Advanced aging is associated with alterations in cardiac muscle function and reductions in skeletal muscle mass and performance.1–4 Reversal of those processes responsible for age-related functional decline is an important area of investigation and might be termed fountain of youth research. A recent series of studies from the Lee/Wagers laboratories5–7 suggest that age-related cardiac, skeletal muscle, and central nervous system abnormalities can be rapidly reversed if an old animal shares its circulation (parabiosis) with a young animal. These studies also suggest that an age-related reduction in growth differentiation factor 11 (GDF11), or its closely related family member growth differentiation factor 11, actually impairs skeletal muscle repair in old animals. One possible explanation for what seems to be mutually exclusive findings is that the original reagent used to measure GDF11 levels also detected many other molecules so that age-dependent changes in GDF11 are still not well known. The more important issue is whether increasing blood [GDF11] repairs old skeletal muscle and reverses age-related cardiac pathologies. There are substantial new and existing data showing that GDF8/11 can exacerbate rather than rejuvenate skeletal muscle injury in old animals. There is also new evidence disputing the idea that there is pathological hypertrophy in old C57b16 mice and that GDF11 therapy can reverse cardiac pathologies. Finally, high [GDF11] causes reductions in body and heart weight in both young and old animals, suggestive of a cachexia effect. Our conclusion is that elevating blood levels of GDF11 in the aged might cause more harm than good. (Circ Res. 2016;118:1143-1150. DOI: 10.1161/CIRCRESAHA.116.307962.)

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Do GDF11 and GDF8 Have Distinct Effects on Target Tissues

One important aspect of the current debate is whether myostatin/GDF8 and GDF11 have identical or distinct effects on target tissues. GDF8 and GDF11 are secreted proteins that are members of the transforming growth factor-β superfamily. The expression of these 2 proteins has distinct tissue specificity; GDF8 is largely expressed in skeletal muscle,12,14,15 with lower expression in heart and adipose tissue,16 whereas GDF11 is expressed in many tissues, including the pancreas, intestine, kidney, skeletal muscle, heart, developing nervous system, olfactory system, and retina.17,18 Studies with global genetic disruption demonstrates that GDF11 regulates anterior–posterior regionalization and kidney development,15–19 whereas GDF8 negatively regulates muscle growth.14 These results suggest distinct developmental roles for GDF8 and GDF11 that may provide some insights into distinct roles within adult tissue.

GDF8 and GDF11 are highly homologous with 89% amino acid sequence identity in the mature, active protein. Similar to other transforming growth factor-β family members, GDF8 is expressed as a 375 amino acid polypeptide that is cleaved into an N-terminal latency-associated protein and a C-terminal mature protein. Additional proteolytic processing at the RSRR site results in a 12.5 kDa C-terminal mature peptide, which forms the biologically active GDF8 dimer and the N terminus propeptide. Both forms, active dimer and propeptide, have been detected in mouse and human serum.20 Because of its high similarity with GDF8, GDF11 most likely follows a similar pattern of maturation; however, to our knowledge, this has never been clearly elucidated.

GDF8 and GDF11 signal through the identical activin type II receptors and activate the canonical SMAD2/3 signaling pathway.21 Indeed, Egerman et al14 documented that both GDF8 and GDF11 equally activate this signaling pathway. These findings are entirely consistent with studies that demonstrate that both GDF11 and GDF8 inhibit skeletal myogenesis and muscle regeneration.17,20,22 These results show that GDF8 and GDF11 signal through identical pathways. Therefore, tissue-specific GDF8 or GDF11 effects are likely because of local differences in their expression. However, the current reviews are specifically discussing circulating levels of active forms of GDF8/11 rather than tissue-specific expression, local protein processing, or local activation.

Genetically induced loss of GDF8 function induces dramatically increased muscle mass and reduced fat pad mass in multiple species.14,18 These findings clearly demonstrate that GDF8 negatively regulates skeletal muscle growth. In contrast, loss of GDF11 results in homeotic transformations throughout the axial skeleton and posterior displacement of the hind limbs during embryonic development.23 GDF11−/− mice die perinatally presumably because of developmental defects in kidney, GI tract, stomach, and the palate.19,24 Analysis of mutant mice suggests that GDF11 has little involvement in skeletal muscle development.19

Collectively, these results suggest that GDF8 and GDF11 have distinct biological roles, but these roles seem to be caused by differences in the developmental pattern of expression or tissue specificity of expression. It does not seem that distinct GDF8 and GDF11 effects results from molecular signaling specificity because all known data show that the 2 molecules bind to identical receptors and activate identical downstream signaling mechanisms.

Does GDF11 Decrease With Age and Disease?

One aspect of the current controversy is whether circulating levels of either GDF8 or GDF11 change with age. One group reports reductions of GDF11 with age,5–8 and a second group reports increasing GDF11 with age.9 Because GDF11 and GDF8 are 89% identical, assessing their relative levels is challenging. Also contributing to the discrepancies between the groups are differences in detection methods, as well as the use of different reagents. Reliably and reproducibly documenting age-related changes in circulating GDF11 is critical to the central hypothesis of the Lee and Wagers studies.5–7

It is now well established that the antibody and SomaLogic technology used to identify and isolate GDF11 as a singular factor responsible for age-related disorders in the original Lee/Wagers report in Cell6 does not have high GDF11 specificity. This reagent binds both GDF11 and GDF8,9,25,26 and because the circulating levels of GDF8 are many fold higher than GDF11,27 the original data published by Lee/Wagers are clearly inadequate to support the central hypothesis of their work. This issue has not yet been resolved, and the Lee/Wagers groups no longer differentiate between GDF11 and GDF8.8 This seems to be an approach taken by several laboratories that are exploring GDF11/GDF8 as a contributing factor.
to age-related disorders. Our conclusion is that data obtained using nonselective reagents is inadequate to support the initial hypothesis that GDF11 is an important factor responsible for age-related cardiac, muscle, and cognitive disorders.

These problems with reagent specificity contribute to the uncertainty regarding GDF11 concentration changes with aging and directly impact the assertion that supplemental GDF11 restores youthful functionality. Importantly, using GDF8 null mice, Rodgers et al. have shown that circulating GDF11 levels are 500× lower than GDF8. If true and if circulating GDF11 and GDF8 have equal access to their common receptors in target tissues, the functional relevance of circulating levels of GDF11 is likely inconsequential.

The Glass group and the Houser laboratory working with Boehringer Ingelheim (BI) developed sensitive, GDF11-specific methods to determine concentrations of free, homodimeric GDF11 in serum from both young and aged mice, as well as in humans. No significant age-related differences in GDF11 for either species were found at BI (data not shown), similar to the results reported by the Glass laboratory. Importantly, Glass’s group reported a trend for increased GDF11 levels in rats and humans. Therefore, using GDF11-specific assays, there is no evidence that GDF11 declines in serum during aging. It is clear that the specificity and sensitivity of the reagents are key factors in the discrepant findings published to date. Other contributing entities include the expression of and GDF11 binding to endogenous protein partners (e.g., GASP-1, GASP-2, and follistatin), as well as the impact of sample handling techniques on the kinetics of that binding.

We conclude that there is not yet sufficient evidence to know if the circulating levels of GDF11 (and GDF8) are reduced in aging. This is an area where new studies with reliable, easily repeatable approaches would help move the field forward.

Cardiac Muscle

This section will address the hypothesis that restoring youthful levels of GDF11 in old mice rescues their pathological cardiac hypertrophy (PCH) and restores normal cardiac performance. The effects of GDF11 on the heart are not well known. The report published in Cell by the Lee/Wagers laboratories suggested that circulating levels of GDF11 fall in old mice, and restoring it to youthful levels reverses age-related PCH. However, a subsequent study by the Houser laboratory, using a well-characterized lot of recombinant GDF11 (rGDF11), at the doses reported to be beneficial by Lee/Wagers groups was unable to validate the original findings.

In healthy adult animals, the heart weight (HW) changes in proportion to changes in body weight (BW). PCH, on the other hand, is an increase in HW that is disproportionate to the BW. PCH usually results from acquired diseases, such as chronic hypertension and ischemic heart disease, or from genetic defects. PCH characteristics include increased HW/BW, increased cardiomyocyte size, altered myocyte Ca²⁺ handling properties, decreased cardiomyocyte number because of myocyte death, and increased fibrosis. Some of these features can be present in the aged heart, but there is little conclusive evidence that PCH develops as part of the normal aging process. In fact, age-related cardiomyopathy is often secondary to the acquisition of cardiovascular disease. Molecular causes of true age-dependent cardiomyopathy, in the absence of disease, are not well defined. There is a need for studies that better explain the mechanisms of aging-related cardiomyopathy to better define strategies to prevent or reverse these defects.

The remainder of this section will discuss the idea that restoring youthful levels of GDF11 reverses age-related PCH. This area of investigation began with a parabiosis experiment in which old mice shared a circulation with young mice. The HW/tibial length (TL) of old mice was rapidly reduced (within a month), whereas in the control animals (old mice sharing a circulation with another old mouse), both the HW/TL and myocyte size were unchanged. The authors reported GDF11 as a circulating factor that was reduced in the blood of old animals that returned to normal levels in old mice sharing a circulation with young mice. In the second portion of this study, old animals received daily injections of what turned out to be poorly characterized rGDF11 (0.1 mg/kg) for one month. This amount of GDF11 therapy reduced HW/TL and myocyte size without changing the BW. The Lee/Wagers laboratories suggested that GDF11 could be a novel therapeutic to reverse the adverse cardiovascular consequences of aging.

Needless to say, others should have been able to validate these results because the relevant translational approaches were simple: daily injection of rGDF11 at the dose of 0.1 mg/kg. The Houser laboratory, collaborating with investigators from BI, set out to validate and then extend these provocative findings. BI first performed extensive in vitro and then in vivo testing of rGDF11 obtained from R&D Systems. BI documented the stability of the recombinant protein in solution at body temperature and ensured it maintained its bioactivity at 37°C for ≤4 weeks.

Given the importance of using well-characterized recombinant protein, we next discuss some of the studies that were performed to characterize rGDF11 for the study published by the Houser laboratory. Like many transforming growth factor-β family members, the active form of GDF11 is not soluble at physiological pH. Therefore, before injection of rGDF11 into animals, an extensive biophysical analysis was performed on purified rGDF11 from R&D Systems. These studies showed that rGDF11 exists as a disulfide-linked dimer in solution but only when the pH is 5.0 or lower. Aggregation and precipitation was observed in rGDF11 samples neutralized from pH 5.0 to pH 7.0, with transient nanoparticles ≤50 nm (200 million Daltons) and larger species observed. At pH 7.0, residual smaller species were undetectable using ultraviolet absorption at 280 or 230 nm. These control studies showed that because of variability in the efficiency of reconstitution from lyophilized powders, the best practice was to solubilize rGDF11 in an acidic buffer and measure the absorbance at 280 nm to document protein amount. These studies showed that assuming that the lyophilized powder is 100% protein (or even a certain percentage was protein by weight) generates
incorrect data and is not a recommended way to ensure correct protein concentration.

When handled carefully using acidic buffers to avoid precipitation, rGDF11 was a stable and well-behaved homogenous solution of disulfide-linked dimers with unremarkable hydrodynamic properties. Analytic ultracentrifugation was used to characterize 2 samples of rGDF11 (from PeproTech and R&D Systems) before and after incubation at 37°C for 1 month. The purity and hydrodynamic sedimentation coefficient were examined using analytic ultracentrifugation showing minor loss of purity and total protein, and essentially identical shape and weight for the main species. The Figure demonstrates the biological activity of rGDF11 from PeproTech versus R&D Systems in the SMAD2/3 cell-based assay. No difference in activity was observed between proteins from these 2 vendors. Additionally, activity of rGDF11 (R&D) was maintained for ≤4 weeks when stored in NaAcetate Buffer, pH 4.5. Collectively, these data document that the rGDF11 protein used in the study by the Houser laboratory was well characterized and stable for the dosing interval of 1 month. The Lee/Wagers group cautions others to be careful when preparing rGDF11. However, they do not describe precautions taken in their studies to ensure reliable dosing or rGDF11 bioactivity. Their published reports suggest that they weigh the recombinant protein supplied by the vendor, place it in saline, and inject it into animals.5,8

Once fully characterized, rGDF11 (0.1 mg/kg) or vehicle was injected daily into old mice for a month.25 This blinded study25 showed that rGDF11 injections raised circulating GDF11 to detectable levels. In this regard, BI developed an assay that could discriminate between GDF8 and GDF11, rather than the reagent used by the Lee/Wagers group that we and others showed readily detects both GDF11 and GDF8.9,25,26

The first important finding of the Houser laboratory/BI study was that there is no evidence for the existence of PCH or deranged cardiac function in 23-month-old C57BL6 mice. The putative pathological hypertrophy reported in both reports by the Lee/Wagers laboratories is simply an artifact of using HW/TL rather than HW/BW to document pathological hypertrophy in mature adult mammals. A recent editorial by McNally37 regarding the new GDF8/GDF11 study by the Lee/Wagers groups also identifies this concern.5 The Houser laboratory found that daily injections of GDF11 (0.1 mg/kg) had no significant effect on HW, BW, cardiac structure, or cardiac function.25

The Lee/Wagers groups have recently published a follow up study in which they perform an rGDF11 dose–response experiment in young and old mice.6 In these experiments, they suggest that the rGDF11 dose (0.1 mg/kg) used in their original report is actually too low to cause decreases in heart size. Because they documented a reduction in heart and myocyte size with a dose of GDF11 in the original report,6 we assume this means that they do not know the rGDF11 concentration used in their original study. Given that they now caution others to properly reconstitute the recombinant protein, it is likely that their original methodology was flawed. It is unclear when they realized that they did not know the actual rGDF11 dose used in the original studies. It is unfortunate that this problem was not promptly reported to the scientific community. As discussed earlier, the Houser and BI laboratories properly characterized rGDF11,25 and we are confident that an rGDF11 dose of 0.1 mg/kg was used in our validation study. The unknown GDF11 dosage used in the original study from the Lee/Wagers laboratory was reported to cause a decrease in HW and myocyte size with no change in BW in their old mice.5

So what are the key differences between these studies and how might they be explained? First, is there any PCH in old (23–24 months of age) C57Bl6 mice? In both GDF11 reports from the Lee/Wagers groups,5,6 they start with the idea that C57Bl6 mice have aging-induced PCH. We were unable to document any PCH in our old mice.25 As discussed earlier and in a recent editorial by McNally,37 the putative pathological hypertrophy is an artifact of using HW/TL as a measure of cardiac pathology. Disease-free mature adult humans often gain and lose weight, and their HW changes proportionally with BW. This is just the normal biology of adult mammals and does not reflect any pathological process.

The parabiosis experiments in the original Lee and Wagers’ study are consistent with our reinterpretation of their data.5 The data required for us to reach this conclusion can be found in Online Table III that is in the version of this report uploaded to the National Institutes of Health.

Figure. Functional activity of recombinant growth differentiation factor 11 (rGDF11) was determined by measuring GDF11 dose-dependent activation of Smad2/3 signaling in HepG2 reporter cells using a luciferase assay as reported in Smith et al. A, Similar activity was observed for both PeproTech and R&D Systems rGDF11. B, Biological activity of rGDF11 (R&D) was maintained for ≤4 weeks when stored in NaAcetate Buffer at 37°C, pH 4.5 at a concentration of 1 mg/mL.
after the article was accepted for publication (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3677132/). These critical data are not found in the version of the report published in Cell. The data in Online Table III show that the BWs of old animals were 25% to 30% greater than the young animals before parabiosis. After a month of parabiosis, the old animals lost almost 25% to 30% of their BW and now were almost the same size as their youthful counterparts. These data strongly suggest that there was no change in the HW/BW of old animals after parabiosis; however, the raw data needed to determine this were not in the published studies. The reduction in HW/TL reported in these animals, in our view, is adequately explained by the parabiosis-induced reduction in BW. However, an unexplained aspect of this study is that when an old animal shared a circulation with another old mouse (the obvious control experiment), both old mice lost 25% of their BW, but no reduction in HW/TL was reported. We have no scientific explanation for how an animal can lose 25% to 30% of its BW without having a corresponding reduction in heart mass.

In our GDF11 study, we used HW to BW ratios (HW/BW) to define the presence or absence of pathological hypertrophy; we reported HWs and BWs along with the HW/BW ratios. We found no age-related changes in HW/BW ratio, and daily rGDF11 injections (0.1 mg/kg) had no effect on HW/BW or myocyte size. In addition, we showed that standard markers of pathological hypertrophy were not different in young and old mice or in old mice before and after GDF11 treatment.

In a new follow-up study from the Lee and Wagers groups, a rGDF11 dose response experiment was performed in both young and old animals. The authors again state that rGDF11 reversed PCH in old animals and surprisingly even in young animals. In this report, the critical heart and BW data are included in Online Table I. In this new study, the influence of age and rGDF11 dose was tested in both young and old animals. The significance of these new data is difficult to evaluate because of the statistical approaches used. In our view, an analysis of variance was needed to document age and treatment effects, and if this is established, then the appropriate post hoc analyses could be performed. Instead, the authors performed an unpaired t test, within each age group, between saline treatment and each of the rGDF11 doses tested. Another concern is that there is an apparent mistake in the reported delta body weight % in the old saline-treated mice (the comparator group for the unpaired t test statistical analysis) that seems to invalidate the major conclusions of these experiments. Readers are referred to Online Table I where the saline-induced BWs decrease (average BW reported), but the % change is listed as an increase. Once this mistake is corrected, there are no apparent rGDF11-induced changes in BW. Regardless of the questionable statistical approach and the discordance of raw BW and % BW changes in the old saline treated mice, there was clearly no effect of rGDF11, at any dose, on HW/BW, documenting that rGDF11 does not reverse pathological hypertrophy.

It is worth noting that the new data from the Lee/Wagers groups show that daily injections of high levels of rGDF11 in young mice, that should raise circulating GDF11 well above normal levels, causes weight loss and reduced HW. These young, lean animals have no cardiac pathologies. In our view, a rapid (9 days) reduction in heart and BW in young, lean mice suggests toxic effects of GDF11—certainly not a desired effect in young, healthy animals. Finally, the dose-dependent GDF11 effects reported were identical in young and old animals. These findings seem to invalidate the original hypothesis because there is clearly no pathological hypertrophy or cardiac dysfunction in young mice, and an identical dose–response relationship would not be expected if old animals truly have reduced circulating GDF11 levels.

Our conclusion is that the data from the Lee/Wagers and the Houser laboratory studies do not support the hypotheses that there is PCH in old C57Bl6 mice, (2) that reduced circulating levels of GDF11 are responsible for this PCH, and (3) that raising GDF11 levels with daily injections of rGDF11 rescues cardiac pathologies. Additional work with carefully characterized animal models and rGDF11 should help the field determine whether raising circulating GDF11 levels in old age repairs or damages the heart.

**Skeletal Muscle**

The extensive repair capacity of adult skeletal muscle becomes progressively reduced during aging in large part from effects on the muscle myofibers, losses of satellite cells (SCs), and losses of SC function. Many laboratories have demonstrated that aged SC function and muscle repair can be improved by exposure to a young systemic environment or the inhibition of progeronic factors. Novel therapeutics to restore skeletal muscle repair mechanisms could improve the quality of life in old individuals.

A recent study from the Lee/Wagers laboratories suggested that reduced circulating levels of GDF11 are responsible in part for the age-related decline in skeletal muscle function, and restoring normal GDF11 levels rejuvenates skeletal muscle. rGDF11 (0.1 mg/kg; Peprotech) was administered daily over 4 weeks to restore normal GDF11 levels in old mice. Multiple assays, including muscle regeneration, transplantation, and in vitro SC cultures, were used to examine whether restoring normal GDF11 levels rescues the defective regenerative capacity of SC. The authors reported a significant improvement in skeletal muscle structure and function in GDF11-treated aged mice. GDF11-treated mice had increased mean muscle fiber size in regeneration assays; SCs had improved engraftment capability in transplantation assays and increased myogenic clonogenic capacity in cell culture assays. Adult mice were unresponsive to rGDF11 therapy. No mechanistic studies were performed to address how GDF11 could function in a manner completely opposite to its highly conserved ortholog GDF8.

A subsequent study from the Glass group was designed to reevaluate the idea that GDF11 can restore the reparative/regenerative properties of aged muscle tissue. They instead found that exogenously administered rGDF11 protein, delivered at same dose and duration (0.1 mg/kg; R&D Systems) as reported by the Lee/Wagers groups, did not improve aged muscle regeneration or increase the number of SCs in vivo.
Moreover, Egerman et al. showed that administration of higher levels of GDF11 (0.3 mg/kg; RnD) to 16-week-old adult mice (for 3 days prior to and for 14 days after injury) actually inhibited muscle repair. In addition, signaling studies and gene expression analysis confirmed that GDF11 and GDF8 were virtually identical in their activity.

The 2 reports seem to reach mutually exclusive conclusions, with one study showing a beneficial antiaging effect of exogenously administered GDF11 and the other showing that increasing GDF11 actually enhances age-related skeletal muscle damage. These findings are difficult to reconcile. Although the experiments involving muscle injury and SC cultures are similar, they are not identical. Differences in the form of muscle injury, source of recombinant protein, and culture media conditions in vitro are potential sources of variability. Below we address each of those potential factors.

**Types of Muscle Injury**

Different forms of muscle injury (thermal versus cardiotoxin) were used in the 2 conflicting studies discussed earlier. Both injury models are commonly used methods to induce muscle repair. Cardiotoxin is usually administered via injection into the targeted muscle, whereas thermal injury is conducted after surgery to expose the muscle. The extent of injury can vary significantly between laboratories because of the type and amount of cardiotoxin used and the method used to apply the thermal injury. The different injury paradigms result in somewhat different rates of muscle repair possibly because of differences in the inflammatory response and extent of cellular death. The Lee/Wagers groups reported improved regeneration of rGDF11-treated muscle after thermal injury and improved engraftment of adult SCs into rGDF11-treated muscle after cardiotoxin injury. Thus, it does not seem that the mode of injury is likely to explain the difference in findings. However, this idea can be directly tested.

Individual muscle fiber size varies considerably within a muscle tissue during the repair process. This variability is compounded if preexisting, that is, uninjured muscle fibers are factored into the analysis. Egerman et al. provided a detailed description of the rigorous methods used to quantify fiber size and accounted for uninjured fibers in the analysis. They did not see any enhancement in myofiber size no matter how the data were analyzed. Unfortunately, Sinha et al. did not fully describe the methods for myofiber size quantification, whether uninjured fibers were excluded, whether the analysis was blinded, or if samples were excluded. In addition, it is also impossible to determine whether appropriate statistical analyses were performed because the raw data are not reported.

**Satellite Cell Cultures**

The Glass group showed that rGDF11 causes a dose-dependent reduction in the expansion of both normal adult and aged SCs grown in culture or on single fibers (the niche). The Lee/Wagers groups showed an rGDF11 dose-dependent increase in aged SC expansion and differentiation with no effect on adult SCs. These disparate findings are also difficult to reconcile because one group found that rGDF11 reduced aged SC expansion at all rGDF11 doses, whereas the other found increased expansion as the exclusive effects.

Importantly, the Lee/Wagers groups reported that myostatin (GDF8), a known inhibitor of SC expansion, decreased expansion of adult and aged SC cultures. As discussed earlier, a major issue in this debate is the fact that GDF11 and GDF8 share significant homology and activate the same signaling mechanism through the same ActRIIB receptors. Tissue-specific effects of GDF11 versus GDF8 could come about by tissue-specific processing of inactive forms of these molecules. However, this point is irrelevant to the current controversy because the debate centers on circulating levels of mature, active forms of these molecules that were obtained from commercial providers.

The difficulty the Lee/Wagers groups have had in defining the actual GDF11 concentrations/activity in their studies also do not easily explain the disparate results in the literature. The Glass and Houser laboratories carefully characterized different lots of GDF11 from different suppliers and only used well-characterized and well-solubilized recombinant protein. Both groups showed dose-dependent GDF11 signaling through SMAD2/3 reporter assays and then used the characterized protein in in vivo studies. The Glass group clearly shows that all rGDF11 doses restricted expansion of SCs from both normal and aged animals.

Egerman et al. used 2 complimentary assays to examine SC function, one that purifies SCs based on enzymatic digestion of muscle tissue, cell surface marker expression, and flow cytometry and the other that isolated single muscle fibers which retains the SCs within their own niche. Sinha et al. assessed SCs in isolation only. Both groups show that the effects of GDF11 on isolated SCs are relatively modest. Egerman et al. reported a more robust phenotype using single muscle fiber assays, suggesting that the muscle fiber or a component of the niche is important for the inhibitory action of GDF11 on SC function. Somewhat counter intuitively, Sinha et al. reported numerous beneficial effects of GDF11 treatment of aged muscle, including reduced DNA damage, rejuvenated mitochondrial function, and muscle force production. Again, no mechanistic studies were performed to elucidate the rejuvenating effects of rGDF11.

**Media Composition**

The notion that different media and serum composition could influence GDF11 activity in cell culture experiments is a possibility. Indeed, serum contains proteases that could impact processing and thus activity of GDF11. However, the recombinant protein used in all of the experiments by both groups was in the mature form, and therefore, it is hard to imagine that protease processing was an issue. GDF11 signaling could be altered if soluble inhibitors such as follistatin and activin were present at different quantities within the media used in the different laboratories. Although this cannot be excluded, in vitro experiments performed in the laboratories of Dr Glass and Dr Brack produced the same in vitro phenotypes with different media. This suggests that GDF11 induces a robust inhibitory response in muscle cells in vitro.
Conclusions

The idea that normal skeletal and cardiac muscle properties can be restored in old animals by daily injections of rGDF11 is not well supported by the studies that have been published to date. This field would benefit from studies with well-documented, easily repeatable approaches, well-characterized reagents, and the presentation of and appropriate analysis of all data needed to form conclusions.

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Disclosures

None.

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We agree with Harper et al that reliable and specific detection methods for growth differentiation factor 11 (GDF11) protein are sorely needed and that, currently, one cannot conclude unequivocally that GDF11 protein levels increase or decrease with age. Assays are also needed that account for ligand status (free, latent, or in complex with extracellular antagonist) to differentiate biologically active from total GDF11. We further agree that rigorous quality control measures are critical when working with recombinant GDF11 (or GDF8) and that batch-to-batch variability and differences in protein source can introduce variation in experimental results. We have instituted such quality control measures in our own laboratories, including spectrophotometry, gel electrophoresis, and bioassay (described in Sinha et al). Finally, we agree that differences in study design likely explain at least some of the discrepant results and further suggest that dose responses are key because even with the most rigorous matching of experimental design and protein-directed quality control, batch-to-batch differences in protein refolding efficiency and aggregation (clearly apparent in Egerman et al; see Online Figure IIB) can lead to differences in ligand activity. Thus, matching protein dose (mg/kg) is inadequate to ensure equivalency of exposure to active ligand. Such activity differences almost certainly played a role in the controversies under discussion here.

We also wish to correct several erroneous statements made by Harper et al: (1) Egerman did not show that GDF11 causes muscle abnormalities in aging—aged mice treated with GDF11 in that study showed no adverse effects on muscle; (2) the Rodgers study referenced by Harper et al only inferred levels of circulating GDF11, using an ELISA assay specific for GDF8 that in our hands does not detect or interact with GDF11 protein (Wagers Lab, unpublished), consistent with the product datasheet (https://resources.rndsystems.com/pdfs/datasheets/dgdf80.pdf); (3) our quality control measures and tent with the product datasheet (https://resources.rndsystems.com); (4) our quality control measures and tent with the product datasheet (https://resources.rndsystems.com); (5) we have investigated mechanisms of GDF11 action (implicating enhanced autophagy, metabolic remodeling, and resolution of DNA strand breaks).

We thank Harper et al for pointing out a minor error in the Online Table of our prior publication. We have submitted a correction for this mistake to the journal. To clarify, the previously reported percent change in body weight is correct, but the average body weight value at day 9 for saline-injected aged mice was reported incorrectly (it should have been 40.73±6.05 g, not 38.35±6.21 g). These data, which we have replicated in subsequent experiments, continue to support the conclusion that GDF11 injection influences body weight. We agree that specific studies are needed to understand whether GDF11 directly affects cardiac tissue or whether other peripheral tissues, such as adipose, represent additional or even primary targets of GDF11 that can subsequently initiate a physiological response that indirectly regulates cardiac mass and hypertrophy in aged mice.

Finally, regarding the potential for distinct biological actions of GDF11 and GDF8, which exhibit only 11% amino acid divergence in their mature domains, we note that although the amino acid substitutions that distinguish GDF11 and GDF8 are few, they are absolutely conserved across multiple vertebrate species, suggesting strong evolutionary pressure to maintain these differences. Moreover, there exist multiple examples in which even single amino acid substitutions can have profound functional consequences (eg, the K153R variant of GDF, discussed in our review).

Is Growth Differentiation Factor 11 a Realistic Therapeutic for Aging-Dependent Muscle Defects?

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