Recent portrayals of growth differentiation factor (GDF)-11 as an endocrine fountain of youth fail to consider the competitive influences of myostatin, a homologous protein with true endocrine action that binds the identical receptor, activin receptor IIb (ActRIIb). Subsequent studies have disproven the original premise that circulating levels of GDF11, but not those of myostatin, decline with age as the exact opposite seems to be true. These latter studies also seriously question the validity of data presented in the previous reports and indicate that myostatin circulates at levels 500× greater than those of GDF11; yet, both ligands share nearly identical affinities for ActRIIb. The compromised striated muscle function, neurogenesis, and vascular remodeling that occur with advanced age are, therefore, not caused by reduced circulating GDF11 as it could never outcompete myostatin for shared receptor-binding sites.

Controversial Claims

Demonstrating that a single circulating factor reverses the effects of aging, particularly in the heart, muscle, and brain, is beyond transformative as it could revolutionize geriatric medicine and the treatment of sarcopenia, cancer cachexia, heart failure, and even stroke. Such a story was recently told. Unfortunately, however, it is founded on a misunderstanding of competitive ligand interactions and on questionable science.

With 2 high-profile publications in Science, Katsimpardi et al1 and Sinha et al,2 and another in Cell, Loffredo et al,3 the same Harvard group suggested that the age-associated decline in circulating levels of GDF11 can induce cardiac hypertrophy, skeletal muscle dysfunction, and compromised neurogenesis.1-3 They also suggested that restoring GDF11 in aged circulation rejuvenates these tissues and induces vascular remodeling. The cornerstone of these conclusions is their reported observation that circulating GDF11, but not myostatin (also known as GDF8), declines with age. This is an extremely important distinction because both ligands bind the identical receptor, ActRIIb, with identical picomolar affinities and induce identical signaling pathways.4 Therefore, they should have identical actions assuming that receptor access is similar; yet, the null phenotypes for each ligand differ substantially with minimal redundancy occurring only in bones.5,6

Two subsequent reports by Egerman et al7 and Smith et al8 and our own, Rodgers and Eldridge,9 directly refute the Harvard group’s results and question the validity of reagents critical to their studies.7-9 In the study by Poggioli et al10 an article recently published in this journal, the Harvard group attempted to address these concerns by collectively assessing GDF11 and myostatin as a single factor “GDF11/8.”10 This conflicts with their foundational claim that reductions in circulating GDF11, but not myostatin, are responsible for the age-related decline in striated muscle function, neurogenesis, and vascular remodeling. More confusing is the fact that Poggioli et al10 assessed the wrong antibody in their critique of Egerman et al.7

Antibody Cross-Reactivity

Using Western blots without loading controls, Loffredo et al3 reported that plasma GDF11 levels were lower in aged versus young mice. However, Egerman et al7 demonstrated that the antibody used in this study (anti-BMP11 recently renamed anti-BMP11+GDF8/GDF11 antibody, Abcam catalog number, ab124721) cross-reacted with myostatin and that it even recognized levels of myostatin that were beyond detection for GDF11. Egerman et al7 further demonstrated nonspecificity of the GDF11 SOMAmer (Slow-Offrate Modified Aptamers) used by Loffredo et al3; all of which is hardly surprising as GDF11 and myostatin are nearly identical homologous proteins that share a common orthologous ancestor.11 Egerman et al7 then validated a novel GDF11-specific immunoassay using a different antibody (antihuman GDF11; R&D Systems, clone 743843, catalog number, MAB19581) and determined that in rats and humans, circulating GDF11 levels rise, not fall, with age.

Poggioli et al10 published a challenge to Egerman et al7 claiming that the Abcam antibody cross-reacts with circulating immunoglobulin10 despite the fact that Egerman et al7 validated their westerns using recombinant GDF11 as a positive control. Even if Egerman et al7 accidentally quantified changes in circulating immunoglobulin, these data still do not refute the immunoassay results with rat and human sera as they were generated with the R&D Systems mouse monoclonal, not the Abcam rabbit monoclonal. Indeed, this immunoassay was generated specifically because the Abcam antibody cross-reacted with myostatin. Conclusions by Egerman et al7, therefore, were not based on Western blotting data but on the immunoassay. None of this was discussed in the study by Poggioli et al10 which also contained several inaccurate representations of the literature. This includes claims that both myostatin and GDF11 influence skeletal muscle patterning when in fact only skeletal patterning is regulated by GDF11 as skeletal muscle size, fiber number, and fiber type are all
unaffected in muscle-specific GDF11 null mice with either wild-type or myostatin null backgrounds.\(^5\)

There is no GDF11/8. These are 2 different factors that regulate different developmental and physiological processes. The collective assessment of both factors is not only confusing and inaccurate, more importantly, it also ignores the original claim that reduced circulating GDF11 alone is responsible for the age-associated changes discussed. Furthermore, the literature discussion by Poggioli et al\(^10\) is misleading and this is particularly true of their representation of our recent study.

**Competing Ligands**

In the study by Sinha et al\(^2\), the Harvard group used a sandwich immunoassay (R&D Systems catalog number, DGDF80) to quantify circulating myostatin in young and aged mice and although levels in the latter were 20% lower, this difference was not significant, leading them to conclude that circulating myostatin levels do not change with age. We used the same assay and determined that it cross-reacts with GDF11 as the addition of recombinant GDF11, also from R&D Systems, produces a false low.\(^2\) We demonstrated GDF11 interference using purified myostatin standards and with serum samples and have since determined that assay interference occurs even when samples are acid treated, a protocol requirement that eliminates interference from follistatin and other myostatin-binding proteins. Most importantly, our study in no way confirms “selective detection of GDF8 (not GDF11)” as Poggioli et al\(^10\) claims because our study suggests that the exact opposite is true.

We also detected serum myostatin immunoreactivity in myostatin null mice, which cannot be due to myostatin, but to GDF11. Levels were 500x lower than those in wild-type mice and are consistent with those reported by Egerman et al\(^7\), further indicating that the assay itself does not selectively detect myostatin, but that GDF11 levels are simply too low to normally cause interference. We then documented a gradual age-related decline in serum myostatin consistent with the previously reported age-related decline among human subjects.\(^12\) Poggioli et al\(^10\), therefore, actually refute the Harvard group’s previous claims as the reduction in circulating GDF11/8 is due to changes in myostatin and not in GDF11. In other words, their new results disprove their original conclusions.

Although 2 commentaries recently summarized this controversy,\(^13,14\) this most salient of points, the fact that circulating GDF11 cannot possibly outcompete circulating myostatin, was never discussed. Without empirical evidence suggesting otherwise, we can, therefore, only objectively conclude that circulating GDF11 is immaterial. All 3 ActRIIb ligands—GDF11, myostatin, and activin—bind with nearly identical affinities. This means that receptor occupancy, activation, and biological activity are dependent on ligand availability, and circulating myostatin is 500x more available than GDF11. In fact, activin is well known to function as an autocrine and paracrine factor; yet, it normally circulates at levels similar or 10-fold higher than those of GDF11.\(^15\) By contrast, several studies suggest that endocrine myostatin attenuates the hepatic growth hormone/IGF1 axis, contributes to the skeletal muscle atrophy that occurs with heart failure, and regulates the normal growth and development of adult skeletal muscle.\(^16–18\)

**Data Analysis Concerns**

Studies by the Harvard group presume that what can occur actually does occur. The cardiac actions attributed to GDF11, for example, could very well be due to myostatin, which inhibits cardiac hypertrophy and excitation–contraction coupling and cardiomyocyte growth.\(^19–21\) Furthermore, the in vitro studies by Sinha et al\(^2\) do not distinguish ActRIIb ligand–specific actions especially as many assessments incorporated highly flawed statistics with type 1 errors.\(^2\) This includes pseudo-replication sampling in Figure S3B and inappropriate use of t tests and Mann–Whitney tests with ≥2 independent variables (Figures 1–3, S2, S4, S6, S11, S14, and S19). Individual P values are indicated in their figures and most do not meet the minimal significance threshold of 0.0083 or 0.0042 when applying a Bonferroni correction, depending on the figure and panel. This is especially true of Figure S19 where 9 of 10 differences noted lack statistical significance, refuting the claim that GDF11 stimulates the proliferation of muscle satellite cells from aged, but not young mice. Sinha et al\(^2\) also failed to consider age and treatment interactions (Figures S13C and S11A-C) and to account for repeated measures and unequal sample size (Figure S14). Moreover, it is likely that different regression analyses (eg, logistic or Poisson) should have been performed in the many instances where categorical dependent variables (eg, percentages/frequencies and cell counts in Figures 1, 2, S2, S4, S6, and S8) were influenced by multiple independent variables and when accounting for measurement interdependence (Figures 2E and 9A–9C). Finally, Sinha et al\(^2\) seem to have misinterpreted serum lactate and glucose data by suggesting the coordinated decline in both supports evidence of improved mitochondrial function in aged rGDF11-treated animals. Serum lactate and glucose are in general inversely related as lactate is a product of glycolysis in type II muscle fibers, whereas mitochondrial activity reflects anaerobic metabolism in type I fibers. Furthermore, mitochondrial dysfunction is well known to increase lactate and reduce glucose levels in blood because of a greater reliance of glycolysis in type II fibers. Thus, the lactate and glucose data do not support the claimed enhancement of mitochondrial function by GDF11 administration (Figure S13).

The flawed statistical design and other serious problems question the reliability of the Sinha et al study.\(^2\) Moreover, the demonstration that circulating myostatin levels are demonstrably higher than those of GDF11 and that levels of the former, but not the latter, decline with age refutes the fundamental claim that declining GDF11 compromises striated muscle function, neurogenesis, and vascular remodeling. Katsimpardi et al\(^1\), Loffredo et al\(^1,13\) and Sinha et al\(^2\) are, therefore, highly suspect. The same can also be said of Poggioli et al\(^10\) and its erroneous critique of Egerman et al\(^7\) and misleading use of
“GDF11/8.” Autocrine or paracrine GDF11 or even activin may in fact regulate some of these processes as the functional significance of any particular ActRIIb ligand is dependent on its competitive availability. In aging animals, however, circulating myostatin, not GDF11 and especially not GDF11/8, is the relevant hormone.

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None.

**References**


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