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8. Sera were numbered and frozen. GluR and β4-nAChR *trpE* fusion protein constructs were produced and analyzed as described (*4, 7*), except we used 2% dry milk (Blotto) to block blots. Blots were incubated overnight at 4°C in primary serum diluted 1:1000 in Blotto, then in goat antibody to human IgG conjugated to alkaline phosphatase diluted at 1:750 in Blotto for 1 hour at 25°C, and then developed (*4*). We removed *Escherichia coli* background by incubating a suspension of lysed, *trpE*-expressing bacteria in the serum sample for 2 hours at 25°C and then centrifuging it. In another examination, each protein immunoblot was photographed, coded, and interpreted blindly by three investigators; interpretations were congruent in 73 of 76 samples (*9*).
9. S. W. Rogers *et al.*, unpublished data.
10. The single control individual whose serum exhibited immunoreactivity to GluR3 fusion protein by protein immunoblot analysis was sampled 1 week after open heart surgery. Cardiac bypass under hypothermic anesthesia may be a nonspecific activator of the immune system [J. R. Utley, *J. Cardiac Surg.* **5**, 177 (1990)]. Her serum was not immunoreactive with GluR3 expressed in transfected cells, suggesting that the immunoreactivity detected by protein immunoblot analysis may not be to native GluR3.
11. HEK 293 cells were transfected with expression plasmids containing the cDNA for either GluR1, 3, or 6 or the bacterial protein β-galactosidase (*4, 7*). The serum from individual AT exhibited nuclear immunoreactivity that we removed by adsorbing the serum samples with HEK 293 cells transfected with the parent expression plasmid without an insert (*4*). Sera were coded and tested in two laboratories independently (T.E.H. and S.W.R.). Sera from immunized rabbits served as the positive controls.
12. ELISA assays used either GluR3 or β4-nAChR fusion proteins that were solubilized and adsorbed to microtiter plates (Immulon) (*4, 7*). Wells were blocked with Blotto for 2 hours at 25°C before we added sera at various dilutions for 1 hour at 25°C. We observed immunoreactivity using goat antibody to human IgG, conjugated with peroxidase, and 2,2′-azino-di-(3-ethylbenzthiazoline-6-sulfonic acid) (1 mg/ml) in McIlvain's buffer (pH 4.6) and 0.0005% hydrogen peroxide. Duplicate samples were scanned at 405 nM with an ELISA reader (Teritek). We determined GluR3-specific immunoreactivity by subtracting β4-nAChR reactivity from GluR3 reactivity.
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Variable Gearing During Locomotion in the Human Musculoskeletal System

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Human feet and toes provide a mechanism for changing the gear ratio of the ankle extensor muscles during a running step. A variable gear ratio could enhance muscle performance during constant-speed running by applying a more effective prestretch during landing, while maintaining the muscles near the high-efficiency or high-power portion of the force-velocity curve during takeoff. Furthermore, during acceleration, variable gearing may allow muscle contractile properties to remain optimized despite rapid changes in running speed. Force-plate and kinematic analyses of running steps show low gear ratios at touchdown that increase throughout the contact phase.

Toes were present in the earliest tetrapods (*1*) and occur in all modern tetrapods except those that are highly specialized for limbless or aquatic locomotion. Feet and toes form an adaptable interface between the animal and its environment. They provide traction and a means for grasping the substrate, function as various tools and weapons (*2*), and help to maintain balance (*3*). In this report, we suggest that feet and toes improve locomotor performance by varying the gear ratio (that is, the velocity ratio) between the ankle extensor muscles and the point of application of the force on the ground (the center of force) during the course of the contact phase of a running step.

The proposed mechanism is easily visualized during running at a steady speed (Fig. 1). The foot is analyzed as a simple Type 1 lever of zero mass with the fulcrum at the ankle by means of the equation $R \times F_r = r \times F_m$, where R is the ground force moment arm, F_r is the ground reaction force, r is the muscle force moment arm, and F_m is the muscle force. During the contact period of a step, the point at which force is applied to the ground (the center of force) moves from under the heel or middle portion of the foot at touchdown to the tips of the toes at takeoff. This forward translation could increase the length of the moment arm between the ankle and the force exerted on the ground (R) and, therefore, increase the gear ratio (R/r) of the ankle extensor muscles and tendons.

Variable gearing would be advantageous in running, as it is in the automobile, because in both cases the motors (cross-bridges in muscle and pistons in engines) have a limited speed range over which they operate at peak power or efficiency (*4*). In order to maintain a narrow range of optimal engine speeds despite varying drive speeds, the ratio of engine speed to drive speed must be changed by a variable gear ratio. Furthermore, muscles have unique properties that can benefit from variable gearing within the contact phase of a running step. Active muscles that are forcibly stretched just before shortening are able to do more work during the shortening. This nonelastic enhancement of the contractile properties of the muscle increases, within limits, with increasing stretch length (*5*) but is effective over relatively small shortening distances. If a runner were to land at a low gear ratio and take off at a higher gear ratio, both the prestretch and the subsequent shortening of the muscles could be optimized. Thus, variable gearing could reduce the need for locomotor specialization, allowing individuals to move about more efficiently, accelerate more quickly, run faster, and jump higher.

To test this hypothesis, five people (3 males and 2 females) ran over a Kistler 9281B force plate. Four of these people also accelerated maximally over the force plate, starting just off the plate so that the first step landed on the plate. A lateral view of limb position was recorded with a Peak high-speed video camera at 120 images per second (Fig. 1). Recordings of forces on the ground allowed calculation of the magnitude and orientation of the ground reaction force and the position of the center of force under the foot. For each video image taken during foot support (at 8.33-ms intervals), the ground force moment arm (R) was calculated by dividing the moment at the ankle by the resultant of the horizontal and vertical ground forces. Similarly, the mus-

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cle force moment arm (r) was calculated as the perpendicular distance from the Achilles tendon to the ankle.

In these people during running at constant speed, the center of force moved from under the arch of the foot just after touchdown to the tips of the toes at takeoff (Fig. 2A, solid points) (6). This forward translation of the center of force increased the length of the moment arm between the ankle and the center of force and, therefore, increased the gear ratio (R/r) of the extensor muscles of the ankle (Fig. 2B, solid points). In four people, the gear ratio was less than 1 at the beginning of support and increased to 3 to 4 by the time of takeoff. In the fifth person, the gear ratio also started off at less than 1 and increased during the first half of support but leveled off at a peak value of just over 2.5 at midsupport.

A different pattern was observed when the subjects accelerated maximally from a standing start (Fig. 2A, open points). In this case, the center of force reached the ball of the foot sooner and remained there longer, not moving under the toes until just before takeoff. The gear ratio was higher early in the first half of the contact phase but was always lower in the second half of the contact phase (Fig. 2B) (7).

The observed variation in gear ratio resulted largely from changes in the length of the lever from the ankle to the center of force on the ground (R). Whereas R often changed by as much as a factor of 20, the muscle lever (r) changed by less than 15%. Changes in R can result from two separate causes: movement of the center of force along the length of the foot (Fig. 2A) or changes in the orientation of the ground reaction force relative to the position of the ankle (Fig. 1). Our results indicate that these factors influence R to varying degrees in different individuals and during different locomotor activities. However, during running at a constant pace, the change in R due to translation of the center of force was usually 10 times greater than the change due to changes in the orientation of the ground reaction force. Furthermore, during rapid acceleration, R changed very little, and the center of force remained stationary under the ball of the foot (Fig. 2A). These observations indicate that the long foot and toes of human runners are primarily responsible for the gearing mechanism by allowing the position of the center of force to vary (8).

We looked at the effect of variable gearing on the shortening velocity of the calf muscles by comparing the kinematically measured shortening velocity of the muscle-tendon system to what that velocity would have been in the absence of variable gearing (9). To determine the velocity of the muscle-tendon system, we multiplied the angular velocity of the foot around the ankle by the length of the muscle lever (r). The muscle-tendon system in our participants showed negative velocities during the first half of foot support (Fig. 3), which indicated that the muscle-tendon system was stretched as the ankle was flexed dorsally. During the second half of foot support, when the ankle was extended, shortening velocity increased rapidly to a maximum at takeoff. We then calculated the muscle-tendon velocity required, in the absence of variable gearing, to maintain the same ankle-to-toe velocity (that is, the same contribution by the ankle and foot to the motion of the body). The gear ratio, fixed at the value recorded at midstance (10), was divided into the ankle-to-toe velocity in order to calculate the muscle-tendon velocity. Two important differences emerged from this analysis (Fig. 3). First, a constant gear ratio would result in much less stretching of the muscle-tendon system on landing, and the potentiating prestretch of the muscle would therefore be reduced. Second, as predicted, a constant gear ratio would require higher shortening velocities of the muscle-tendon system during takeoff.

These observations show that during the first part of the contact phase of a running step, the gear ratio of the calf muscles is quite low (less than 1) (Fig. 2B). This increased the stretch applied to the muscles (Fig. 3) and therefore the work the muscles could do during subsequent shortening (5). During the middle part of the contact phase, the gear ratio remained relatively low (about 2). Thus, the mechanical advantage of the muscles was relatively high during the period when the force exerted on the ground was the greatest and the muscle shortening velocity was the lowest. And finally, during the latter part of the contact phase, as the force exerted on the ground decreased and the velocity of the foot rapidly increased, the gear ratio increased to 3 to 4. This would tend to keep the muscle operating at a lower velocity and in the

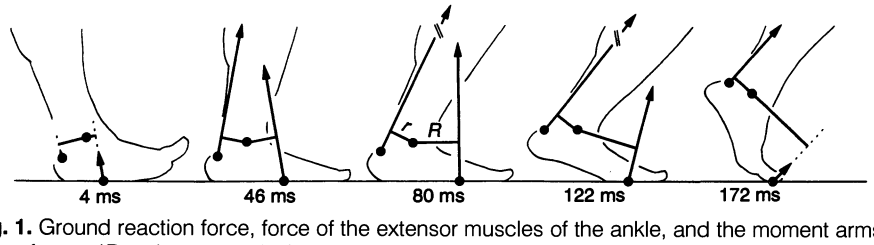

Fig. 1. Ground reaction force, force of the extensor muscles of the ankle, and the moment arms of these forces (R and r, respectively, labeled in the center panel) during foot support while running at a constant speed. The ground force moment arm, R, is the perpendicular distance from the ground reaction force to the ankle. The muscle force moment arm, r, is the perpendicular distance from the Achilles tendon to the ankle. The dot between the foot and the ground is the position of the center of force.

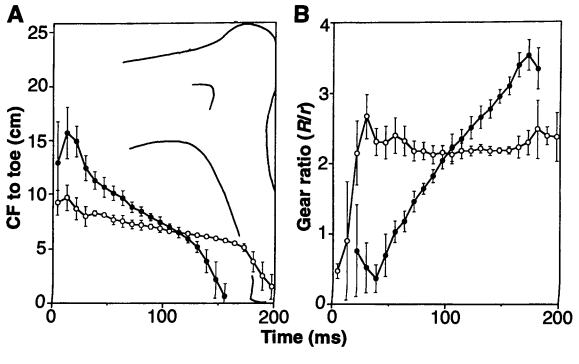

Fig. 2. (**A**) Distance between the center of force (CF) exerted on the ground and the tip of the first (big) toe during the ground contact phase in a representative individual. The tracing of the foot on the right side of the graph provides a reference. Solid circles show the mean and standard deviations of four trials of running at a constant speed; open circles show the mean and standard deviations of three trials of rapid acceleration from a standing start. (**B**) Gear ratio (R/r) during ground contact phase. Subject and indications are as in (A).

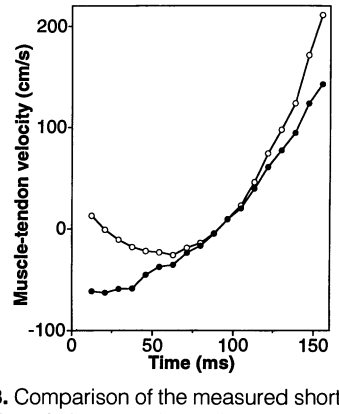

Fig. 3. Comparison of the measured shortening velocity of the muscle-tendon system (solid circles) to the calculated shortening velocity when the gear ratio was fixed at the value recorded at mid-stance (open circles). Same subject as in Fig. 2.

higher force portion of the force-velocity curve and quite likely would tend to maintain the muscles at a relatively high power and efficiency (*11*). Further evidence that gearing may enhance the contractile performance of the calf muscles comes from the observation that during rapid acceleration the gear ratio remained relatively low (Fig. 2B). Again, this is what would be expected if the system were to function so as to maintain the muscles at high power.

Morphologists and muscle physiologists have long recognized that synergistic muscles often have different mechanical advantages around joints, and they have therefore suggested that different muscles use different gears (*12*). Leverage around joints has also been shown to vary with body size (*13*) and during ontogeny (*14*). But these separate muscle systems and allometric patterns are not variable gearing mechanisms for individual muscles within an individual. Variable gearing has been suggested to result from the unusual organization of the ankles of artiodactyls and lagomorphs (*15*) and has been documented in the flight system of blowflies (*16*). Additionally, humans have been shown to shift mechanical advantage around the hip, knee, and ankle when they change gaits from a walk to a run (*17*), presumably using mechanisms similar to those proposed here.

The concept of variable musculoskeletal gearing has received relatively little attention from physiologists. Given the variety of species specialized for terrestrial locomotion, and the grace, skill, and speed with which many animals run and jump, it would be surprising if variable gearing had not evolved.

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7. The difference in gear ratio between running and acceleration was significant at the 0.0001 level, as measured by repeated multivariate analysis of variance (MANOVA), for the four people from whom we have recordings of both running and acceleration.
8. In contrast to these results from humans, preliminary data we have collected from a dog show a pattern of decreasing gear ratio during ankle extension. The center of force translates forward during foot support in this dog as it does in humans, but the more vertical orientation of the foot results in the ankle catching up to the ground reaction force during late foot support. This results in a decrease in the length of R and a decrease in the gear ratio.
9. Our kinematic technique is limited by the fact that an unknown portion of the measured change in length resulted from the stretch of the tendon in series with the muscles.
10. This is an appropriate value of gear ratio to use for comparison, because mechanical advantage at midstance is often used in calculations and modeling by biometricians.
11. It is also possible that the observed gearing could have a detrimental effect by increasing the gear ratio too quickly, in a manner analogous to trying to accelerate a car rapidly while in third or fourth gear. This would keep shortening velocities below those at which peak muscle power was produced.
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Characterization of a Functional $GroEL_{14}(GroES_7)_2$ Chaperonin Hetero-Oligomer

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Chaperonins GroEL and GroES form two types of hetero-oligomers in vitro that can mediate the folding of proteins. Chemical cross-linking and electron microscopy showed that in the presence of adenosine triphosphate (ATP), two $GroES_7$ rings can successively bind a single $GroEL_{14}$ core oligomer. The symmetric $GroEL_{14}(GroES_7)_2$ chaperonin, whose central cavity appears obstructed by two $GroES_7$ rings, can nonetheless stably bind and assist the ATP-dependent refolding of RuBisCO enzyme. Thus, unfolded proteins first bind and possibly fold on the external envelope of the chaperonin hetero-oligomer.

Chaperonins, also called cpn60 and cpn10, belong to a ubiquitous class of sequence-related chaperone molecules in mitochondria, chloroplasts, and bacteria. In the cell, they are implicated in the folding of proteins (*1*) and in the molecular response to cellular stress (*2*). In vitro, chaperonins assist in the correct refolding of proteins by preventing aggregations (*3, 4*). As determined by electron microscopy, cpn60 from bacteria (GroEL) is an oligomer of 14 identical 57.3-kD subunits, with a structure of two stacked heptameric rings (*5–7*) arranged around a twofold axis of symmetry. This oligomer, $GroEL_{14}$, appears as a hollow cylinder, with a cavity that spans the sevenfold axis of symmetry of the molecule (*7–9*). The cpn10 from bacteria (GroES) is a heptameric ring of identical 10.4-kD subunits (*10*).

The molecular mechanism by which chaperonins assist the folding of a large array of proteins (*11*) remains obscure (*12, 13*). Central to this issue is the molecular architecture of the GroEL-GroES hetero-oligomers and of the $GroEL_{14}$ core oligomer which can spontaneously bind unfolded proteins (*3*). The step leading to the dissociation of the protein-$GroEL_{14}$ complex and the subsequent correct refolding of the assisted protein is coordinated by the co-chaperonin $GroES_7$ and requires Mg-ATP (*3, 12*). Electron microscopy reveals that one $GroES_7$ ring can asymmetrically bind on either end of the $GroEL_{14}$ cylinder and thus obstruct one end of the central cavity (*7, 8*). Refolding proteins may compete with $GroES_7$ for the same binding sites on either end of the $GroEL_{14}$ cylinder (*14, 15*). However, electron micrographs of $GroEL_{14}$ molecules previously incubated with denatured proteins indicate that proteins bind directly within the central cavity (*9, 16*). Biochemical analysis suggested that the asymmetric $GroEL_{14}GroES_7$ complex is a functional chaperonin hetero-oligomer (*8, 17, 18*). Hence, a model for the chaperonin reaction cycle is an asymmetric $GroEL_{14}GroES_7$ hetero-oligomer that assists the folding and release of a protein from within the central cavity through the unobstructed end of the $GroEL_{14}$ cylinder (*13, 19*).

In contrast, we now show that the asymmetric $GroEL_{14}GroES_7$ molecule is only one of two active forms of chaperonin hetero-oligomers. In solution, there exists a second symmetric $GroEL_{14}(GroES_7)_2$ chaperonin that can stably bind and efficiently assist the refolding of the RuBisCO enzyme. Both the asymmetric $GroEL_{14}GroES_7$ and the symmetric $GroEL_{14}(GroES_7)_2$ hetero-

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