscle spindle and provide preparatory stiffening of the muscle before excessive loading.\textsuperscript{68} Clearly, more research is needed to determine the efficacy of these proprioceptive feedback mechanisms in stabilizing the joint under sudden loading conditions. In addition, determining whether males and females differ in neuromuscular activation or mechanics in response to proprioceptive feedback may provide a potential explanation for the disparity in ACL injury rates. To that end, sEMG is one tool that has been and can be used to study neuromuscular response mechanisms and potential sex differences in muscular activation and recruitment.

\textit{Physiologic Basis and Characteristics of the Recorded EMG Signal}

In order to quantify and characterize reflexive muscular responses, electromyography is used to record and analyze the electrical activity of the muscle. Electromyography is concerned with the development, recording, and analysis of myoelectrical signals derived from motor unit activity.\textsuperscript{85} The most basic signal that can be obtained from the electromyogram is the action potential resulting from the depolarization and repolarization of a single muscle fiber membrane.\textsuperscript{85,86} As the nerve action potential arrives at the motor endplate, it is propagated out and away in both directions along each muscle fiber within the motor unit.\textsuperscript{57,60} In order to observe a single muscle fiber action potential, an indwelling microelectrode must be inserted within the muscle fiber. sEMG, on the other hand, uses electrodes applied to the surface of the skin and represents the extracellular recording of the muscle fiber action potentials at the skin surface.\textsuperscript{60}
In order to fully appreciate what is contained in the sEMG signal, it is important to know what these potentials look like graphically (Figure 1). With sEMG, 2 electrodes are typically used, and the electrical (voltage) difference or fluctuation between the 2 electrodes is recorded on the oscilloscope. In the absence of a motor unit action potential, the voltage difference between the 2 electrodes is zero and the signal is at baseline on the oscilloscope. However, when the motor unit is activated and an action potential is propagated along the muscle membrane, an electrical change is recorded by the overlying electrodes that coincides with the depolarization and subsequent repolarization of the muscle membrane. Since the potential is recorded extracellularly, the surface of the muscle membrane, which is normally electropositive, becomes electronegative as depolarization occurs (Figure 2). As this electronegativity reaches the first (proximal) electrode, it becomes negative with respect to the second (distal) electrode, and a positive deflection is recorded on the oscilloscope. As the action potential continues down the membrane across the second electrode and repolarization occurs at the first electrode, a negative deflection will be seen on the oscilloscope. Therefore, as an action potential passes by the 2 recording electrodes, a biphasic waveform results from the changing polarity (Figure 3).
Due to the increased distance of the electrodes from the muscle fiber and the larger recording area inherent in sEMG, the signal that is picked up consists of activity from multiple muscle fibers arising from one or more motor units. Therefore, as Figure 1 demonstrates, rather than a clean biphasic waveform, the myoelectric signal derived from sEMG provides a more general representation of muscular activity, recording one or more motor unit action potentials arising from all discharging units within the vicinity of the electrodes.60,78,85,86 The more isolated a given motor unit and the synchronization of individual muscle fiber firing, the more biphasic and higher the amplitude waveform that will be observed.57,87 However, as the force output of the muscle increases and the number of motor unit action potentials within the recording area increases, waveforms can vary considerably and identifying a single action potential becomes very difficult.57 Furthermore, only the superficial musculature can be assessed, since it is impossible to record deeper layers of the muscle without also recording the activity of the muscles lying superficially.56,88

**Limitations of sEMG**

Although a useful tool, electromyography is not without limitations, and, therefore, the methods and interpretations reported in the literature should be critically evaluated. A number of factors can seriously affect reliability issues by greatly influencing the quality and content of the myoelectric signal. DeLuca56 and others57,58,85,86,88,89 have identified several factors, both intrinsic and extrinsic, that can greatly influence the signal that is detected and recorded by the differential electrodes.

Intrinsic factors refer to the physiologic, anatomical, and biochemical characteristics of the muscle, which are typically outside the control of the researcher. These factors include 1) the number of active motor units at any one time that can affect signal amplitude and duration; 2) the fiber-type composition of the muscle, which can influence firing rate; 3) fiber diameter, which can influence amplitude and conduction velocity; 4) depth and location of the active fibers relative to the electrodes, which will determine the extent of spatial filtering, amplitude, and the frequency of the signal; 5) the amount of tissue (skin and adipose) between the electrodes and the muscle affecting spatial filtering; and 6) the variations in impedance and electrical properties within and between muscle tissue, fatty tissue, and skin that can affect the propagation of the current.56,57,89,90

Extrinsic factors are more within the control of the investigator and refer to the actual electrode configuration, which can largely determine the quality and quantity of the detected signal.56,58 The area and shape of the electrodes used will determine how large an area of the muscle is being recorded.56,88 Area is affected by both the size of the electrode used57,85 and the interelectrode distance that is chosen.56,57,86,88 The position of the electrodes over the muscle is also an important factor. Surface electrodes are limited to the detection of motor unit action potentials that arise from fibers in close proximity to the electrode site and will record muscle
fiber activity only within 1 to 2 cm of their locations.\textsuperscript{58,86} Furthermore, the longitudinal and horizontal placement of the electrode in relation to the muscle can have considerable impact on the acquired signal.\textsuperscript{56,57} It becomes apparent that even slight changes in electrode configuration or orientation can significantly alter the signal obtained both within and across subjects. Unfortunately, no standards exist as to the proper configuration or dimensions of surface electrodes for a given purpose.\textsuperscript{56} Therefore, to insure reliability of data and comparisons across trials and subjects, the application method and size, type, spacing, and location of electrode placement must be standardized, adequately reported, and controlled.\textsuperscript{85,88,91}

Signal artifact can also greatly affect the fidelity of the signal and, thus, signal reliability and interpretation. Artifact is defined as the components of the EMG signal that are not produced by the electrical activity of the intended muscle and are instead produced by crosstalk (activity of nearby muscles not under study),\textsuperscript{56,57,85,88,92,93} electrical interference from nearby electrical devices,\textsuperscript{57,59,85,88,92} or movement artifact.\textsuperscript{57,85,88,92} Signal artifact can be detected anywhere within the EMG instrumentation and at any point during the recording process.\textsuperscript{89} However, the influence of artifact can be greatly minimized with proper electrode configurations, adequate skin preparation, high-quality instrumentation, stabilization of electrodes and lead wires, and an electromagnetically shielded or quiet-environment recording.\textsuperscript{57,85,88,92}

Clearly, there are a number of anatomical, physiologic, and technical factors that must be considered and controlled when conducting EMG research, since they can greatly influence the EMG signal.\textsuperscript{56,85} Additionally, how the signal is processed and analyzed can result in serious measurement error. Therefore, appropriate documentation and reporting of the instrument parameters, detection methods, and processing techniques are essential if one is to truly understand and accurately interpret or replicate research findings. To that end, the Ad Hoc Committee of the International Society of Electrophysiological Kinesiology was developed in 1980 to address recommendations for the standardization of EMG instrumentation, documentation, and data reporting.\textsuperscript{91} These recommendations are outlined in each issue of the Journal of Electromyography and Kinesiology and can be accessed from the Journal of Athletic Training web page at http://www.nata.org/jat.

**Temporal Measurement of Reflexive Activation**

The most basic information that can be derived from the EMG signal is whether or not a muscle is active or at rest.\textsuperscript{93} The timing and phasing of this muscular activity has been used to determine muscular response characteristics such as reaction time,\textsuperscript{14,23,24,55,65,71} electromechanical delay (EMD),\textsuperscript{27,28} and firing patterns* in response to a stimulus.

**Muscle reaction time.** Muscle reaction time is a valuable tool in determining how well the joint detects a disturbance and how quickly the muscles respond to a stimulus or perturbation. Muscle reaction time or latency refers to the time it takes from the onset of the stimulus for the action
potential to reach the intended target muscle, as indicated by electrical activity recorded in the EMG signal. For time-response studies, a contact switch or similar mechanical device can be interfaced with the EMG to accurately mark when the stimulus occurs and thus provide reliable measures.

**Electromechanical delay.** Electromyography has also been used extensively to quantifiably measure the time lapse between the change in electrical activity and the actual force generation in the muscle. It is important to realize that the EMG signal reflects only the electrical activity of the muscle, which is not synonymous with the production of tension. In fact, a natural EMD exists between neural activation of the muscle as recorded electrically by EMG and the actual generation of force. EMD can be measured using a force transducer (or similar device) interfaced with the EMG to detect and quantify when muscular tension is developed after neural activation (Figure 4). This delay can be quite variable due to factors such as fiber-type composition and firing rate dynamics of the muscle, velocity of movement, viscoelastic properties and length of the muscle and tendon tissues, activity state, and coactivity of other muscles. EMDs reportedly vary anywhere from 30 to 50 milliseconds to as much as a few hundred milliseconds. Considering this additional time lapse and the need to develop sufficient muscular tension rapidly enough to provide dynamic joint stability, EMD should be considered when evaluating muscular responses to an imposed perturbation or injurious stress.

![Figure 4. Monosynaptic (M1) and voluntary (V) myoelectric activity and force-generation recordings of the quadriceps following a reactive patellar tendon tap. EMD represents the time lapse between start of EMG activity and force generation for each response.](image)

**Recruitment and coactivity patterns.** When muscle reaction time and EMD measures are collected on more than one muscle or muscle group, activation patterns such as recruitment order and coactivity around a joint can also be evaluated. To measure muscular firing patterns, such as the order of activation of various muscle groups about a joint, all that is required of the measurement device is to determine when one muscle is active in relation to the other muscles under observation. In addition to the order of recruitment, the relative extent to which a muscle responds or contributes to joint stabilization under a given condition can also provide useful information. This relative response can be determined by comparing the percentage of each

*References 23, 30, 39, 49, 52, 53, 55, 94, 95.*
muscle's activity to its maximal voluntary isometric contraction (% MVIC). The % MVIC is typically calculated by dividing the amplitude of the EMG signal during the activity under study by the amplitude obtained during a controlled MVIC of the muscle.

**Determining onset time.** Determining the exact time a muscle becomes activated after a stimulus can be influenced by a number of factors. With sEMG, the onset of myoelectric activity will reflect the combined latency (both nerve and muscle) of the fastest muscle fibers in the vicinity of the electrodes.\(^{57,85}\) While the average conduction velocity of the motor neuron is around 100 m/s, the average conduction velocity of the muscle fiber is approximately 4 m/s.\(^{56,58,85}\) Therefore, latency is dependent on the length of the peripheral nerve (ie, the distance the action potential must travel before reaching the motor endplate), nerve conduction velocity, terminal nerve fiber conduction velocity, transmission delays at the neuromuscular synapse, and muscle fiber conduction velocity.\(^{57}\) As a result, factors such as electrode placement and distance from the motor point, individual physical characteristics, and mechanical or physiologic characteristics of the muscle can significantly influence the relative or absolute differences found in latency measures.\(^{73,75,76,87,88,96,97}\) Furthermore, the methods used to determine the onset of muscle activity can also greatly influence the latency measures obtained.\(^{98,99}\)

To accurately determine the onset of muscle activity, the clinician or researcher must be able to confidently and consistently identify when EMG activity begins or significantly deviates from static or baseline activity. To do so, the EMG signal must exceed a threshold that can be defined in some way, either visually (subjective) or by a statistically predetermined level (objective).\(^{94}\) As is true in most EMG methodology, while there is no universally accepted method for determining precisely when muscle activity onset occurs, a number of methods have been used to aid in this determination.\(^{93}\)

One subjective method is to use the raw signal along with visual recognition, using subjective criteria to determine when muscle activation occurs or to mark the point at which EMG activity begins or changes abruptly from baseline activity.\(^{93,98}\) The subjectivity of this assessment poses serious threats to measurement reliability, particularly between investigators.\(^{98}\) Furthermore, under conditions where the muscle is already contracting and considerable baseline activity is present, the exact moment muscle activity deviates from baseline is often obscured and difficult to determine visually.\(^{59}\)

An alternative, more objectively defined method is to use a computer-assisted analysis program to identify a muscular event based on statistical criteria.\(^{98}\) An example of a computer-assisted analysis is to take a representative sample of the baseline activity, statistically determine the mean value and standard deviation of the signal, and then use 2 or 3 standard deviations from average baseline activity as the threshold for detection.\(^{56,59}\) Using a 2-standard deviation threshold allows the researcher to be 95% confident that a significant change has occurred in
muscle activity that is not a result of random occurrence. However, while these computer-assisted methods yield more reliable measures, they are unable to confirm the validity of the measure or event. As such, some level of visual recognition by an experienced investigator is still required. Paramount to any onset detection or statistical analysis method used, it is essential that the chosen method be well defined and consistently used if measurement reliability is to be achieved. This applies equally to any signal-processing techniques that can also influence measurement reliability and validity.

**Signal processing.** Oftentimes, the raw signal must be processed in order to more clearly distinguish and separate meaningful or significant events. Processing techniques usually involve some type of filter or mathematical average in order to reduce the number of data points and provide a clearer representation of signal activity. Two common signal-processing techniques often used are root mean square smoothing and signal averaging. EMG data is typically collected at 1000 Hz or one data point every millisecond. When processing the raw signal with a root mean square, all data points are converted to a singular polarity (rectified) by squaring them (Figure 5A) and then averaging over a user-defined time interval. By choosing longer time intervals (ie, time duration over which data points are averaged), fewer data points will be produced, which will result in a smoother signal over a given time series (Figure 5B). This method effectively filters the signal to provide a more general representation of muscular activity. Signal averaging takes this a step further by superimposing multiple trials or tracings on one another to produce a composite or averaged signal that is representative of activity across

![Figure 5. Raw rectified (A), root mean square (B), and averaged (C) EMG signal tracings for repeated reflex activation trials.](image)
all trials (Figure 5C). However, in order to use signal averaging, data must be acquired at the same precise time and duration across all trials. This can be accomplished through a trigger-sweep data-collection mode using a mechanically reliable triggering device to clearly define when a trial begins or ends.\textsuperscript{100}

When processing methods are used before the determination of muscle activity onset, it is important to realize that, any time the raw signal is processed or filtered, a loss of EMG information results and the actual rise time of the signal may be significantly altered, affecting the researcher’s ability to determine the exact time of muscle activity onset.\textsuperscript{89,93} Therefore, while processing may be necessary to assist the researcher in yielding more consistent and systematically accurate measures, statistically significant changes may occur from processing alone. To exemplify this fact, Gabel and Brand\textsuperscript{99} studied the effects of various processing methods on measurement variation and statistical significance, comparing left and right differences in EMG signals for the vastus lateralis and medial gastrocnemius during gait. Their purpose was to determine whether the number of gait cycles averaged or the degree of filtering (smoothing) had any effect on the statistical results obtained from the variance ratio, coefficient of variation, Pearson $r$, analysis of variance, and $t$ test. Their results demonstrated that all statistical tests were affected to some degree (with some influenced more than others) by the degree of filtering or averaging, or both. Given their findings, the ability to compare results across studies using different processing techniques would seem questionable. Furthermore, since this study was carried out on 2 healthy subjects with no lower limb clinical pathology, their findings also demonstrated that statistically significant variations can be found in the absence of clinically significant differences. Therefore, in order for results of sEMG data to be clinically meaningful, to be accurately interpreted, and to allow comparisons across studies, it is essential that investigators justify and report in detail the type and method of signal processing used, as well as the statistical test used to determine muscle activity onset time.

In summary, the absolute measurement of muscle response times via sEMG can be influenced by a number of factors. Each of these factors alone can result in significant variations in latency measures that may obscure or confound clinically significant variations. Unfortunately, the manner in which EMG has been used to assess neuromuscular response characteristics in terms of instrumentation, signal processing, and data acquisition is varied and at times quite confusing and poorly understood; no standardized procedures currently exist in this regard. Additionally, many research papers fail to adequately report their procedures, which prevents others from being able to replicate or validate their findings.\textsuperscript{92} What appears then to be the most important factor when assessing neuromuscular response characteristics with EMG is not necessarily which methods are used, but whether the methods are consistent, well defined, and well controlled for all trials and tests to insure that a measure is reliable, valid, and comparable with other studies.\textsuperscript{56,59,93}
RESEARCH APPLICATIONS ASSESSING SEX DIFFERENCES IN NEUROMUSCULAR RESPONSES

Given the role of musculature in maintaining joint equilibrium and stability at the knee, there has been considerable interest in investigating neuromuscular response characteristics and their association with ACL injury. A number of research models have employed sEMG to evaluate activation patterns at the knee after joint loading or perturbation (ie, a mechanical stress placed on the joint either internally or externally). However, most of these models have evaluated this relationship from a postinjury, rehabilitative reference point rather than a preinjury, predictive one. Very few studies to date have specifically addressed potential sex differences in neuromuscular response characteristics. We found only one published study that specifically addressed this relationship at the knee.

Sex Differences at the Knee

Huston and Wojtys appear to have been the first to assess sex differences in neuromuscular responses at the knee. Their purpose was to identify potential physiologic differences between males and females with regard to anterior tibial laxity, isokinetic measures (strength, endurance, and time to peak torque at 60°/s and 240°/s), and neuromuscular responses (muscle reaction time and muscle recruitment order) after anterior tibial translation. An anterior tibial translation device, first described by Wojtys and Huston, was designed to apply an unanticipated, anteriorly directed force to the posterior aspect of the lower leg with the subject in a semiseated, partial weightbearing position and the knee flexed to 30°. Potentiometers placed on the patella and tibial tuberosity were used to quantify the relative tibial displacement in relation to the femur. sEMG electrodes were placed over the midbelly of the medial and lateral quadriceps, medial and lateral hamstrings, and the gastrocnemius muscles to record spinal, intermediate, and voluntary response times and recruitment patterns in response to the perturbation. These response times represented the time delay between the initiation of the anterior tibial translation force stimulus and the onset of the monosynaptic reflex, long-loop reflex, and voluntary responses, respectively. Onset for each response was determined based on time of occurrence and signal shape characteristics.

Female athletes participating in Division I basketball, field hockey, gymnastics, and volleyball were compared with Division I football players and nonathlete male and female controls. The findings identified no differences in spinal, intermediate, or voluntary response times after anterior tibial translation. However, a different muscle recruitment order was observed at the intermediate reflex response levels in the female athlete group compared with all other groups. At this response level, female athletes more often initiated the quadriceps first, while the male athlete and control groups preferentially activated the hamstrings first in response to anterior tibial translation. No difference in recruitment patterns at the spinal and voluntary response level were found between sexes. Sex differences were also found with isokinetic testing, in that female athletes took significantly longer to reach peak torque in their hamstrings compared with male

References 14, 23, 24, 30-32, 39, 48, 49, 52, 55, 71.
athletes, both at 60°/s and 240°/s. While no correlation was found between muscle strength and response times, the 5 strongest female athletes used a voluntary muscle recruitment order favoring initial activation of the hamstrings, while the 5 weakest favored initial activation of the quadriceps.

This model effectively demonstrates an objective and well-controlled method by which to quantify dynamic muscular activation in response to an unanticipated knee perturbation. Furthermore, it demonstrates the use of isokinetic dynamometers to provide a measure of mechanical force delay within the muscle. While time to peak torque is not a true measure of EMD, this measure does account for delays in mechanical force production not accounted for in the myoelectrical activation time recorded via EMG. However, it would seem reasonable that true EMD could also be measured if EMG data were collected simultaneously with an isokinetic dynamometer or other force transducer and if the precise time at which force was initiated (rather than peaked) could be determined.

This study also demonstrates some of the previously discussed limitations and reliability concerns associated with EMG measures. Inadequate reporting of instrumentation, of signal processing, and of the method used for determination of muscle activity onset time makes it difficult for others to replicate their findings. In addition, the time delays reported by Huston and Wojtys for spinal and intermediate reflexes appear to be substantially longer than those reported by others. Therefore, reporting whether the signal was processed and the method by which muscle activity onset time was determined is essential if one is to adequately interpret the findings of Huston and Wojtys or compare their results with others.

*Sex Differences at Other Joints*

Force plates (and similar force transducers) have been used with EMG at other joints in order to measure sex differences in EMD in addition to myoelectric response times. Winter and Brookes measured both myoelectric and electromechanical response delays in males and females during a rapid, voluntary plantar flexion movement. With the subject seated, the ball of the foot was positioned on a force platform to record muscular force generation, and the heel was placed over a pressure pad to record initiation of joint movement. Surface recording electrodes were placed over the lateral surface of the soleus to monitor myoelectric activity. In response to an auditory stimulus, subjects were asked to plantar flex the foot as quickly as possible. Time delays from stimulus to EMG activity, EMG activity to initial force generation, EMG activity to initiation of heel movement, force generation to heel movement, and total reaction time (stimulus to heel movement) were quantitatively measured. Their results indicated no differences in myoelectric response times, but they did find significant differences in EMD, both in time from force generation to heel movement and in EMG activity to heel movement. The methods were well reported in this study, and standard error values as well as test-retest coefficients of variation were also reported. Reporting these error coefficients provides a sense of how variable
the data are between tests and provides a basis with which to compare statistically significant findings. While the authors did not include in their discussion the relationship of these measures to their findings, it appears that the statistically significant differences obtained were only slightly greater than the standard measurement error.

Similarly, Bell and Jacobs\(^\text{27}\) compared myoelectric response times and EMD in males and females during a maximal contraction of the elbow flexors after a visual stimulus. Subjects were assigned to one of 4 groups based on sex and maximum biceps force generation (ie, weak and strong males, weak and strong females). Subjects were asked to quickly and maximally contract the biceps in response to a light stimulus while holding a bar attached to a force transducer. With the light stimulus acting as a trigger to begin data recording, EMG activity (over the belly of the biceps) and force measures were recorded for a 2-second interval. Both onset of EMG and force generation were determined via computer software using threshold-detection methods. Results indicated no difference in myoelectric response time among the 4 groups. However, the EMD in both male groups was significantly shorter than in both female groups. No correlation was found between response times and strength.

These studies suggest that intrinsic, mechanical properties within the muscle may differ between males and females, with males having the ability to initiate a more immediate stiffening response after muscular activation.\(^\text{28}\) However, the relevance of these findings to the knee musculature is not known. Furthermore, these studies evaluated muscular response characteristics under voluntary conditions with the muscle at rest before the stimulus. These conditions are not representative of the dynamic and reflexive responses that may occur with joint perturbations or during sport activity, where the muscle may already be contracting. Research models assessing sex differences in reflexive stiffening and EMD at the knee under dynamic conditions and after unexpected joint perturbations are needed.

DIRECTIONS FOR FUTURE RESEARCH

From the limited research available, it appears that males and females may differ in some aspects of neuromuscular responses. However, more research is needed to draw firm conclusions regarding these differences and their potential roles as possible predisposing factors to the higher incidence of ACL injury in females.

When considering previous studies that have measured reflexive activation patterns after unanticipated joint loading or perturbation, the response stimulus has been typically applied in an open chain or partially loaded lower extremity under resting conditions.\(^\text{14,23,24,48,55,71}\) Unfortunately, these conditions do not mimic the environment of the joint during the activities when these injuries are likely to occur. Research indicates that hamstring activation patterns and their ability to stabilize the knee can vary substantially depending on weight-bearing status, joint angle, and trunk position.\(^\text{33,36,54}\) Furthermore, whether a muscle is actively contracting before the
perturbation may greatly influence immediate stiffening responses and reflexive activity patterns. Therefore, there is a need to assess neuromuscular responses using functional, full weightbearing activities and perturbation models. Studies by Gauffin and Tropp and Branch et al assessing dynamic activation patterns in ACL-deficient subjects during jumping and cutting activities may provide potential models to assess sex differences during similar activities.

Research should also address sex differences in intrinsic stiffening responses and delays in force production not accounted for in EMG measures alone. In order for the neuromuscular system to be effective in preventing ligament strain, muscular tension must be developed in a timely fashion to limit joint deformation. Measures of intrinsic stiffening before reflexive muscular activation, as well as the EMD after myoelectric activation, provide essential information regarding the adequacy (or inadequacy) of protective neuromuscular response mechanisms. Furthermore, given recent evidence suggesting that estrogen levels may affect collagen metabolism and tissue compliance, the influence of this hormone on intrinsic and electromechanical response characteristics in females deserves attention.

Future studies should also consider assessing sex differences while controlling for skill level and training across subjects. Both specificity of training and level of conditioning may significantly impact muscle reaction time and coactivity patterns, and thus the ability to provide dynamic stability and adequate joint protection. While the study by Huston and Wojtys appears to be the first to specifically address potential sex differences at the knee, it should be noted that the female and male athlete groups participated in different sports. The different skill and training backgrounds required for various sport activities could potentially confound results, making it difficult to determine whether differences were due to sex or training. Therefore, training variables should be considered when developing future research models to explore potential sex differences in neuromuscular response characteristics.

Finally, it is apparent from the literature that the manner in which sEMG has been used to assess neuromuscular response characteristics has been quite varied and has been, at times, inadequately reported. Given the multiple factors that can influence the detection and interpretation of the EMG signal, lack of standardization and reporting make it difficult to interpret the findings and compare results between studies. Moreover, given the inherent variability in EMG data, including reliability estimates and reporting the standard error of measurement would seem prudent and would provide the reader with the information needed to critically evaluate the clinical versus statistical significance of a study's results. In order to detect true differences between the sexes, any statistically significant difference must reasonably exceed the expected variability in scores that can be evaluated only with repeat testing and reliability studies. Of the studies discussed previously, only Winter and Brookes reported test-retest measurement variance with their data. However, Huston and Wojtys did report expected
measurement variability in previous work and stated that this variation was accounted for in their statistical analysis. Future investigators should subject their data to the scrutiny of this measurement analysis if truly valid and clinically relevant conclusions are to be made. Shrout and Fleiss and Denegar and Ball provide excellent discussions on the issues and computation methods associated with measurement reliability and standard error of measurement.

CONCLUSIONS
The speed at which muscular activation can be generated may be an important determinant in providing dynamic stability and potential injury prevention. Whether or not a difference in muscular response characteristics exists between the sexes may be a significant finding in assessing ACL injury risk in females. However, more research is clearly needed in this area. Future studies should address sex differences at the knee under functional, weightbearing conditions while controlling for training and other confounding variables. Other associated factors, such as hormone levels and their influence on muscular mechanics and activation patterns in females, should also be addressed.

To that end, sEMG can provide a useful tool to assess potential sex differences in the timing, recruitment order, and coactivity patterns of the knee musculature in response to an imposed perturbation. However, the appropriate application, as well as limitations, of this instrument must be fully realized if quality research is to be conducted and if valid and reliable results are to be obtained. Although this review is far from exhaustive regarding the many technical aspects of sEMG, our hope is that the information presented here will enhance the reader's appreciation for the use of this evaluative tool and generate further interest in and research on this timely topic.

REFERENCES


