# Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2010 - 12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA

4. **Grant Contact Person's Telephone Number:** 814 935 1081

5. **Grant SAP Number:** 4100050904

6. **Project Number and Title of Research Project:** 29. Body Weight- and Nutrition-Sensitive Regulation of Skeletal Muscle Composition in Health and Obesity

7. **Start and End Date of Research Project:** 9/1/2010 - 8/31/2011

8. **Name of Principal Investigator for the Research Project:** Scot Kimball, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 30,900

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

| Last Name | Position Title | % of Effort on Project | Cost |
|---|---|---|---|
| Kutzler | Research Support Asst | 23% | 9051.94 (sal+fr) |

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

| Last Name | Position Title | % of Effort on Project |
|---|---|---|
| Kimball | Professor | 2% |
| Schilder | Assistant Professor | 5% |

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

| Type of Scientific Equipment | Value Derived | Cost |
|---|---|---|
| NONE | | |

**10. Co-funding of Research Project during Health Research Grant Award Period.** Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No___X_____

If yes, please indicate the source and amount of other funds:

**11. Leveraging of Additional Funds**

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes___X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

| A. Title of research project on grant application | B. Funding agency (check those that apply) | C. Month and Year Submitted | D. Amount of funds requested: | E. Amount of funds to be awarded: |
|---|---|---|---|---|
| Macronutrient Regulation of Alternative Pre-mRNA Splicing | ■NIH R01DK094141<br>☐ Other federal (specify:_____)<br>☐ Nonfederal source (specify:) | 11/12 | $1,923,910 | $330,103 |
| Macronutrient Regulation of Alternative Pre-mRNA Splicing | ■NIH R56DK094141<br>☐ Other federal (specify:_____)<br>☐ Nonfederal source (specify:_) | 3/11 | $1,923,910 | $153,000 |

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes__X_____ No_____

If yes, please describe your plans:

We will continue to apply for funding as circumstances permit.

**12. Future of Research Project.** What are the future plans for this research project?

Future plans for this project include the delineation of the mechanism through which troponin T pre-mRNA alternative splicing is impaired in response to consumption of a high-fat diet, and to identify other pre-mRNAs that are regulated in a similar manner.

**13. New Investigator Training and Development**. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes_____ No____X_____

If yes, how many students? Please specify in the tables below:

| | Undergraduate | Masters | Pre-doc | Post-doc |
|---|---|---|---|---|
| Male | | | | |
| Female | | | | |
| Unknown | | | | |
| **Total** | | | | |

| | Undergraduate | Masters | Pre-doc | Post-doc |
|---|---|---|---|---|
| Hispanic | | | | |
| Non-Hispanic | | | | |
| Unknown | | | | |
| **Total** | | | | |

| | Undergraduate | Masters | Pre-doc | Post-doc |
|---|---|---|---|---|
| White | | | | |
| Black | | | | |
| Asian | | | | |
| Other | | | | |
| Unknown | | | | |
| **Total** | | | | |

14. **Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

    Yes_____ No_____X_____

    If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. **Impact on Research Capacity and Quality**. Did the health research project enhance the quality and/or capacity of research at your institution?

    Yes___X_____ No___ _____

    If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

    The results of the studies performed under the CURE award have resulted in the awarding of 2 NIH grants.

16. **Collaboration, business and community involvement.**

    16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No____X_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____X_____

If yes, please describe involvement with community groups that resulted from the research project:

**17. Progress in Achieving Research Goals, Objectives and Aims.**

List the project goals, objectives and specific aims (as contained in the grant application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance

Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

**There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.**

A. Goals, objectives, and specific aims

Troponin T (*Tnnt)* splice form expression affects muscle calcium sensitivity, force output, and ATP consumption rate during activity, and therefore this newly recognized regulatory mechanism may be important for both resisting gravitational forces and maintaining energy homeostasis. We hypothesize that this mechanism is impaired in obesity, causing a mismatch between body weight, muscle performance, and energy expenditure. Consequently, body-weight inappropriate skeletal muscle *Tnnt* splicing will result in chronic muscle weakness, reduction in exercise participation, and reduction in the rate of energy consumption. The proposed studies will more firmly establish body weight- and nutrition-dependent causes of variation in mammalian skeletal muscle *Tnnt* expression, and the generality of a mismatch between body weight, *Tnnt* splicing, and skeletal muscle performance and energy expenditure during obesity. Elucidation of mechanisms controlling body weight- and nutrient sensitivity of *Tnnt* expression in skeletal muscle will ultimately allow interventions that may restore *Tnnt* homeostasis, overall mobility, and body weight-appropriate energy expenditure in obese humans. The following specific aims are proposed:

*Specific Aim 1. Troponin T homeostasis in a diet-induced model of obesity.* To determine the generality of obesity-associated (i.e., in both genetic and non-genetic disease models) impaired *Tnnt* splicing response to body weight, we will induce obesity in otherwise healthy rats by means of a high-fat feeding diet, and examine the time course of *Tnnt* splicing responses to changes in body weight. We expect that as body weight increases, *Tnnt* splicing responses will be progressively attenuated in diet-induced obesity compared to control rats, resulting in *Tnnt* splice form profiles that are poorly matched to body weight.

*Specific Aim 2: The effect of caloric restriction on Troponin T expression in rat skeletal muscle.* How nutrition may affect *Tnnt* alternative splicing in mammalian skeletal muscle is presently unknown. Therefore, in a fashion similar to our recent work in insects, we will assess how caloric restriction affects *Tnnt* splicing in skeletal muscle from adult rats. We expect that caloric restriction will positively affect body weight-specific skeletal muscle performance and that the *Tnnt* splice form profile in muscles from caloric restricted rats therefore will resemble that of control rats of higher body weight.

B. Studies related to Specific Aim 1

B.1 Comparison of the effects of diets containing 10 or 30 kcal% fat on *Tnnt* pre-mRNA alternative splicing

In a recent study (*J. Expt'l. Biol.* 214:1523-32, 2011), we reported that the mRNA encoding

fast troponin T (*Tnnt3*) undergoes alternative splicing in gastrocnemius muscle in response to changes in body weight. Importantly, body weight-induced changes in *Tnnt3* mRNA alternative splicing were impaired in a genetic model of obesity (i.e. the obese Zucker rat). In Specific Aim 1 of the original application, the overall goal was to assess the generality of obesity-associated impairment of the *Tnnt3* splicing response to body weight through induction of obesity in otherwise healthy rats by means of feeding a high-fat diet. In these studies, alternatively spliced forms of *Tnnt3* mRNA were quantitated by real-time polymerase chain reaction (RT-PCR) using fluorescently tagged primers followed by capillary electrophoresis. As described in greater detail below, the results support the hypothesis that body weight-induced changes in *Tnnt3* mRNA alternative splicing are impaired in skeletal muscle in obese rats, and that the impaired response may lead to a mismatch between body weight, muscle performance, and energy expenditure.

Methods

Animals. All studies using rats were reviewed and approved by the institutional animal care and use committee (IACUC) of The Pennsylvania State University College of Medicine. Obesity-prone male Sprague-Dawley rats (OP-CD; Charles River) weighing approximately 200 g were randomly divided into two groups. Rats in the first group were fed a control diet containing 10% of total energy (kcal%) from fat (Research Diets, Inc.) and rats in the second group were fed a diet with 30 kcal% from fat (Research Diets, Inc.) for up to 13 weeks. At various times, body composition was measured using a Bruker Minispec LF90 NMR Analyzer (Bruker Optics, Inc., Billerica, MA). Briefly, rats were weighed, immobilized in a Plexiglas cylinder, and placed inside the Minispec for approximately 1 min. During this time, measurements of whole body fat and lean (muscle) mass content were obtained using manufacturer-recommended rat-specific acquisition parameters. Prior to removal of the gastrocnemius, rats were deeply anesthetized using isoflurane.

Quantitation of *Tnnt3* mRNA splice form relative abundance. *Tnnt3* mRNA splice form abundance in gastrocnemius was quantitated as described previously (*J. Expt'l. Biol.* 214:1523-32, 2011). Briefly, total RNA was extracted from muscle using Trizol reagent (Invitrogen, Carlsbad, CA, USA), and precipitated in isopropanol, according to the manufacturer's instructions. Total RNA was reverse transcribed using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA) using a fluorescein-labeled forward primer, and two unlabeled reverse primers. Using this amplification strategy, all of the possible alternative *Tnnt3* mRNA splice form amplicons have a unique size, with a minimum length difference of three nucleotides. PCR was performed using HotStart GoTaq polymerase (Promega, Madison, WI, USA). For quantitative analyses of *Tnnt3* splice form relative abundance, fluorescein-labeled PCR products were analyzed by capillary electrophoresis (ABI DNA Analyzer, Applied Biosystems). The relative abundance of each amplicon in the PCR reaction was determined by dividing its peak height by the total of all peak heights. Amplicon fragment size was determined using an internal size standard and Genemapper® (Applied Biosystems) fragment analysis software.

Results

When outbred Sprague-Dawley rats are placed on a high-fat diet, only about 50% of them

overeat and develop diet-induced obesity, while the remainder of the animals gain no more weight than ones fed a control, low-fat diet (*Am. J. Physiol.* 285:R1184-91, 2003). Consequently, in the present study, obesity-prone, male Sprague-Dawley rats (OP-CD; Charles River) were used because we reasoned that it might be difficult to distinguish animals responsive to the high-fat diet from non-responders at the shorter time points. Surprisingly, over the 13-week time course of the present study, rats gained similar amounts of body weight, regardless of the diet consumed (Figure 1A). To determine whether consumption of the high-fat diet led to increased adiposity compared to consumption of the control diet, at various times after the start of high-fat feeding fat and lean body mass were measured by NMR analysis using a Bruker Minispec. As shown in Figure 1, the proportion of fat (Figure 1B) and lean (Figure 1C) body mass was the same in rats consuming the high-fat diet (red bars) as those consuming the control diet (blue bars) up until the 8 week time point.

To identify potential high-fat diet-induced changes in splicing of the *Tnnt3* mRNA, a combination of quantitative RT-PCR and capillary electrophoresis was used to assess the relative distribution of the mRNA in the various splice forms in gastrocnemius muscle. A total of 12 *Tnnt3* splice forms were detected (Figure 2). Based on the criteria described in our recent study (*J. Expt'l. Biol.* 214:1523-32, 2011), we refer to the forms as *Tnnt3* β1-9 (contains exon 17) and *Tnnt3* α1-3 (contain exon 16). Notably, even though feeding a high-fat diet had no detectable effect on total body weight or fat or lean body mass, specific splice forms were differentially expressed in muscle from high-fat fed rats compared to controls at the time points examined. For example, in muscle of rats fed a high-fat diet (red symbols and line), the relative expression of the *Tnnt3*α1 splice form was significantly less than in rats fed a control diet (blue symbols and line), even after only one week (Figure 3). In contrast, the relative expression of *Tnnt3*β5 and *Tnnt3*β9 splice forms was significantly increased at all time points in rats consuming a high-fat compared to a control diet. However, as shown for the *Tnnt3*β3 splice form (Figure 3), not all splice forms exhibited diet-induced alterations in expression. Indeed, the relative expression of 7/12 forms was found to be altered in muscle of rats consuming a high-fat compared to a control diet.

## Discussion

In agreement with results presented in our recent study (*J. Expt'l. Biol.* 214:1523-32, 2011), *Tnnt3* mRNA splice form expression changed with alterations in body weight, such that the relative expression of some splice forms was upregulated whereas others were downregulated with increasing body weight. However, in rats fed a diet containing 30%kcal from fat, the pattern of splice form expression was altered compared to animals fed a control diet with 10%kcal from fat, with some splice forms exhibiting increased, and others decreased, relative expression. Interestingly, the high-fat diet-induced changes in expression manifested without a detectable increase in adiposity until 13 weeks of treatment. This finding suggests that the observed changes in *Tnnt3* mRNA alternative splicing were a result of diet *per se* rather than to obesity. It is also interesting that some, but not all, of the *Tnnt3* splice forms found to change in the present study (e.g. *Tnnt3*α1 and *Tnnt3*β5) were shown to be differentially expressed in muscle from obese compared to lean Zucker rats (*J. Expt'l. Biol.* 214:1523-32, 2011). This finding suggests that the commonalities between the models may represent splice forms that were differentially modulated in response to the hyperphagy

that occurs in obese Zucker rats, rather than to a change in fat mass.

Overall, when combined with the results of our previous study (*J. Expt'l. Biol.* 214:1523-32, 2011), the results of the present study suggest that excess calorie consumption, particularly of a diet containing a high proportion of calories from fat, interferes with the normal regulation of alternative splicing engendered by increases in body weight. It is also tempting to speculate that, because Troponin T is involved in modulating muscle force and power output, the mismatch between body weight and *Tnnt3* splice form expression in high fat-fed animals may contribute to pathologies common in human obesity, such as chronic muscle weakness, increased load-induced muscle injury, and unwillingness to participate in exercise.

B.2. Comparison of the effects of diets containing 10, 30, 45, or 60 kcal% fat on *Tnnt* pre-mRNA alternative splicing

The goal of the experiments described in this study was to extend those described in section B.1 above to establish the time course and dose response for the diet-induced changes in alternative splicing.

Methods

The methods used in the studies described below are the same as those above under section B.1, with the following exceptions. First, instead of feeding rats diets containing either 10 or 30 kcal% fat, rats were fed one of four diets containing either 10 (control), 30, 45, or 60 kcal% fat for eight weeks. In a separate study, rats were fed either the control diet or a diet with 32 kcal% fat for either one or four weeks.

Results

To assess the role of fat content on alternative splicing of *Tnnt3* pre-mRNA, male Sprague Dawley rats were randomly divided into four groups and were fed diets containing either 10, 30, 45, or 60 kcal% fat (all diets were purchased from Research Diets, Inc.) for eight weeks. At the end of the study, rats were deeply anesthetized and the gastrocnemius muscle was removed and homogenized. RNA was extracted, reverse transcribed using a fluorescein-labeled forward primer and two unlabeled reverse primers, and the products were analyzed by capillary electrophoresis as described in greater detail in the previous progress report. As shown in Figure 4, the effect of the high-fat diets on *Tnnt3* pre-mRNA splicing was similar, with various splice forms exhibiting either decreased (e.g. *Tnnt3*α1) or increased (e.g. *Tnnt3*β3 and *Tnnt3*β5) relative expression, whereas others were unchanged (not shown) compared to rats fed a control, 10 kcal% diet. Notably, the effect of the 60 kcal% diet was greater compared to either the 30 or 45 kcal% diets on most (e.g. *Tnnt3*α1 and *Tnnt3*β3) but not all (e.g. *Tnnt3*β5) *Tnnt3* splice forms.

To assess the time course for high-fat diet-induced alterations in alternative splicing of *Tnnt3* pre-mRNA, rats were fed either a 10 or 32 kcal% fat diet for either one or four weeks. RNA was extracted from the gastrocnemius muscle and analyzed by real-time polymerase-chain reaction (RT-PCR) and capillary electrophoresis as described in the previous paragraph. Representative results are shown in Figure 5. Specifically, expression of some *Tnnt3* splice forms (e.g. *Tnnt3*α1) were found to be upregulated as early as one week after the rats were placed on the high-fat diet and remained elevated for at least four weeks. Other *Tnnt3* splice

forms (e.g. *Tnnt3*β3) were unchanged after one week of feeding a high-fat diet, but were changed by the four week time point. Interestingly, over the 4-week time course of the present study, rats gained similar amounts of body weight, regardless of the diet consumed. Moreover, lean body mass was unchanged (lean mass/total body mass ratio: 0.704±0.035 vs. 0.692±0.004 for high-fat compared to control diet, respectively) at 1 week, and only slightly (5%) but significantly decreased at 4 weeks.

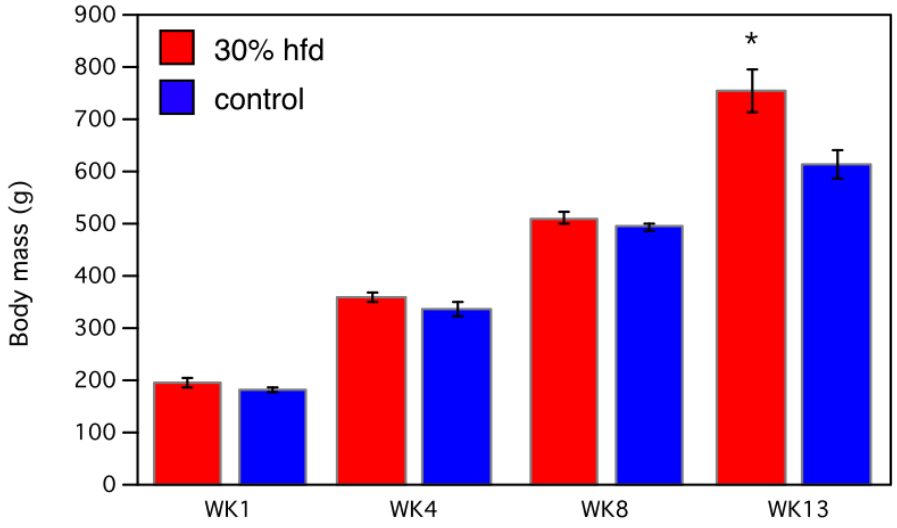
Discussion

In agreement with data presented in section B.1 above, alternative splicing of *Tnnt3* pre-mRNA was altered in rats consuming a high-fat diet, with some splice forms exhibiting upregulated relative expression, some downregulated relative expression, and others were unchanged in rats fed a high-fat compared to low-fat diet. The current studies extend the previous ones to show that feeding rats a diet with 30 kcal% fat is sufficient to induce the changes observed previously, and that for most splice forms, feeding a diet with 60 kcal% fat elicits changes greater in magnitude compared to feeding diets with either 30 or 45 kcal% fat.

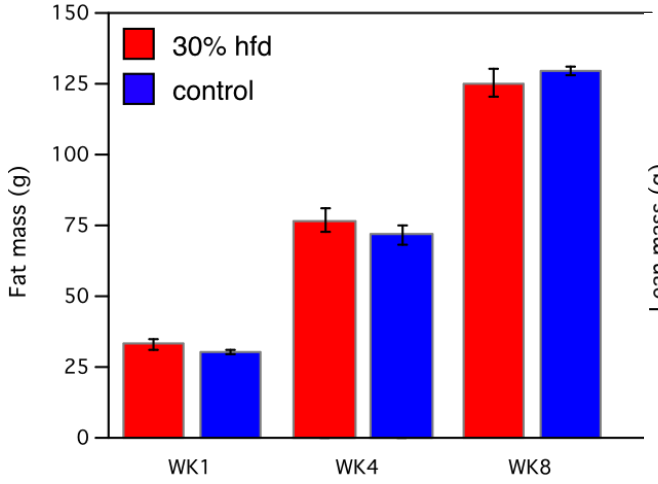
The current studies also extend the ones described above to assess the time course for high-fat feeding-induced changes in alternative splicing of the *Tnnt3* pre-mRNA. In the previous studies, and in the study presented in Figure 4 in this report, rats were fed a high-fat diet for eight weeks. Interestingly, expression of many of the splice forms was altered after only one week of consuming a high-fat diet, although a few splice forms required a longer period (e.g. four weeks) before their expression was significantly altered. It is noteworthy that changes in alternative splicing of *Tnnt3* pre-mRNA occurred prior to detectable changes in either body weight or lean body mass. These findings suggest that high-fat diet-induced changes in *Tnnt3* pre-mRNA alternative splicing were a result of diet *per se* rather than to diet-induced changes in body weight or composition. To our knowledge, this represents the first evidence of a role for diet in the regulation of quantitative variation in alternative splicing in mammals *in vivo*. The results also indicate that high-fat fed rats will be a useful model system to study the regulation of alternative splicing of pre-mRNA by macronutrients.

Figures for Specific Aim 1

A




B




C

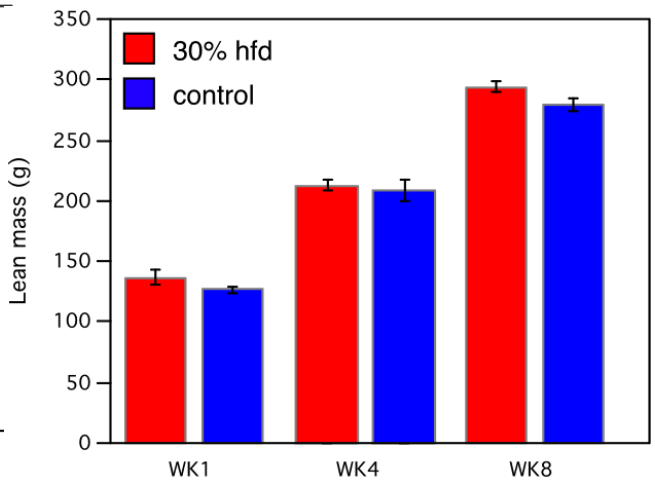



Figure 1. Body mass and composition in rats fed either a control or 30% high fat diet up to 13 weeks. Presented are mean values (N = 4 per treatment at each time point) and associated standard errors. The dietary treatment did not affect body weight until the 13 week time point, and had no effect on fat and lean mass contributions to total body mass.

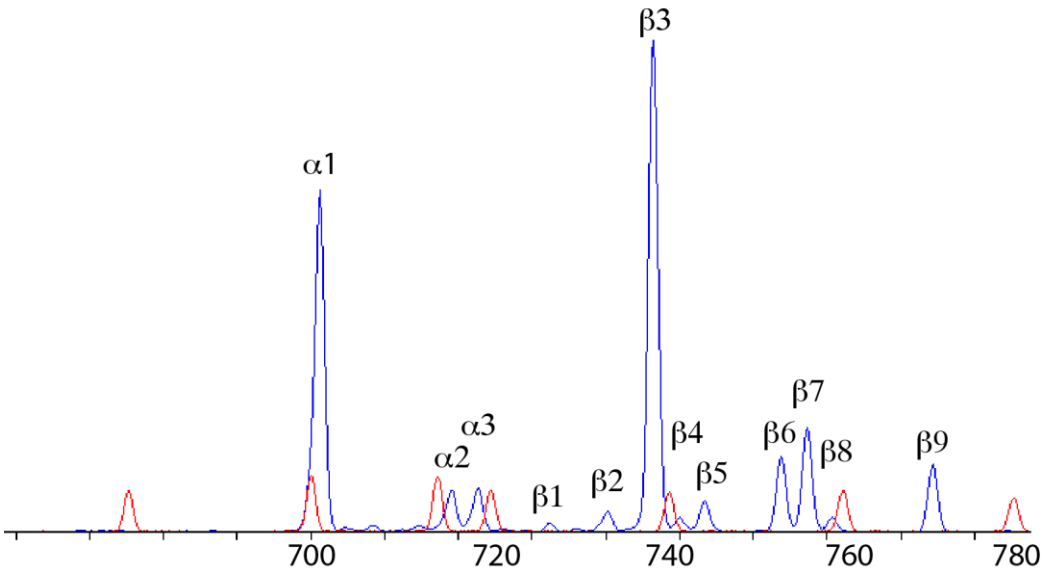



Figure 2. *Tnnt3* DNA fragment analysis. Fluorescently labeled DNA fragment peaks showing Tnnt3 splice form diversity and abundance (i.e. peak height) of each splice form in rat gastrocnemius muscle. Internal size standards are represented by red traces. Relative abundance of specific troponin T mRNA splice forms is defined as the ratio of its peak height and total peak height of all splice forms.

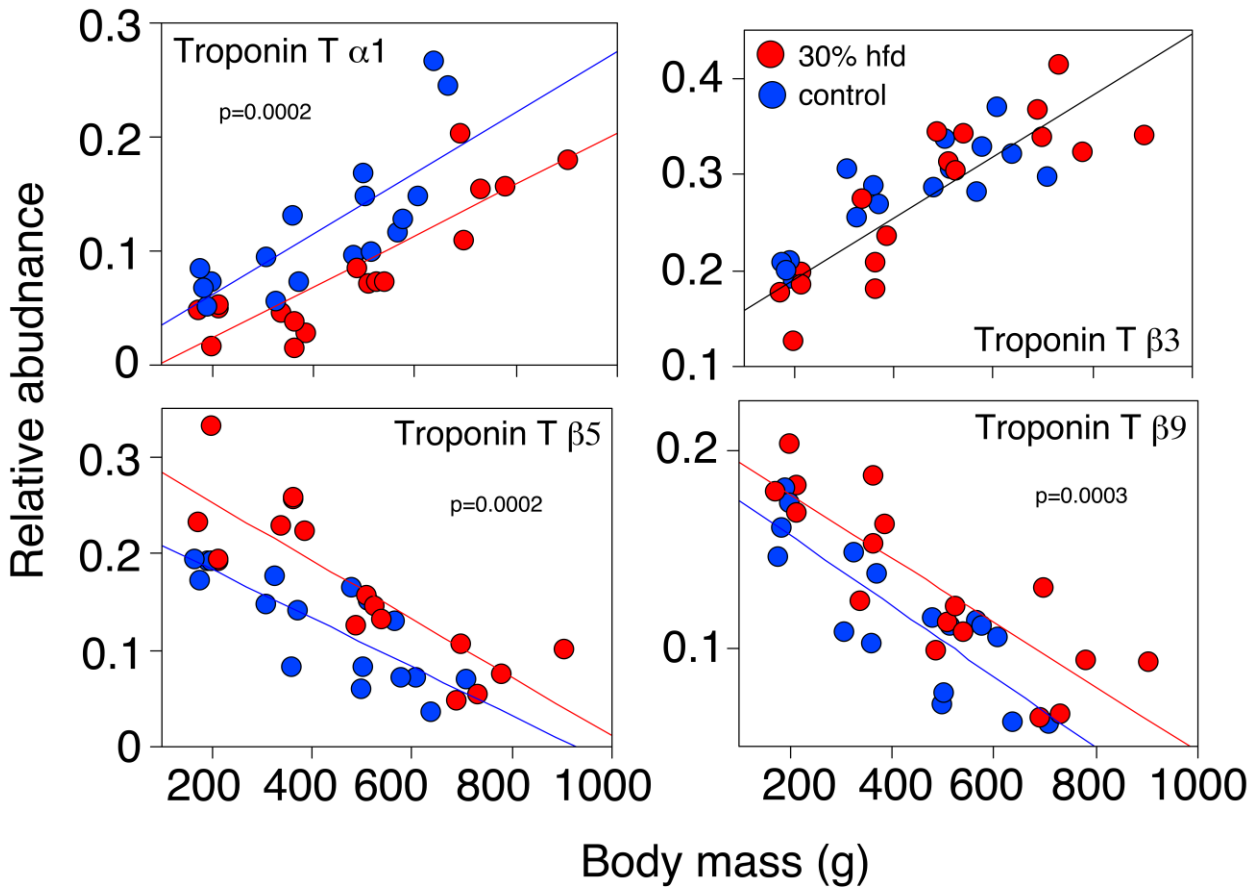



Figure 3. Effect of 30% high fat diet on gastrocnemius muscle alternative Tnnt3 pre-mRNA splicing. Presented are the relative abundances of Tnnt3 splice forms α1, β3, β5, and β9 (the 4 most abundant splice forms in gastrocnemius muscle) as a function of body mass. Solid lines depict linear regression fits to data for control and 30% high fat diet fed animals separately. Data shows significant depression (i.e. Tnnt3 α1), and elevation (i.e. Tnnt3 β5, β9) of the slope of fits relating relative splice form abundance to body weight. No effect of the dietary treatment was observed for Tnnt3 β3.

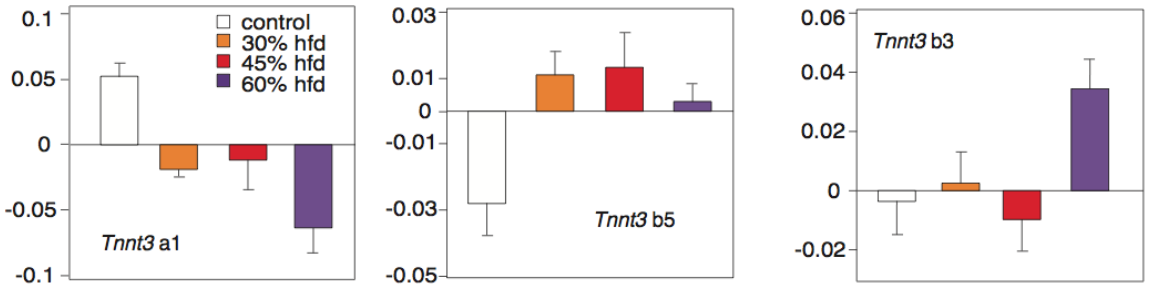



Figure 4. A high-fat diet affects alternative pre-mRNA splicing. DNA fragment analysis of RNA isolated from the gastrocnemius of rats fed a control (10 kcal% fat) or 30, 45, or 60 kcal% fat diet for eight weeks. Three representative splice forms of fast troponin T (*Tnnt3*) are shown.

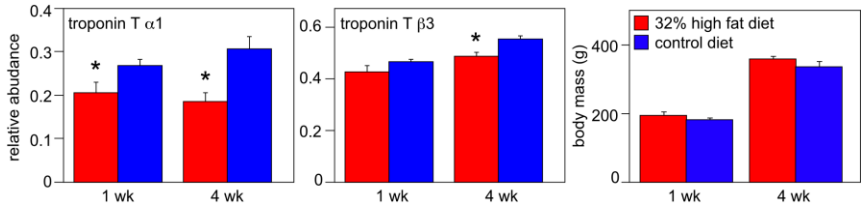



Figure 5. A high-fat diet affects alternative pre-mRNA splicing. DNA fragment analysis reveals that male Sprague-Dawley rats show body weight inappropriate levels of troponin T α1 and β3 splice forms in gastrocnemius after 1-4 weeks of high-fat diet, versus rats on a low-fat diet. Rats from different treatments did not differ in body weight. Asterisks indicate significant differences at $\alpha = 0.05$.

C.1. Studies related to Specific Aim 2

The budget included with the original application was cut, and therefore, the scope of the project had to be reduced. Because the cost of purchasing and maintaining animals was a significant portion of the budget for the studies proposed in the original Specific Aim 2, we decided to revise the aim to include an investigation of the signaling pathways involved in regulating *Tnnt3* pre-mRNA splicing in cells in culture, rather than the originally proposed animal studies.

Methods

Cell culture and mechanical stretch conditions. C2C12 myoblasts were purchased from ATCC (Manassas, VA, USA). The cells were maintained in high-glucose (i.e. 4.5 g/L) DMEM with 10% FBS and 1% P/S under standard cell culture conditions (i.e. 5% $CO_2$, 95% $O_2$, T = 37°C). They were seeded at 100,000/well in type I collagen-coated, BioFlex® flexible-bottomed six-well culture dishes (Flexcell International, Hillsborough, NC, USA) in "medium-glucose" (i.e. 2.75 g/L) DMEM with 10% FBS and 1% P/S. The cells were then allowed to proliferate to approximately 80% confluency (~48 h post-seeding), and the medium was replaced with low-glucose DMEM with 10% FBS and 1% P/S at 24 h post-seeding. Myoblasts were induced to differentiate to myotubes in low-glucose DMEM, with 2% HS, and 1% P/S for 6 days. The medium was refreshed every 48 h, and was changed to low-glucose (i.e. 1 g/L), serum-free DMEM with 1% P/S 12 h prior to the onset of cyclic stretch. For the Akt and ERK1/2 inhibition studies, a final medium replacement with low-glucose, serum-free DMEM with 1% P/S containing 0.4 uM Akt_KI or 10 µM U0126, respectively, was performed 2 h prior to the onset of mechanical stretch. Cells were kept in medium containing the inhibitors throughout the 24 h experiment.

The BioFlex® culture plates were placed on the Flexcell FX-5000 Tension system (Flexcell International, Hillsborough, NC, USA) 2 h prior to the onset of the actual experiments. The Flexcell FX-5000 Tension system provides equibiaxial, cyclic strain by means of vacuum-controlled stretching of the flexible rubber bottom of BioFlex® culture plates around cylindrical loading posts. The loading posts are kept in an incubator under standard cell culture conditions (37°C, 5% $CO_2$, 95% $O_2$). Mechanical stretch was applied by means of a sinusoidal (0.5Hz), 15% strain wave for 24 h. Cells were harvested at 0.5, 1, 2, 4, 8, and 24 h, except for the Akt and ERK1/2 inhibition experiments, in which case cells were harvested

at 8 and 24 h only. Control cells were maintained in similar fashion on type I collagen-coated BioFlex culture plates, but did not receive mechanical stretch.

Characterization and quantitation of *Tnnt3* pre-mRNA splice form abundance. Total RNA was extracted from cell lysates using Trizol reagent (Invitrogen), and precipitated in isopropanol, according to the manufacturer's instructions. The RNA and non-template (i.e. $H_2O$ instead of RNA) samples were reverse transcribed using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems). *Tnnt3* amplicons were amplified by PCR using fluorescein (FAM)-labelled forward primer fTnt_F1 (5'-FAM-CCCCCAACCTTCTCAGACT-3'), and two unlabeled reverse primers, fTnt_R2 (5'-CCTTCTTGCTGTGCTTCTGG-3') and fTnt_R4 (5'-CGGACAGTCATGATATCGTATTT-3'). PCR was performed using HotStart GoTaq polymerase (Promega) under the following cycling conditions: 2 min at 95°C, followed by 4 cycles of 30s at 94°C, 30s at 65°C (-1.0°C/cycle), followed by 1 min, 15s at 72°C. This was followed by 33 cycles of 30s at 94°C, 30s at 60°C, 1 min, 15s at 72°C, and ending with a final 15 min at 72°C. Negative control samples were included in this analysis for each experiment.

To quantify effects of mechanical stretch on *Tnnt3* alternative splicing, irrespective of overall gene expression patterns (see Figure 6A), we determined quantitative changes within the *Tnnt3* splice form mixture for each sample. Thus, FAM-labelled PCR products were diluted 1:10 and 1 µL was analyzed by capillary electrophoresis (ABI Hitachi 3730XL DNA Analyzer; Applied Biosystems), which allows precise determination of PCR fragment size and quantity in a PCR amplicon pool. Samples with fragment peak heights exceeding the linear detection range of the instrument were further diluted and run again. The relative abundance of each *Tnnt3* amplicon in the PCR reaction was determined by dividing its peak height by the total of all *Tnnt3* amplicon peak heights (see also Figure 6). The use of this relative abundance measure is a method of normalizing for different starting amounts of template. For our purposes therefore, no further normalizing method is necessary**.** Amplicon fragment size was determined using the GS1200 LIZ internal size standard and Genemapper® (Applied Biosystems, Carlsbad, CA, USA) fragment analysis software.
To examine *Tnnt3* splice form exon composition, amplicons were extracted from an agarose gel using a QiaQuick gel extraction kit (Qiagen), cloned using a TOPO-TA cloning kit (Invitrogen), and sequenced (ABI Hitachi 3730XL DNA Analyzer).

Data analysis. All *Tnnt3* splice form relative abundance data were arcsine transformed to meet normality assumptions of the statistical tests applied. Untransformed data means are presented in figures and tables. Specific statistical tests applied to the data are indicated in the text, or figure and table legends. All statistical analyses were performed using JMP™ software (The SAS Institute).

## Results

We first set out to characterize C2C12 *Tnnt3* splice form expression by means of PCR using FAM-labeled primers followed by DNA fragment analysis. For this purpose, C2C12 myotubes were differentiated for 4 days as described in the Methods section and harvested at the start of differentiation (i.e. timepoint 0; at this point myoblasts were at 80% confluency), and 24, 48, 72, and 96 hours post-differentiation. Total RNA was extracted from cell lysates, reverse transcribed, and *Tnnt3* splice forms were amplified by PCR. DNA fragment analysis

of the fluorescently-labeled PCR amplicon pool resulted in the identification of 15 constitutively present (i.e. present in each individual sample, but not in negative control samples) DNA fragment peaks (Figure 6). Cloning and sequencing of PCR amplicon pools resulted in the identification of 13 of the expected 15 alternative *Tnnt3* mRNA splice forms. The predicted exon composition of the remaining two amplicons (i.e. *Tnnt3* mt1 and mt4) is indicated in Figure 6.

As shown in Figure 7A, mechanical stretch significantly decreased overall inclusion of *Tnnt3* exon 4 in C2C12 myotubes at 24 h of treatment. This effect was driven by increased levels of *Tnnt3* mt1 and mt2, which lack exon 4, and decreased levels of *Tnnt3* mt14 and mt15, which contain exon 4 (Figure 7B). Notably, overall exon 4 inclusion decreased despite a significant increase in the relative abundance of *Tnnt3* mt4, which does contain exon 4. Mechanical stretch had no effect on the relative abundance of 7 out of 15 splice forms (*Tnnt3* mt5-6, mt9-13), including one of the most abundant splice forms *Tnnt3* mt12. Considerable baseline (i.e. in the absence of mechanical stretch) variation in the relative abundance of 12 out of 15 *Tnnt3* splice forms was also detected. Finally, acute (i.e. after 30 min) changes in the relative abundance of *Tnnt3* mt3-4, and mt7-8 were detected, but these were not sustained at subsequent timepoints. These acute changes are responsible for the increased levels of *Tnnt3* splice forms containing exon 4 observed at the 30 min timepoint in Figure 7A. Effects of mechanical stretch on *Tnnt3* alternative splicing were not associated with changes in total *Tnnt3* mRNA levels (Figure 7C) or apparent cell numbers or protein content, as determined by Western blotting of GAPDH levels (Figure 7D).

To examine the possible roles of Akt and ERK1/2 signaling in the regulation of *Tnnt3* splicing we first examined how the mechanical stretch paradigm affected Akt and ERK1/2 phosphorylation. Phosphorylation of both kinases increases rapidly (i.e. within 30 minutes; Figure 8) in response to mechanical stretch. While Akt phosphorylation decreased to control levels after 2 h, ERK1/2 phosphorylation followed a biphasic pattern, with a second significant activation event occurring at 8 hours, and maintained at 24 hours. Similar rapid onset activation patterns for Akt and ERK1/2 signaling were previously reported for these kinds of mechanical stretch paradigm, and our findings provide further evidence for the role of these protein kinases (and associated signaling pathways) in translating mechanical stimuli to intracellular functional events.

We next performed experiments in which C2C12 myotubes were mechanically stretched in the presence of Akt_KI, a specific inhibitor of Akt phosphorylation, or the MEK signaling inhibitor U0126. In these experiments, myotubes were harvested only at 8 and 24 hours of stretch, as these were the time points where most significant mechanical stretch induced effects on *Tnnt3* splicing were observed. In confirmation of the results depicted in Figure 8, no effect of mechanical stretch on Akt phosphorylation was observed at either 8 or 24 hours (Figure 9A). In contrast, addition of Akt_KI to the medium significantly blunted Akt phosphorylation at 8 and 24 hours in both control and mechanically-stretched cells, whereas addition of U0126 did not affect basal levels of Akt phosphorylation. Addition of U0126 significantly blunted ERK1/2 phosphorylation at 8 and 24 hours in both control and mechanically-stretched cells (Figure 9B), whereas Akt_KI did not affect basal levels of ERK1/2 phosphorylation. As observed in Figure 8 mechanical stretch caused increased ERK

phosphorylation in both untreated and Akt_KI-treated, mechanically-stretched cells (although no statistically significant increase was observed for the Akt_KI treated, mechanically-stretched cells; Figure 9B).

In the study depicted in Figure 10, mechanical stretch caused alterations in *Tnnt3* pre-mRNA alternative splicing that qualitatively recapitulated those presented above. In other words, in both untreated, and U0126-treated C2C12 myotubes, mechanical stretch caused decreased overall *Tnnt3* exon 4 inclusion at 24 h of treatment (Figure 10A). Importantly, Akt_KI treatment prevented this stretch-induced effect. More specifically, Akt_KI treatment prevented the stretch-induced increase in *Tnnt3* mt1 relative abundance, and decreases in *Tnnt3* mt14-15 relative abundance (Figure 10B) demonstrated earlier, while the stretch-induced increases in *Tnnt3* mt2 and mt4 relative abundance were unaffected by Akt_KI treatment.

## Discussion

In the studies described above, we demonstrate how mechanical stretch of C2C12 muscle cells in culture results in changes to *Tnnt3* pre-mRNA alternative splicing that are qualitatively similar to those observed in response to added weight in rats. Moreover, inhibition of Akt signaling, but not that of ERK1/2, prevent the stretch-induced effect on *Tnnt3* pre-mRNA alternative splicing. These findings suggest that effects of muscle load on *Tnnt3* pre-mRNA alternative splicing are controlled by a cell-autonomous mechanism, rather than systemically. They also indicate that, in addition to its regulatory role in protein synthesis and muscle mass plasticity, Akt signaling may regulate muscle sarcomere composition by modulating alternative splicing events in response to load. Manipulation of *Tnnt3* pre-mRNA alternative splicing by mechanical stretch of cells in culture provides a model to investigate the biology of weight sensing by skeletal muscles, and facilitates identification of mechanisms through which skeletal muscles match their performance and experienced load.

C.2. Figures for Specific Aim 2

A

| 5' UTR | 1 - 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 -15 | 16 | 17 | 18 | 3' UTR | Relative abundance (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| mt1 | | | X | | | | X | | X | | | | 5.0 (0.7) |
| mt2 | | | X | X | X | X | | | X | | | | 1.1 (0.2) |
| mt3 | | | X | | | X | | | | X | | | 2.9 (0.4) |
| mt4 | | X | X | | | | | | | X | | | 12.9 (1.1) |
| mt5 | | | X | X | X | | | | | X | | | 1.1 (0.3) |
| mt6 | | | X | X | | X | | | | X | | | 1.4 (0.3) |
| mt7 | | X | X | | | X | | | | X | | | 15.6 (0.4) |
| mt8 | | | X | | | | X | | | X | | | 4.3 (0.3) |
| mt9 | | | X | X | X | X | | | | X | | | 1.7 (0.2) |
| mt10 | | X | X | X | X | | | | | X | | | 2.7 (0.7) |
| mt11 | | | X | | | X | X | | | X | | | 2.6 (0.2) |
| mt12 | | X | X | | | | X | | | X | | | 19.0 (1.0) |
| mt13 | | | X | X | | X | X | | | X | | | 2.2 (0.2) |
| mt14 | | X | X | | | X | X | | | X | | | 21.0 (1.4) |
| mt15 | | X | X | X | X | | X | | | X | | | 3.9 (0.4) |

B

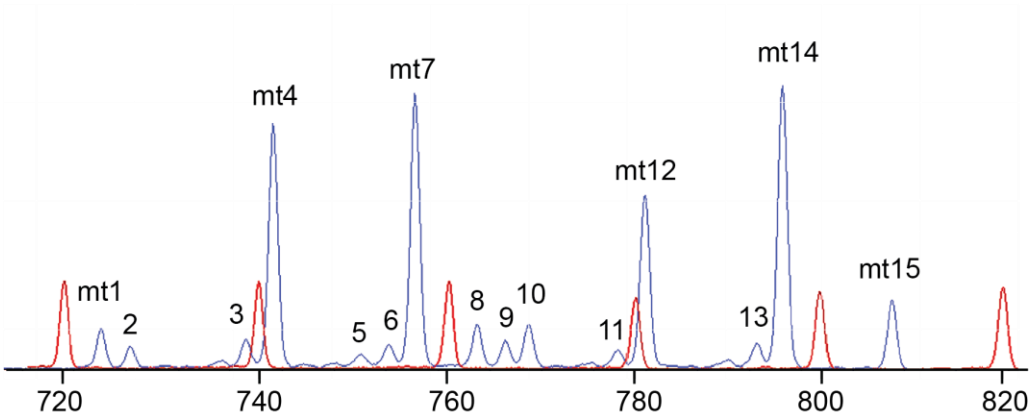



Figure 6. Characterization of C2C12 myotube *Tnnt3* splice forms. A. Mouse *Tnnt3* pre-mRNA contains 18 exons. While the 5'-end alternative exon cassette (i.e. exons 4 -9) combined with mutually exclusive 3'-end exons 16 and 17 can give rise to potentially 124 different splice forms, only 15 *Tnnt3* splice forms (labeled mt1 - mt15) were detected. *Tnnt3* splice forms highlighted in red have not yet been verified by cloning and sequencing. The relative abundance of each *Tnnt3* splice form at day 7 of differentiation is indicated on the right (values represent means (SE)). B. Fluorescently labeled *Tnnt3* PCR amplicons from C2C12 myotubes separated by means of capillary electrophoresis (blue trace). Intensity (height) of peaks is proportional to the mRNA splice form abundance in the original RNA sample. Internal size standards are represented by the red trace.

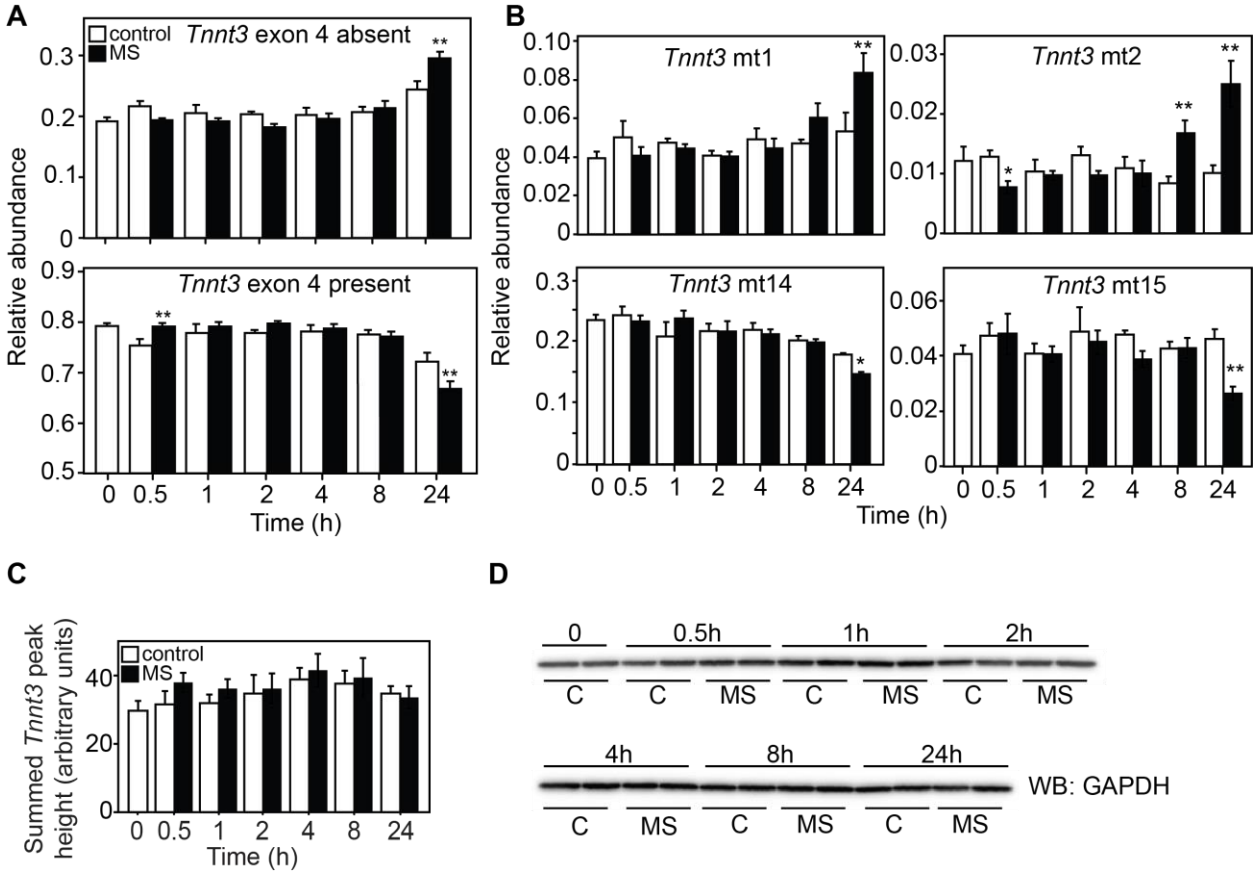



Figure 7. Mechanical stretch induces changes in alternative splicing of *Tnnt3* pre-mRNA. A. Mean summed relative abundances of *Tnnt3* splice forms missing (upper panel) or including (lower panel) exon 4, as a function of the duration of mechanical stretch. B. Mean relative abundance of individual *Tnnt3* splice forms (*Tnnt3* mt1-2, and mt14-15) affected by mechanical stretch. *Tnnt3* mt4 was also increased by mechanical stretch (Figure 8) but not displayed here. Error bars represent standard error of means. Asterisks represent statistically significant effects of mechanical stretch at $\alpha = 0.05$ (*), or $\alpha = 0.01$ (**), as determined by two-way ANOVA, followed by LS Means Contrasts Student's t-tests at a given time point (N = 8 per treatment for each time point, obtained from four (N = 2 per treatment and timepoint) independent experiments). Mechanical stretch did not affect overall *Tnnt3* mRNA levels (C) or protein levels as indicated by GAPDH expression (D) across the experimental timecourse. C = control, MS = mechanical stretch.

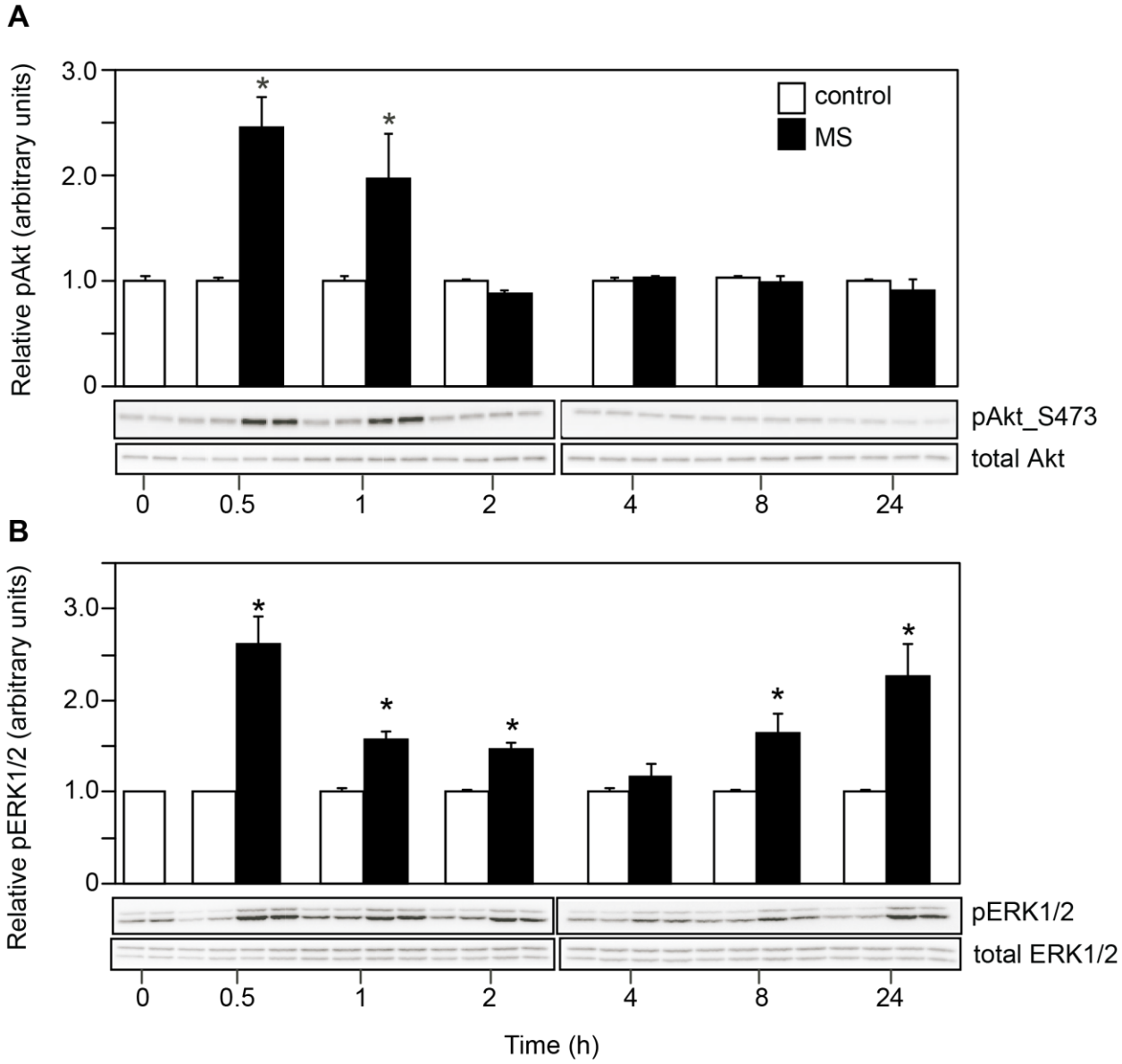



Figure 8. Mechanical stretch activates Akt and ERK1/2 protein kinases. Western blot analysis showing increased mean phosphorylation levels of Akt (top panel), and ERK1/2 (bottom panel) kinases in C2C12 myotubes in response to mechanical stretch. Representative blots (duplicates for each treatment) are presented below the quantitative results. In contrast to ERK1/2, for which phosphorylation was increased throughout the experiment, Akt phosphorylation was acute and preceded the effects on *Tnnt3* pre-mRNA alternative splicing. Means of samples from mechanically-stretched cells are presented relative to means in control cells, with the latter set to 1.0. Error bars represent the standard error of means, and asterisks represent statistically significant differences at $\alpha = 0.05$ (N = 4 per treatment for each time point, obtained from two independent experiments), as determined by Student's t-tests at each time point. MS = mechanical stretch.

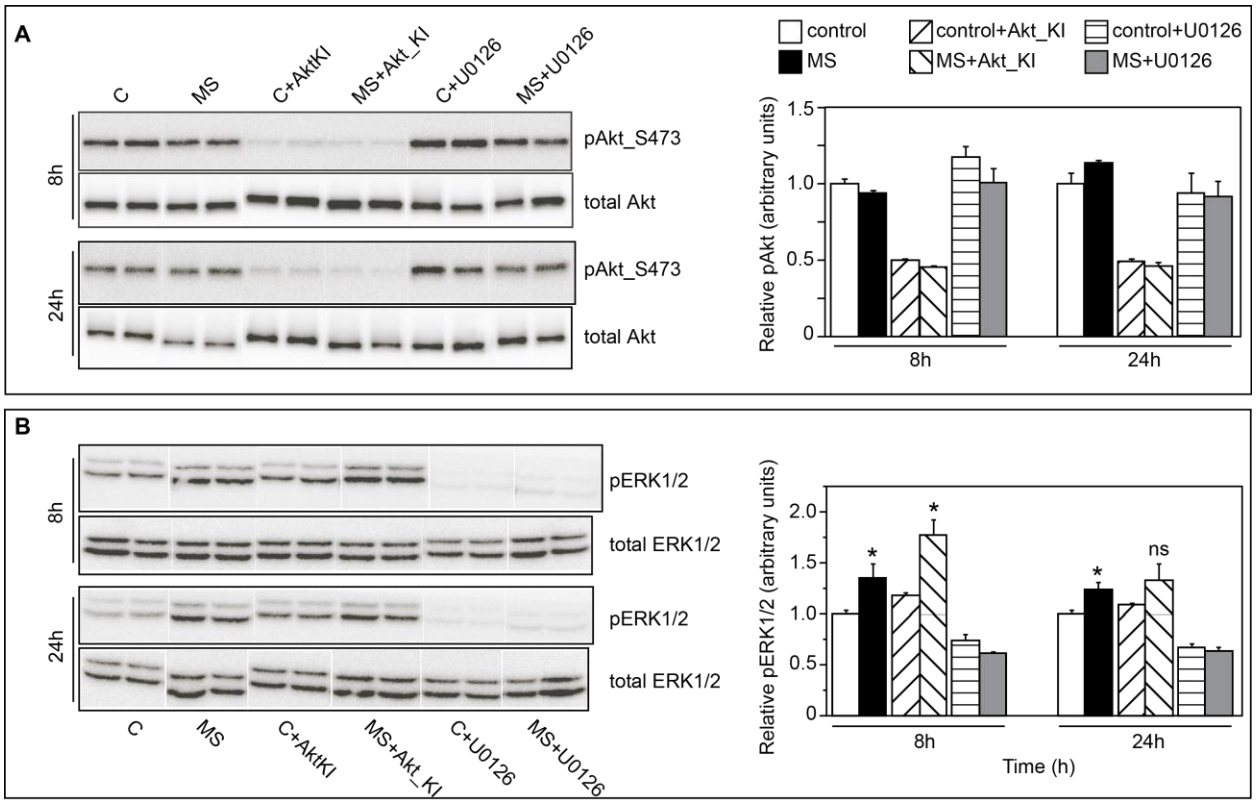



Figure 9. Effects of mechanical stretch in the presence of pharmacological inhibitors of Akt or ERK1/2. Western blot analyses of phosphorylation levels of Akt and ERK1/2 in mechanically-stretched (MS) and control (C) cells, in the presence or absence of inhibitors Akt_KI or U0126 (see Methods), at 8 h and 24 h of treatment. Representative blots (duplicates per treatment) are displayed in the left panels, while quantification of relative phosphorylation (with respect to total Akt and ERK1/2 levels respectively) are displayed in the right hand panels. In both cases, the effects of mechanical stretch on Akt and ERK1/2 phosphorylation displayed in Figure 8 were completely blocked by the respective inhibitors, while no adverse effects of Akt_KI on ERK1/2 phosphorylation, or U0126 on Akt phosphorylation were observed. Error bars represent standard error of means, and asterisks indicate significant differences between control and mechanically-stretched cells for each treatment at $\alpha = 0.05$ (N = 6 per treatment for each time point; two independent experiments were performed with N = 3 per treatment and time point each), as determined by Student's t-tests. C = control, MS = mechanical stretch.

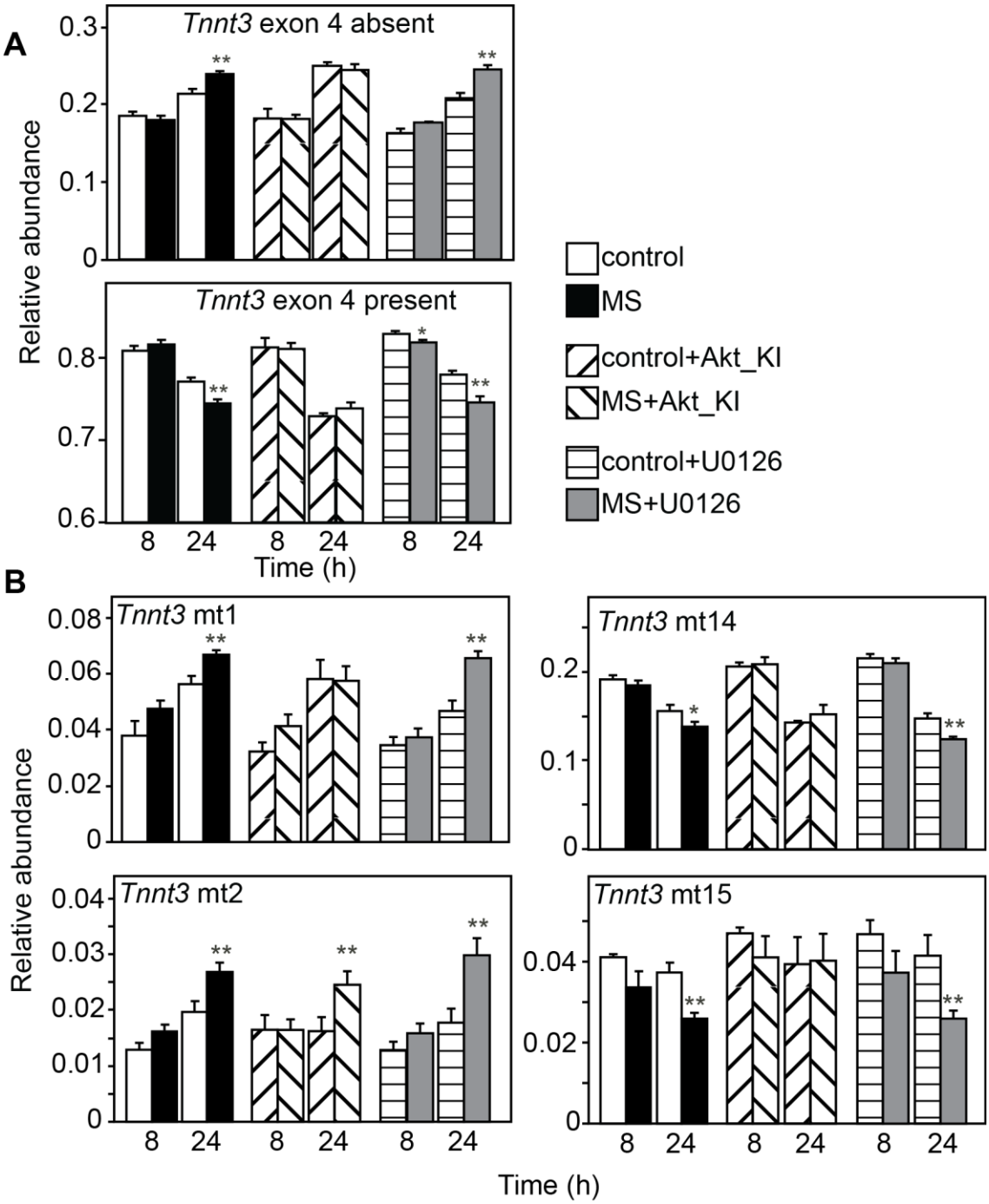



Figure 10. Akt-KI inhibits the effect of mechanical stretch on alternative splicing of *Tnnt3* pre-mRNA. A. Mean summed relative abundances of *Tnnt3* splice forms missing (upper panel) or including (lower panel) exon 4, at 8 h and 24 h of mechanical stretch in the presence or absence of pharmacological inhibitors Akt_KI and U0126. Mechanical stretch caused the same effects on *Tnnt3* exon 4 inclusion for control and U0126 treated cells as displayed in Figure 7. This effect was inhibited by Akt_KI treatment. B. Akt_KI inhibited the response to mechanical stretch for *Tnnt3* mt1, mt14-15, but not for *Tnnt3* mt2 and mt4 (the latter is not displayed here). Error bars represent standard error of means. Asterisks

represent statistically significant effects of mechanical stretch at $\alpha = 0.05$ (*), or $\alpha = 0.01$ (**), as determined by two-way ANOVA, followed by LS Means Contrasts Student's t-tests at a given time point (N = 6 per treatment for each time point; two independent experiments were performed with N = 3 per treatment and time point each). MS = mechanical stretch.

Oral presentations that are related to the work described above

4/11/11 Rudolf J. Schilder, Ph.D. "Body weight sensing by skeletal muscle in health and disease", Brown University, Department of Ecology and Evolutionary Biology, Providence, RI

5/16/11 Rudolf J. Schilder, Ph.D. "Body weight sensing by insect and mammalian skeletal muscle", Arizona State University, School of Life Sciences, Tempe, AZ

8/9/11 Scot R. Kimball, Ph.D. "Nutrient-Mediated Regulation of Alternative Pre-mRNA Splicing", FASEB Summer Research Conference: Nutrient Control of Metabolism and Cell Signaling, Steamboat Springs, CO

Posters presented that are related to the work described above

3/14/11 Rudolf J. Schilder, Scot R. Kimball, Leonard S. Jefferson, "A high-fat diet impairs alternative pre-mRNA splicing in skeletal muscle", Penn State Institute for Diabetes and Obesity Summit 2011, State College, PA

4/9/11 Rudolf J. Schilder, Scot R. Kimball, Leonard S. Jefferson, "Regulation of alternative splicing of skeletal muscle Troponin T in C2C12 myotubes in response to cyclic strain and manipulation of mTOR signaling", Experimental Biology 2011, Washington, DC

4/23/12 Scot R. Kimball, Rudolf J. Schilder, Elisabeth A. Charleston, and Leonard S. Jefferson, "A high-fat diet impairs alternative splicing of the troponin T pre-mRNA in skeletal muscle", Experimental Biology 2012, San Diego, CA

**18. Extent of Clinical Activities Initiated and Completed**. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

**If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed.** (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

**Note**: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

**19. Human Embryonic Stem Cell Research.** Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
__X__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

**20. Articles Submitted to Peer-Reviewed Publications**.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

**Note:** The grant agreement requires that recipients acknowledge the Pennsylvania

Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

| Title of Journal Article: | Authors: | Name of Peer-reviewed Publication: | Month and Year Submitted: | Publication Status (check appropriate box below): |
|---|---|---|---|---|
| 1. Cell-autonomous regulation of fast Troponin T pre-mRNA alternative splicing in response to mechanical stretch | Rudolf J. Schilder, Scot R. Kimball, Leonard S. Jefferson | American Journal of Physiology: Cell Physiology | November 2011 | ☐ Submitted<br>☐ Accepted<br>X Published |

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes___X_____ No_____

If yes, please describe your plans:

The studies involving the effects of feeding a high-fat diet on troponin T pre-mRNA alternative splicing will be included in a manuscript to be prepared for submission to a peer-reviewed journal in the future.

**21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project.** Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

There is no immediate impact of the current research on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures. However, because troponin T isoforms play an important role in determining the efficiency of muscle function, understanding the mechanisms involved in disregulated isoform expression in response to consumption of a high-fat diet has the potential to provide a possible avenue for treating the complications associated with type 2 diabetes and the metabolic syndrome in the future.

**22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment**. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable."

Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

**23. Inventions, Patents and Commercial Development Opportunities.**

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No___

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes__ No___

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No___X_____

If yes, please describe your plans:

**24. Key Investigator Qualifications.** Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

## BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

| NAME | POSITION TITLE |
|---|---|
| Scot R. Kimball | Professor |
| eRA COMMONS USER NAME (credential, e.g., agency login) | |
| skimball | |

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | MM/YY | FIELD OF STUDY |
|---|---|---|---|
| Clarkson College, Potsdam, NY | B.S. | 1977 | Chemistry |
| University of Vermont, Burlington, VT | Ph.D. | 1983 | Biochemistry |
| Penn State College of Medicine, Hershey, PA | Post-doc | 1982-1985 | Physiology |

### A. Personal Statement

I have studied the regulation of mRNA translation for more than 25 years. As a post-doctoral fellow, I purified, characterized, and subsequently cloned a number of mammalian translation initiation factors. After joining the faculty at The Pennsylvania State University College of Medicine I pursued studies designed to delineate mechanisms involved in controlling the translation of mRNAs, both on a global and individual basis. During the course of these studies, I developed assays for assessing the activity of many of the translation initiation factors and their regulatory proteins, as well as the signaling pathways that control their function. With these tools, I have pursued investigations into the mechanisms through which nutrients and hormones act to stimulate protein synthesis in skeletal muscle and the liver. In the past 10 years, my research has expanded to include an investigation of the mechanisms and signaling pathways involved in the upregulation of protein synthesis in skeletal muscle in response to resistance exercise, and, conversely, the mechanisms and signaling pathways involved in the downregulation of skeletal muscle protein synthesis under conditions of muscle wasting, e.g. aging, sepsis, and inactivity. Pursuit of a number of these areas of investigation has led to the establishment of productive collaborations with researchers both at the Penn State College of Medicine and at other institutions. In particular, in recent studies I have collaborated with Drs. Rudolf Schilder and James Marden on assessing the effect of diet and body weight on alternative splicing of the troponin T pre-mRNA in rats, as well as a number of ongoing collaborations with other investigators.

### B. Positions and Honors

Employment

1985-93 Assistant Professor, Department of Cellular and Molecular Physiology, The Pennsylvania State University College of Medicine, Hershey, PA

1993-2000 Associate Professor, Department of Cellular and Molecular Physiology, The Pennsylvania State University College of Medicine, Hershey, PA

2000-Pres. Professor, Department of Cellular and Molecular Physiology, The Pennsylvania State University College of Medicine, Hershey, PA

Honors

1973-77 NY State Regent's Scholarship, Harrington Memorial Scholarship, Clarkson

| | |
|---|---|
| | Trustee Scholarship |
| 1976 | Elected to Gamma Sigma Epsilon (National Chemistry Honor Fraternity) |
| 1977 | Graduated with Honors, Clarkson College, Potsdam, New York |
| 1977-82 | Teaching Assistant, Department of Biochemistry, University of Vermont, Burlington, Vermont |
| 1978 | Muscular Dystrophy Association Scholarship |
| 1983-85 | NIH Postdoctoral Fellowship, Department of Physiology, The Pennsylvania State University College of Medicine, Hershey, PA (with Professor Leonard S. Jefferson) |

Editorial board memberships

International Journal of Biochemistry and Cell Biology
American Journal of Physiology: Endocrinology/Metabolism
Journal of Nutrition

Study sections
2008 EMNR Special Emphasis Study Panel
2009 ad hoc reviewer, NIH Challenge Grants
2010 EMNR Special Emphasis Study Panel
2011 EMNR Special Emphasis Study Panel

**C. Peer-reviewed Publications or Manuscripts (selected from a total of 236)**

1. Whitney, M.L., Jefferson, L.S., and Kimball, S.R. ATF4 is necessary and sufficient for ER stress-induced upregulation of REDD1 expression, Biochem. Biophys. Res. Commun. 379:451-5, 2009 (PMC19114033)
2. McGhee, N.K., Jefferson, L.S., and Kimball, S.R. Elevated corticosterone associated with food deprivation upregulates expression in rat skeletal muscle of the mTORC1 repressor, REDD1, J. Nutri. 139:828-34, 2009 (PMC2714387)
3. Gallagher, J.W., Kubica, N., Kimball, S.R., and Jefferson, L.S. Reduced eukaryotic initiation factor 2B ε-subunit expression suppresses the transformed phenotype of cells overexpressing the protein, Cancer Res. 68:8752-60, 2008 (PMC2664734)
4. Winter, J.N., Fox, T.E., Kester, M., Jefferson, L.S., and Kimball, S.R. Phosphatidic acid mediates activation of mTORC1 through the ERK signaling pathway, Am. J. Physiol. 299:C335-44, 2010 (PMC2928642)
5. Tuckow, A.P., Vary, T.C., Kimball, S.R., and Jefferson, L.S. Ectopic expression of eIF2Bε in rat skeletal muscle rescues the sepsis-induced reduction in guanine nucleotide exchange activity and protein synthesis, Am. J. Physiol. 299:E241-8, 2010 (PMC2928514)
6. Schilder, R.J., Kimball, S.R., Marden, J.H., and Jefferson, L.S. Body weight-dependent troponin T alternative splicing is evolutionarily conserved from insects to mammals and partially impaired in skeletal muscle of obese rats, J. Exptl. Biol. 214:1523-1532 (2011) (PMC3076076)
7. Woo, C.W., Kutzler, L., Kimball, S.R., and Tabas I. Toll-like receptor activation suppresses endoplasmic reticulum stress-induced CHOP and translation inhibition through activation of eIF2B, Nature Cell Biol. 14:192-200 (2012)
8. Schilder, R.J., Kimball, S.R., and Jefferson, L.S. Cell-autonomous regulation of fast Troponin T pre-mRNA alternative splicing in response to mechanical stretch, Am. J. Physiol. Epub ahead of print; PMID 22592404 (2012)

## BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

| NAME | POSITION TITLE |
|---|---|
| Rudolf J. Schilder | Research Associate |
| eRA COMMONS USER NAME (credential, e.g., agency login) | |
| RJSCHILDER | |

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | MM/YY | FIELD OF STUDY |
|---|---|---|---|
| Wageningen University and Research Center, Wageningen, The Netherlands | M. Sc. | 1994-2000 | Biology |
| Pennsylvania State University, University Park, PA | Ph.D. | 2001-2006 | Physiology |
| University of Nebraska, Lincoln, NE | Post-doc | 2006-2008 | Biochemistry |
| Pennsylvania State University, Hershey, PA | Post-doc | 2008-2011 | Physiology |

### A. Personal Statement

I have a broad background in comparative physiology. My work integrates molecular, organ- and whole organism physiology to understand the homeostatic interactions between musculoskeletal design and performance requirements. My Ph.D. work focused on the analysis of body weight, and environmental (i.e. infection, nutritional status) effects on muscle composition and *in vivo* and *in vitro* muscle function. My postdoctoral research examined molecular (genetic) regulatory processes (i.e. gene transcription-, and post-transcriptional mechanisms such as alternative mRNA splicing) important to energy metabolism and muscle performance in insects and mammals. My current work continues to explore the genetic architecture and neurobiology controlling skeletal muscle plasticity in response to body weight variation.

### B. Positions and Honors

Employment

2001-2006 Graduate student, Department of Biology, Pennsylvania State University, University Park, PA

2006-2008 Postdoctoral Scholar, School of Biological Sciences, University of Nebraska, Lincoln, NE

2008-2010 Postdoctoral Scholar, Department of Cellular and Molecular Physiology, Pennsylvania State University College of Medicine, Hershey, PA.

2010-2012 Postdoctoral Fellow, Department of Cellular and Molecular Physiology, Pennsylvania State University College of Medicine, Hershey, PA (American Physiological Society Postdoctoral Fellow in Physiological Genomics).

2011- Research Associate, Department of Biology, Pennsylvania State University, University Park, PA & Department of Cellular and Molecular Physiology, Pennsylvania State University College of Medicine, Hershey, PA.

Honors

| | |
|---|---|
| 2006 | APS Comparative and Evolutionary Physiology Section Scholander Award |
| 2006 | Pennsylvania State University, Dept. of Biology Graduate Student Research Excellence Award |
| 2010 | American Physiological Society Postdoctoral Fellowship in Physiological Genomics |
| 2010 | American Physiological Society Intersociety Meeting Best Poster Award |

**C. Peer-reviewed Publications or Manuscripts**

1. R. J. Schilder, J. H. Marden (2004). A hierarchical analysis of the scaling of force and power output by dragonfly flight motors. *The Journal of Experimental Biology* 207, 767-776.

2. R. J. Schilder, J. H. Marden (2006). Metabolic syndrome and obesity in an insect. *Proceedings of the National Academy of Science USA* 103, 18805-18809.

3. R. J. Schilder, J. H. Marden (2007). Gut microbes trigger metabolic syndrome in an insect. *Journal of Diabetes Science and Technology* 1, 794-796.

4. R. J. Schilder, J. H. Marden (2007). Parasites, proteomics, and performance: effects of gregarine gut parasites on dragonfly flight muscle composition and function. *The Journal of Experimental Biology* 210, 4298-4306.

5. R. J. Schilder, S. R. Kimball, J. H. Marden, and L. S. Jefferson (2011). Body weight-dependent troponin T alternative splicing is evolutionarily conserved from insects to mammals and partially impaired in skeletal muscle of obese rats, *The Journal of Experimental Biology* 214: 1523-1532.

6. R. J. Schilder, A. J. Zera, C. Black, M. Hoidal, and P. Gribben (2011). The biochemical basis of life history adaptation: Molecular and enzymological causes of NADP+-isocitrate dehydrogenase activity differences between morphs of Gryllus firmus that differ in lipid biosynthesis and life history. *Molecular Biology and Evolution* 28: 3381-93.

7. R. J. Schilder, S. R. Kimball, L. S. Jefferson (2012). Cell-autonomous regulation of fast Troponin T pre-mRNA alternative splicing in response to mechanical stretch. *American Journal of Physiology Cell Physiology* Epub ahead of print; PMID 22592404.

8. J. H. Marden, H. W. Fescemeyer, R. J. Schilder, W. R. Doerfler, C. W. Wheat. Polymorphism in an energy metabolism gene affecting the hypoxia inducible pathway, tracheal network development, flight, and mitochondrial integrity. *PloS Genetics* (In Review).