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# Antagonistic functions of LMNA isoforms in energy expenditure and lifespan

Isabel Cristina Lopez-Mejia, Marion De Toledo, Carine Chavey, Laure Lapasset, Patricia Cavelier, Celia Lopez-Herrera, Karim Chebli, Philippe Fort, Guillaume Beranger, Lluis Fajas, Ez Zoubir Amri, François Casas and Jamal Tazi

*Corresponding author: Jamal Tazi, Institut de Génétique Moléculaire de Montpellier*

**Review timeline:**

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## Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

*Editor: Esther Schnapp*

1st Editorial Decision 10 November 2013

Thank you for your patience while your manuscript was peer-reviewed. We have now received the full set of referee reports that is pasted below.

As you will see, while referee 1 raises a number of concerns, referees 2 and 3 acknowledge that the reported phenotypes of the lamin C only mice are interesting. However, both referees also point out that the link between the metabolic phenotypes and increased longevity remains unclear and that it should be strengthened and discussed. They also both request that circulating lipids, glucose and insulin should be examined in the LCS/LCS mice, and referee 2 adds that inflammation and gender differences should be investigated. S/he further pinpoints several technical issues and missing information and statistics that need to be provided. While referee 2 indicates in the referee cross-comments that a strong change of focus is not necessary, s/he agrees with referee 3 that brown fat should be examined by focusing on the classical BAT depots. Both referees also think that data on the lamin A only mouse would be interesting but are not required for publication of the manuscript here. From the referee cross-comments it is clear that neither referee 2 nor referee 3 agrees with the concerns raised by referee 1, which therefore do not need to be addressed.

Given these constructive comments, we would like to invite you to revise your manuscript with the understanding that the referee concerns (as mentioned above and in their reports) must be fully

 

addressed and their suggestions taken on board. Acceptance of the manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions. Also, the revised manuscript may not exceed 30,000 characters (including spaces, references and figure legends) and 5 main plus 5 supplementary figures, which should directly relate to their corresponding main figure. Shortening to an appropriate length may be made easier by the submission of commonly used materials as Supplementary information. However, please note that materials and methods essential to understanding the experiments described in the main body of the manuscript may not be presented in this way. Secondly, we offer the possibility of combining the results and discussion into a single section. This may help to eliminate some redundancy that is inevitable in discussing the same experiments twice. Changing the reference style to the numbered EMBO reports style will also help to reduce the character count. Please let me know if you have questions regarding manuscript shortening.

Regarding data quantification, can you please specify the number "n" for how many experiments were performed, the bars and error bars (e.g. SEM, SD) and tests used to calculate p-values in the respective figure legends? This information is currently incomplete and needs to be provided in the figure legends.

We recently decided to offer the authors the possibility to submit "source data" with their revised manuscript that will be published in a separate source data file online along with the accepted manuscript. If you would like to use this opportunity, please submit the source data (for example entire gels or blots, data points of graphs, additional images, etc.) of your key experiments together with the revised manuscript. Please include size markers for scans of entire gels, label the scans with figure and panel number, and send one file per figure or per figure panel.

We would also welcome the submission of cover suggestions, or motifs to be used by our Graphics Illustrator in designing a cover.

I look forward to seeing a revised version of your manuscript when it is ready. Please let me know if you have any further questions or comments regarding the revision.

REFEREE REPORTS:

Referee #1:

I am highly skeptical of this report. There are several recent papers in the literature on the topic of mitochondrial function and progeria, but those reports are extremely weak and extremely dubious. This report adds to the confusion/pollution. Even if the findings in this report ultimately turned out to be valid, which is extremely doubtful, this report would not valuable because the data is not convincing.

First, the mechanisms by which a nuclear lamin would affect mitochondrial function is unclear. The result is unexpected and unexplained. And whenever one has an unexpected and unexplained result, one has to worry about passenger gene effects. It is noteworthy that the mutation was generated in strain 129 ES cells. Even if the mice have been backcrossed extensively, one still has to worry about passenger genes. You are looking at hundreds if not thousands of genetic differences, and not just to a single targeted mutation. One could overlook that if the results made genetic or physiologic sense, but the mechanisms are completely unclear.

Also, the numbers of mice examined is too low to draw conclusions about longevity. In many cases, the numbers of mice used for other experiments was too low.

There are too many "data not shown" statements in the paper.

 

The authors state that there was a difference in susceptibility to cancer, but there was no data. Is an effect on lymphatic malignancy a general property of metabolic abnormalities in mice?

It is important to show data for each genotype with the associated wild-type littermates. Do not mix the wild-type mice into one group. Also, the numbers of mice in the experiments do not make sense. Clearly, the numbers do not reflect mendelian ratios for expected offspring. Why? Was there some sort of selection?

Comparing the lamin C only mice to the progeria mice does not make sense. You should compare lamin C only mice with the lamin A only mice. According to references in your manuscript, the lamin A only mice have already been generated.

Drawing inferences about mitochondrial function in progeria mice, which have multiple broken bones, is highly dubious.

If you are going to do metabolic and gene expression studies in fibroblasts, you should do the experiments in mice before and after Cre adenovirus and lacZ adenovirus. Similarly, it would be useful to compare metabolism in mice before and after induction of a ubiquitously expressed Cre trangene.

The bioinformatics studies at the beginning of the paper are irrelevant and should be deleted.

Referee #2:

Summary:

1. Does this manuscript report a single key finding? YES.
Mice with exclusive expression of lamin C display prolonged lifespan, increased adiposity and reduced oxidative potential in adipose tissue, in a fashion opposite to the progeroid phenotype of progerin-expressing mice.
2. Is the reported work of significance (YES), or does it describe a confirmatory finding or one that has already been documented using other methods or in other organisms etc (NO)? YES.
3. Is it of general interest to the molecular biology community? YES.
Lamins are of central importance to cell function, and are associated to human diseases and aging, but work on genetic mouse models in particular will be invaluable to gain in-depth understanding of their molecular function at the organismal level.
4. Is the single major finding robustly documented using independent lines of experimental evidence (YES), or is it really just a preliminary report requiring significant further data to become convincing, and thus more suited to a longer-format article (NO)? NO.
There are some statistics issues of key results and the energy balance assessment is incomplete. The observations on lifespan, energy expenditure and adipose tissue phenotype are not consistently linked and may be correlative/unrelated.

Constructive criticism for the authors:

The current manuscript presents the unexpected observation that mice engineered to express only the Lamin C isoform (instead of A and C) display prolonged survival. This mouse model had been previously reported to have no obvious phenotype. In addition, the authors present convincing data on increased adiposity/body weight and reduced oxidative potential in adipose tissue and isolated cells. These results are contrasted to the phenotype of progerin-expressing mice, and raise the intriguing possibility that lamin C and progerin may have antagonistic functions. Given the central importance of lamins in cellular function and their involvement in human disease and aging, this study would be expected to appeal to a broad readership. There are some statistics issues with key results and the writing style should be improved to deliver clarity of the arguments. The major shortcoming of the study, which needs to be addressed, is the very weak link between the lifespan phenotype and the metabolic/adipose tissue phenotype.

Major points:

 

1. There is no plausible link between prolonged lifespan, increased adiposity, reduced energy expenditure and oxidative capacity of adipose tissue in LCS mice. The observations may be unrelated. In fact, reduced oxidative capacity (incl. lower Pgc1a expression) in adipose tissue has been associated with obesity, insulin resistance and type 2 diabetes (e.g. Kusminski and Scherer, TEM, 2012). The reduction in energy expenditure probably cannot be attributed to white adipose tissue oxidative capacity, since the metabolic rate of white fat is rather small compared to skeletal muscle and other organs. Similarly, the reduced RER is not consistent with reduced oxidative capacity. Although the observations in adipose tissue are interesting, the authors should perform a broader analysis of parameters potentially relevant to aging, if they wish to claim a link between metabolism and prolonged lifespan in the LCS mice. This analysis should include:
- Assessment of systemic insulin sensitivity (serum insulin levels, fasting blood glucose, glucose and insulin tolerance tests).
- Inflammation markers in adipose tissue and serum.
- Serum lipid profiles (lipoproteins, triglycerides, cholesterol, free fatty acids).
- Increased adipocyte size has been linked in some mouse models to an increased ability to store away lipids and prevent lipotoxicity. The serum, liver, muscle lipid concentrations should be informative.
2. The interpretation of the alignment/conservation results for Lamin A/C is not clearly presented and it does not become clear how it contributes to the main hypothesis of the manuscript. The authors should consider a clearer description of the results, their interpretation, and how they contribute to the main hypothesis.
3. How were the survival curves calculated in Fig. 2A (Kaplan-Meier)? How were the mean survival times calculated? Which statistical method was used for comparing life span between mouse genotypes (Fig. 2A/B)? The authors should apply a test applicable to survival curves (e.g. log-rank (Mantel-Cox) test) as they cannot be tested by t-test.
4. How was the gender balance in each genotype in the survival study? Did the curves of male vs female differ? Adipose tissue biology and its impact on systemic metabolism is characterized by strong sex-differences.
5. The oxygen consumption data in Figure 4 have been normalized to body weight, which can be misleading because metabolic rate is not simply proportional to body weight (Tschöp et al., Nature Methods 9, 2012, Speakman et al., Disease Models & Mechanisms, 2013). In other words the effects described in this way may even be reversed if raw oxygen consumption data are shown. Given the substantial differences in body weight between genotypes and the central importance of this result for the study, the authors should apply ANCOVA or a generalized linear model to assess the (statistical) differences in oxygen consumption (see Tschöp et al. or Speakman et al.).
6. Data on food intake and physical activity should be provided in order to obtain a more complete assessment of the energy balance.

Minor points:

7. Figure legend/explanation of details in Fig. 1. Which sequences exactly are depicted under C and D? What do the frames 1 and 2 stand for? The details need to be in the figure legend.
8. The description of the alleles used and how they were generated is not clearly written. Since the molecular details are of central importance for the study, a schematic may help the reader.
9. On which tissue extracts were the western blots in Fig. 2C performed?
10. Which pathways do the authors refer to with '...two of the three most regulated pathways involved energy metabolism...' on page 15, and how do they relate to energy metabolism in a direct way?
11. The conclusion on page 11 that '...LMNA splicing isoforms were involved in the regulation of energy expenditure...' cannot be drawn based simply on altered adipocyte size and unaffected differentiation. The sentence should be rephrased.
12. The statement 'This is a feature that has often been associated with a high-fat diet.' on page 11/12 requires a reference to literature.
13. There is no legend for Suppl. Fig. 5.
14. 'These 30 genes are potentially involved in the process of aging' on page 15: Do the authors mean 'could be involved' or is there a concrete link to aging? Clarification is required.
15. The observed changes in leptin expression cannot explain the metabolic phenotypes in a plausible way. E.g. increased leptin signaling in the LCS/LCS mice would result in reduced food

 

intake and increased energy expenditure leading theoretically to weight loss, opposite tot the observed phenotype. The authors should comment on this.

Referee #3:

The work by Lopez-Mejia and colleagues entitled "Antagonistic functions of LMNA isoforms in energy expenditure and lifespan" describes the effect of two different lamin isoforms on longevity and energy homeostasis. The authors demonstrate that mice expressing exclusively lamin C show increased longevity and decreased energy expenditure which is coupled with an increase in weight gain. IN contrast mice expressing progerin in addition to lamin A and C as reported previously develop a wasting syndrome coupled with shortened lifespan.

The study is very well conducted and discovers some novel aspects of lamin A/C biology. In my opinion this is very interesting, the only criticism I have is that the focus is not placed ideally. It is well known that progerin formation causes lipdystrophy and shortens lifespan, however to my knowledge the general opinion is that fat cells cannot be formed correctly (see also data from Xiong et al, Ageing 2013). The authors show here that expression of this isoform rather causes a wasting syndrome similar to cancer cachexia with enhanced brown fat formation, rather than alterations of a lipid storage phenotype. I would suggest to highlight this aspect of the work and focus more on the brown fat formation in progerin expressing mice. The effect of lamin A loss and exclusive lamin C expression is much more mild and does not completely correlate with the phenotype observed. It seems that mice have less capacity to burn lipids and thus gain more weight again dependent on brown fat formation. Why this should increase longevity however is unclear to me and should be discussed in much more detail. Again I would focus more on the weight phenotype and the reduced appearance of brown fat rather than on the longevity. To substantiate the data on adipose tissue remodeling it would also be interesting to include some results on metabolic parameters such as circulating lipids, glucose and insulin especially for the LCS/LCS mice.

1st Revision - authors' response 27 January 2014

Summary of our findings and their significance

Here we present evidence that RNA processing of the *Lmna* gene acts as an active highly conserved mechanism in mammals to produce two isoforms, lamin C and progerin, with antagonistic functions involved in the control of energy expenditure and lifespan. From an evolutionary perspective, the production of lamin C by RNA processing could arise during evolution to conteract deleterious effect associated with inefficient prelamin A maturation. The finding that lamin C only mice have expended lifespan and reduced energy expenditure, enhances the potential of *Lmna* RNA processing pathway as a target for clinical intervention in aging-related metabolic diseases.

The introductory paragraph contains 189 words (1,339 characters including space) and the main text 5073 words (34,442 characters including space).

There are 5 figures, 4 supplementary figures and 1 supplemental Table.

We would like to thank the reviewers for their constructive comments and suggestions for the improvement of the manuscript.

**Reviewer 2**

*General point:*

1. *There is no plausible link between prolonged lifespan, increased adiposity, reduced energy expenditure and oxidative capacity of adipose tissue in LCS mice. The observations may be unrelated. In fact, reduced oxidative capacity (incl. lower Pgc1a expression) in adipose tissue has been associated with obesity, insulin resistance and type 2 diabetes (e.g. Kusminski and Scherer, TEM, 2012). The reduction in energy expenditure probably cannot be attributed to white adipose tissue oxidative capacity, since the metabolic rate of white fat is rather small compared to skeletal muscle and other organs. Similarly, the reduced RER is not consistent with reduced oxidative capacity. Although the observations in adipose tissue are interesting, the authors should perform a broader analysis of parameters potentially relevant to aging, if they wish to claim a link between metabolism and prolonged lifespan in the LCS mice.*

   Although we believe that lamin isoforms are controlling the energy balance and have an effect on lifespan, we fully agree with the reviewer comment that the two observations could be unrelated. We also agree with the reviewer that our study was missing a broader analysis of metabolic parameters relevant to aging to potentially provide the link between prolonged lifespan, increased adiposity, reduced energy expenditure and oxidative capacity of adipose tissue in LCS mice. Therefore, we have performed the experiments suggested by the reviewer as follow:

- *Assessment of systemic insulin sensitivity (serum insulin levels, fasting blood glucose, glucose and insulin tolerance tests).*

  Metabolic tests for insulin sensitivity and fasting insulin indicated that the *Lmna*$^{LCS/LCS}$ mice have a mild insulin-resistant phenotype consistent with their obese phenotype. Surprisingly, old *Lmna*$^{LCS/LCS}$ mice (20 month old mice) are rather hypoglycemic showing that they are able to compensate for obesity and induced insulin resistance. This finding, reinforce the significance of the Lmna RNA processing pathway in controlling the fat storage and glycemia. These data were included as part of a new Figure 3 and supplemental figure 2D and in the results section (p 9-10). While it is still unclear why *Lmna*$^{LCS/LCS}$ mice have increased lifespan, the data show for the first time that obesity and induced insulin resistance will not have a negative effect on lifespan if lamin C isoform is exclusively expressed in the organism. This sentence was included in the concluding remarks p15

- *Inflammation markers in adipose tissue and serum.*

  The microarray analysis of transcriptome in adipose tissue from LCS and progeria mice included 67 inflammation markers (among which 38 have validated functions as anti- (16), pro-inflammation (17) and 5 both anti and pro- inflammation markers). We did not observe any significant changes in the expression of these markers (value <=0.05 and fold >= 1.5). These suggest that there were no inflammation associated with the expression of LMNA isoforms in the adipose tissue.
  We were unable to obtain compelling evidence for variation of inflammation markers in the serum. The small amount of serum collected from each individual proved not to be enough to perform a rigorous analysis. The analysis will need to collect more blood samples from the various genotypes at various ages (including older mice) and at the moment we do not have this material. Inflammation is itself an important and huge topic that deserves more analysis that we believe beyond the scope of this paper, which is mainly focussed on the adipose tissue.

- *Serum lipid profiles (lipoproteins, triglycerides, cholesterol, free fatty acids).*

  This important analysis was performed with serum from 40-weeks-old mice of different genotypes. The results are presented in supplemental figure 2C. Apart from a slight difference in triglyceride levels, we did not detect any difference in the levels of cholesterol, HDL, LDL and NeFa between *Lmna*$^{G609G/+}$, *Lmna*$^{LCS/LCS}$ and WT mice, implying that *LMNA* isoforms have little effect if any on circulating lipids and lipoproteins. These data were included as supplemental Figure 2C and in the results section (p9)

  *- Increased adipocyte size has been linked in some mouse models to an increased ability to store away lipids and prevent lipotoxicity. The serum, liver, muscle lipid concentrations should*

 

*be informative.*

Although we did not measure lipid concentrations in liver and muscle, we have performed histological analysis of these organs and we did not observe any difference between mice of different genotypes.

*2- the interpretation of the alignment/conservation results for Lamin A/C is not clearly presented and it does not become clear how it contributes to the main hypothesis of the manuscript. The authors should consider a clearer description of the results, their interpretation, and how they contribute to the main hypothesis. `*

As suggested by the reviewer the paragraph dealing with the alignment/conservation of both progerin and Lamin C isoforms has been re written in order to make it clear how this analysis contribute to the main hypothesis (results section p 5-6 and concluding remarks p15)

*3- How were the survival curves calculated in Fig. 2A (Kaplan-Meier)? How were the mean survival times calculated? Which statistical method was used for comparing life span between mouse genotypes (Fig. 2A/B)? The authors should apply a test applicable to survival curves (e.g. log-rank (Mantel-Cox) test), as they cannot be tested by t-test.*

The survival curves were completed using the Kaplan Meier curve. The median survival is representative of the survival curves. We use the Log-rank (Mantel Cox) test to perform the statistical analyses of the survival curves. This information was included in the materials and methods section (p18) and in the legend of figure 2

*4- how was the gender balance in each genotype in the survival study? Did the curves of male vs. female differ? Adipose tissue biology and its impact on systemic metabolism is characterized by strong sex-differences.*

We did not observe any difference between genders of the same genotype. Please note that we did not have enough males of $Lmna^{LCS/+}$ mice and therefore the corresponding curve was omitted. However, the results of male and female survival curves of each genotype are presented supplemental figure 1A. This important information was described in the results section p7

*5- The oxygen consumption data in Figure 4 have been normalized to body weight, which can be misleading because metabolic rate is not simply proportional to body weight (Tschöp et al., Nature Methods 9, 2012, Speakman et al., Disease Models & Mechanisms, 2013). In other words the effects described in this way may even be reversed if raw oxygen consumption data are shown. Given the substantial differences in body weight between genotypes and the central importance of this result for the study, the authors should apply ANCOVA or a generalized linear model to assess the (statistical) differences in oxygen consumption (see Tschöp et al. or Speakman et al.).*

When we have normalized the VO2 to body weight and applied an ANOVA statistical test for the homogeneity of the groups, the results are statistically significant. The major difference between the groups is attributed to the adipose tissue (figure 2D), which represents at most 3% of total body weight.
Furthermore, the results obtained with MEFs are consistent with the global energy expenditure phenotypes of both $Lmna^{G609G/+}$ and $Lmna^{LCS/LCS}$ mice, supporting the conclusion that progerin increases the metabolic rate, whereas lamin C reduces overall energy consumption. These results are put together in figure 4.

*6. Data on food intake and physical activity should be provided in order to obtain a more complete assessment of the energy balance*

Food intake was not significantly different between controls and transgenic mice at any age tested; this important information is included in Figure 2C and results section p7.

 

Although we did not measure the physical activity of mice of each genotype, 45-weeks-old mice expressing progerin appear less active than wild type, indicating that their lean phenotype is not due to strong physical activity but rather due to increased mitochondrial biogenesis in the BAT. This increase in mitochondrial biogenesis is potentially inducing weight loss through thermogenesis. The data corresponding to BAT analysis was included in this version figure 3 B and C.

Minor points:

*7. Figure legend/explanation of details in Fig. 1. Which sequences exactly are depicted under C and D? What do the frames 1 and 2 stands for? The details need to be in the figure legend.*

The details to understand the figure are included in the figure legends as suggested by the reviewer

*8. The description of the alleles used and how they were generated is not clearly written. Since the molecular details are of central importance for the study, a schematic may help the reader.*

A schematic representation of structure of the targeted allele after homologous recombination is shown Fig 2A

*9. On which tissue extracts were the western blots in Fig. 2C performed?*

The Western blots in Fig. 2A are performed with extracts from the livers of mice of different genotypes. This information is now indicated in the legend of the figure.

*10. Which pathways do the authors refer to with '...two of the three most regulated pathways involved energy metabolism...' on page 15, and how do they relate to energy metabolism in a direct way?*

The two pathways are arachidonic acid metabolism and glycerolipid metabolism are indicated now in the results section p14

*11. The conclusion on page 11 that '...LMNA splicing isoforms were involved in the regulation of energy expenditure...' cannot be drawn based simply on altered adipocyte size and unaffected differentiation. The sentence should be rephrased.*

This sentence has been deleted and replaced by « these results suggest that lamin C and progerin are more important for the fate of differentiated adipocytes than for preadipocyte differentiation. » in the result section p9 as suggested by the reviewer

*12. The statement 'This is a feature that has often been associated with a high-fat diet.' on page 11/12 requires a reference to literature.*

The reference was included to document this statement p10

*13. There is no legend for Suppl. Fig. 5.*

All the legends of the new supplementary figures are now included.

*14. 'These 30 genes are potentially involved in the process of aging' on page 15: Do the authors mean 'could be involved' or is there a concrete link to aging? Clarification is required.*

As suggested by the reviewer we have changed this sentence in p14 These 30 genes could be involved in the process of aging

*15. The observed changes in leptin expression cannot explain the metabolic phenotypes in a*

 

*plausible way. E.g. increased leptin signaling in the LCS/LCS mice would result in reduced food intake and increased energy expenditure leading theoretically to weight loss, opposite tot the observed phenotype. The authors should comment on this.*

Few sentences were included in p 14-15 to comment on Leptin overexpression in LCS/LCS mice

Reviewer 3:

*The study is very well conducted and discovers some novel aspects of lamina A/C biology. In my opinion this is very interesting, the only criticism I have is that the focus is not placed ideally. It is well known that progerin formation causes lipdystrophy and shortens lifespan, however to my knowledge the general opinion is that fat cells cannot be formed correctly (see also data from Xiong et al, Ageing 2013). The authors show here that expression of this isoform rather causes a wasting syndrome similar to cancer cachexia with enhanced brown fat formation, rather than alterations of a lipid storage phenotype. I would suggest to highlight this aspect of the work and focus more on the brown fat formation in progerin expressing mice. The effect of lamin A loss and exclusive lamin C expression is much more mild and does not completely correlate with the phenotype observed. It seems that mice have less capacity to burn lipids and thus gain more weight again dependent on brown fat formation.*

We are especially grateful to reviewer 3 for the deep understanding and consideration of the questions for the field that our paper creates. As suggested by reviewer 2 and the editor letter, we have included the data on BAT but did not put the main focus of the paper on BAT. As correctly predicted by reviewer 3, there is a lot of mitochondrial biogenesis going on in the BAT of progeria mice but less in LCS mice compared to WT. These important data were included in the results section p11-12, Figure 3 B and C and Figure 4B, and in the supplemental figure 3.

*To substantiate the data on adipose tissue remodeling it would also be interesting to include some results on metabolic parameters such as circulating lipids, glucose and insulin especially for the LCS/LCS mice.*

As suggested by the reviewer we have included data on metabolic tests for insulin sensitivity and fasting insulin. These data indicated that the *Lmna*$^{LCS/LCS}$ mice have a mild insulin-resistant phenotype consistent with their obese phenotype. Surprisingly, old *Lmna*$^{LCS/LCS}$ mice (20 month old mice) are rather hypoglycemic showing that they are able to compensate for obesity and induced insulin resistance. This finding, reinforce the significance of the Lmna RNA processing pathway in controlling the fat storage and glycemia. These data were included as part of a new Figure 3 and supplemental figure 2C and in the results section (p 9-10). While it is still unclear why *Lmna*$^{LCS/LCS}$ mice have increased lifespan, the data show for the first time that obesity and induced insulin resistance will not have a negative effect on lifespan if lamin C isoform is exclusively expressed in the organism. This sentence was included in the concluding remarks.

I hope, that you will find that the revised work now meets the high standards of Embo reports. I will ask you also to

Thank you very much for your time and consideration.

2nd Editorial Decision 04 February 2014

Thank you for the submission of your revised manuscript to our journal. We have now received the enclosed reports from the referees that were asked to assess it.

As you will see, while referee 3 is happy with the revised version, referee 2 does not recommend publication. Given these discrepant reports, I have asked an advisor for another opinion on your study, which is pasted below. The advisor acknowledges the high quality of the data, but also agrees with referee 2 that the metabolic phenotype is not linked to the longevity phenotype. S/he suggests that you improve the discussion and provide explanations for the observed longevity phenotype. In addition, both points of referee 2 (as below) must be addressed. I need to stress that this is a borderline decision, and that the remaining concerns need to be addressed in a satisfactory manner for publication of the manuscript here.

The current character count (more than 38.000) still exceeds our limits, and the manuscript text therefore needs to be shortened by a few thousand characters. You can move the methods sections "Histological Studies" and "Cell culture and extracellular flux analysis" to the supplementary information, but the remaining methods must remain in the main manuscript file.

Please let me know if you have questions regarding the revisions.

I look forward to seeing a new revised version of your manuscript as soon as possible.

REFEREE REPORTS:

Referee #2:

As can be read in my comments on former points 1 and 5 below, I do not feel that the authors have adequately addressed two of the major issues raised in my first review. Therefore, I cannot recommend publication in the current form.

Point 1.
Although the efforts made by the authors to provide data on further metabolic analysis needs to be acknowledged, the results, if anything, make the aging and metabolic phenotypes more unrelated (in fact, LCS/LCS mice had reduced insulin sensitivity). So the study basically presents two unrelated phenotypes, and the authors have not made a further attempt to link the observations, or at least try to discuss possible explanations linking the function of Lamins A and C to current theories of aging. In my opinion, both phenotypes are interesting but would require additional findings to place the major observations into the context of the state-of-the-art and to be adequate for publication in EMBO Reports. Besides this major issue, the authors have not responded to my comments above on white fat-dependent energy expenditure and RER.

Point 5.
I do not think that body weight-normalized VO2 data represent a high modern standard in indirect calorimetry studies. Applying the suggested data processing could have easily been performed by a statistician. The authors could at least have presented the absolute VO2 data in addition, so that the reader can assess the result. This remains a major issue, given the central importance of the VO2 results.

Referee #3:

The addition of the metabolic data as well as the data on brown adipose tissue very much strengthens the paper and also supports the hypothesis the authors put forth. Also the inclusion of the BAT data supports the observed phenotype and this phenomenon in my opinion is the main contributor to the phenotype rather than the alteration of lipid storage. In my personal opinion the observed phenotype is Therefore I would recommend to publish this paper.

Comments from the advisor:

The data that are presented are of rather high quality, but the metabolic phenotype included is superficial and by no means an in-depth characterization. This explains the frustration of reviewer 2,

 

who thinks that for a strong metabolic foundation, more experiments are required. However, I think that even with more experiments the 2 phenotypes may not necessarily become better linked. Therefore, I see two ways forward: (1) either to reject the work - but that would be a pity, because, as stated above, the data are of rather high quality; or (2), ask that the authors provide a more in-depth discussion of why the phenotypes are in apparent contradiction. There are several papers, in worms (e.g. PMID 23698443) and mice (e.g. 17981116), that underscore that repression of oxidative metabolism can be linked to longevity. Those data are only a few high flying exemplars as there are many more studies along those line. If the current work is put in the context of these studies, the disconnect between the metabolic and lifespan phenotypes may be less apparent and ultimately benefit the value of the work. Furthermore, the authors should also comply to point #5 of reviewer 2, as how they express CLAMs data is inappropriate.

Cross-comments from referee 1:

On my point 2: This is about the level of standard. Up to date high quality metabolic studies present not-normalized VO2 data or apply the method for data analysis which I suggested, and this is a published recommendation by leading groups in the field. I am aware that there are still many indirect calorimentry reports with body weight-normalized data, but I cannot recommend EMBO Rep this standard.

On the comment of Reviewer 3 concerning the role of BAT for the metabolic phenotype: In line with reviewer 3, I also assume that differences in BAT activity are an important determinant of the metabolic phenotypes observed. However, this has not been emphasized in the manuscript as requested by Reviewer 3, and most data are on white fat, which may be irrelevant to the energy expenditure phenotype. Importantly, the authors have provided data only on brown adipocyte size and mitochondrial content. In my opinion, this is not sufficient to be able to state that BAT activity is altered and impacts on energy expenditure. So on this point I clearly disagree with Reviewer 3.

On the missing link between the metabolic and aging phenotypes (my point 1): it has now occurred to me that Reviewer 3 had actually made this comment in his first review.
> The effect of lamin A loss and exclusive lamin C expression is
much more mild and does not completely correlate with the phenotype observed. It
seems that mice have less capacity to burn lipids and thus gain more weight again
dependent on brown fat formation. Why this should increase longevity however is
unclear to me and should be discussed in much more detail. Again I would focus
more on the weight phenotype and the reduced appearance of brown fat rather than
on the longevity.
As far as I can see from the rebuttal, the authors have not included this comment (the last two sentences) in their letter, which substantiates my concerns.

I repeat that I find the observations very interesting, but I don't think that the manuscript represents a robust documentation of a single finding.

Cross-comments from referee 3:

I read the manuscript carefully again and the points raised by reviewer 1 are valid, especially point 1. The LCS mice live longer are insulin resistant and have less energy expenditure which is indeed contradicting current models. I have to agree that this point is not adequately addressed. I am not sure whether these problems can be addressed easily. One could argue that adipose tissue is more insulin sensitive and thus protects from deleterious effects but to prove such a point a CLAMP study would be necessary which is clearly beyond the scope of this study.

The point on VO2 normalized to body weight is in my opinion less important nevertheless it would be good to analyze the data independent of body weight as proposed by multiple studies. Such an analysis requires only statistical work and no further experiments.

 

2nd Revision - authors' response 07 February 2014

I have included few sentences in the concluding remarks to answer the concerns of referee 2 to improve the discussion and provide explanations for the longevity phenotypes of LCS mice (p14, 15). I have included the absolute value of VO2 and VCO2 in fig.4 as suggested by the reviewer and I have alluded to this in the text (p9). As you will see the RER will still the same as this value is independent of weight, however if the values are not normalized to the weight of the animals, we do not see any significant differences between different genotypes. Since the results in MEFs are consistent with the results of energy expenditure when normalized to body weight, we are confident that our interpretation is correct. We agree with the reviewer that the reader should get the data of absolute value.

I have shortened the text to essential information but could not bring it to 30 000 characters as requested. It is still 34 000 characters including space. I am sending the the text and figure 4 (the other figures are still the same) and would like to have your opinion.

Correspondence - editor 07 February 2014

Thank you for sending the revised text. I think the discussion you have added is good. You also may want to mention that some of the phenotypes of the lamin C only mouse do usually not support increased longevity, as the referees point out. I have made a few suggested changes, and included comments where I am not sure about the meaning, to the abstract, introduction and concluding remarks, but these regard only the language. The abstract needs to be written in present tense. Can you please check and make the changes as you feel appropriate and upload the revised version on our website?

3rd Editorial Decision 11 February 2014

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. As you are aware, this File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

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