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Loss of mitochondrial protease OMA1 alters processing of the GTPase OPA1 and causes obesity and defective thermogenesis in mice

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1st Editorial Decision 08 November 2011

Thank you for submitting your manuscript for consideration by The EMBO Journal. Let me first of all apologise for the delay in getting back to you with a decision. Unfortunately, the referees were not able to return their reports as quickly as initially expected.

Your manuscript has now been evaluated by three referees whose comments are shown below. As you will see, all three referees consider the study as interesting and important and would support its publication here after appropriate revision. We will thus be happy to consider a revised version of this study that addresses the referees' criticisms in an adequate manner. One key point will be to provide some more direct, siRNA-based evidence that the phenotype you observe indeed depends on loss of Oma1-dependent Opa1 processing. Along these lines and point 1 raised by referee 3, together with a recent publication on the role of Opa1 as AKAP on lipid droplets (Pidoux et al. 2011, PMID: 21983901), I think that it would strengthen the study immensely if you could provide some initial data on whether loss of function of Oma1 affects the AKAP function of Opa1 at lipid droplets. Please do not hesitate to contact me at any time in case you would like to discuss any aspect of the revision further.

I should add that it is EMBO Journal policy to allow only a single round of revision, and that acceptance or rejection of your manuscript will therefore depend on the completeness of your responses in this revised version as well as on the final assessment by the referees.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Peer Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website: http://www.nature.com/emboj/about/process.html

We generally allow three months as standard revision time. As a matter of policy, competing manuscripts published during this period will not negatively impact on our assessment of the conceptual advance presented by your study. However, we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed.

We now encourage the publication of source data, particularly for electrophoretic gels and blots, with the aim of making primary data more accessible and transparent to the reader. Would you be willing to provide files comprising the original, uncropped and unprocessed scans of the (key) gels used in the figures at revision stage? We would need 1 file per figure (which can be a composite of source data from several panels) in jpg, gif or PDF format, uploaded as "Source data files". The gels should be labelled with the appropriate figure/panel number, and should have molecular weight markers; further annotation would clearly be useful but is not essential. These files will be published online with the article as a supplementary "Source Data". Please let me know if you have any questions about this policy.

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

Yours sincerely,

Editor
The EMBO Journal

REFEREE COMMENTS

Referee #1

The manuscript by Quiros P. et al. is a very interesting and important work that uncovers for the first time a role for a mitochondrial quality control protease (OMA1) in the regulation of lipids metabolism and thermogenesis. Recent experimental evidence unveiled a role for PARL (Civitarese et al., 2010) in cell metabolism beyond their role in apoptosis and/or mitochondrial morphology. The work of Quiros and colleagues settles very well into this scenario demonstrating for the first time that OMA1 participates in fatty acid metabolism in vivo.

In this manuscript the authors describe a knock-out mouse model for OMA1 that could be a powerful tool to investigate the role of OMA1 in other physiological and pathological conditions. They describe in detail, by using hystochemistry and by measuring several metabolic parameters, that OMA1-/- mice accumulate fatty acids and develop liver steatosis. Moreover, these animals are not able to regulate their body temperature in response to cold. Taken together, these data suggest that OMA1-/- mice carry defects in the fatty acids metabolism. In order to characterize the molecular mechanism, authors analyze mitochondrial morphology and respiration in OMA1-/- animals, and report that the OMA1-Opa1 axis participates in a stress response that allows the adaptation of mitochondria to stress, including cold stress.
The manuscript is very interesting and describes an unexpected link between a mitochondrial protease and lipid metabolism; the main problem that shall be addressed before publication is that an experimental proof that the phenotype depends on changes in Opa1 processing is missing. While this reviewer fully appreciates the difficulty in obtaining such a proof in vivo (it requires the generation of a mouse model expressing a mutant Opa1 that can't be cleaved by Oma1), it is conversely important to show that for example the reduced oxidation of palmitate in Oma1-/- BAT is directly dependent on Opa1. In fig. 5H authors show that the pattern of basal Opa1 cleavage is very much different in Oma1-/- BAT also in basal conditions; this appears to be the only change in mito dynamics proteins (Drp1 and Mfn2 whose levels are altered by qPCR in HFD seem conversely

to be normal in control diet at the protein level). Thus, the authors should capitalize on this exact experiment to provide a direct link between Oma1 and Opa1 in the control of palmitate oxidation. I suggest that BAT cells should be transfected with a siRNA for Opa1 and a mutant of Opa1 not cleavable by Oma1 (an S1 mutant) re-expressed and palmitate oxidation analyzed. This experiment will allow to establish a direct link between Oma1, Opa1 and the metabolic phenotype observed.

Referee #2

The authors generated mice which was null for the OMA1 gene. The protein has been implicated in the processing of the OPA1 gene. The generation of the knockout mouse provides novel insight into the actions of OMA1 on OPA1 processing, mitochondrial biology, and systemic energy metabolism.

Interestingly the OMA1 null mouse provides data that demonstrates a reduction in several important oxidative genes and an increase in lipogenic genes. The observations on mitochodrial morphology and fusion are of interest.

Some questions/points

1) Review the oil red O data in figure 2, there appears to be an increase in oil red O staining in the livers of OMA1 CD- not on a high fat diet. Is this true and if so should be noted in text?

2) I am not sure why adiponectin increases with high fat feeding
This is somewhat surprsing as the literature often demonstrates a reduction in adiponectin with obesity. Not sure of reason here unless it relates to genetic background

3) on p8 the authors refer to PPAR genes Are these PPAR alpha or beta or gamma genes?

4) The authors note a granuloma in mesenteric fat tissues- Does this relate to the observations of Saverio Cinti in Journal of LIpid Research that obesity is associated with adipocyte death and macrophage infitration?

5) The observed reduction in fat oxidation coupled with reduced energy expenditure argue for an alteration in oxidtiave metabolism as the authors suggest. The loss of heat and inability to maintain heat may relate primarly to brown fat dysfunction as confirmed by the cold studies. C Ronald Kahn with Dr. Saverio Cinti have demonstrated that the 129 mice is relatively protected against obesity due to expression of ectopic fat(I believe PNAS article). Since these mice were generated on C57BL6J and then crossed to C57BL6J only once they have a signficant complement of 129sJ genes and therefore ectopic brown fat. The complement of brown fat might even enhance the potential for the increase in obesity althought of course skeletal muscle and liver are affected as well. This might be wortwhile mentioning in the text briefly

Referee #3

This is a very interesting study on a topic of interest. However, some of the conclusions are not justified and some of the approaches need revisions.

1. It is hard to conclude that the absence of the isoform c of OPA1 is the direct cause for decreased beta oxidation and that this is the mechanism for impaired metabolism.

2. The conclusion that there are no age-related effects is not justified since the authors do not show mice at 15 and 24 months. Effects on ageing can also be acceleration of age-related diseases or more degeneration.

3. At 8 weeks there is a marked increase in body weight in KO with HFD. Are the different effects in brown fat and liver also seen at 8 weeks? This is important as the defects in liver and BAT could be a consequence of the inflammatory response caused by obesity.

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4. How do the authors explain the early increase in WAT by HFD in the KO? Do the authors suggest that it is related to decreased beta oxidation (although it is thought that does not play any important role in WAT)?

5. Measurents of insulin and ITT at 8weeks of high fat diet would be informative. Is the obesity triggered by higher insulin sensitivity or by a primary defect in the white adipocyte?

6. The authors should perform respiration analysis in isolated mitochondria with palmitoyl carnitine?
This is an important experiment in order to understand if the defect in beta oxidation is in the mitochondria per se (cpt translocation and ETF). It would also be valuable to do the same experiments in MEFS or primary cultures, with labeled palmitate or inhibition of respiration by etomoxir in the presence of palmitate and/or oleate.

7. Authors did not show if the body composition was the same in KO and control mice before HFD. This is especially important since the analysis of insulin, leptin and adiponectin plasma levels were different even under standard diet.

8. The molecular weights of the OPA1 isoforms shoul be labeled in each of the figures were they appear.

9. In multiple figures the n is not clear and the age of the mice is not stated. The number of mice, number of litters and age should be mentioned in each experiment.

10. Authors normalized the food intake to the total weight of mice, which is not appropriate; Authors should have normalized it to their lean body mass.

11. Authors should address the possibility that reduced heat production in the KO mice is due to reduced fuel availability rather than uncoupling of the BAT.

12. For the heat production experiment, the Authors exposed the mice to cold for 3 hours, and in the first hours thermo genesis is for the most due to muscle shivering which is ignored here. Authors should have exposed the mice for a day or more to the cold to see the mere BAT thermogenesis effect.

13. The RQ measurement is not clear, Authors did not mention what diet the mice were on. Authors should also provide the RQ for both conditions before and after HFD.

1st Revision - authors' response 25 January 2012

Please find enclosed a revised version of our manuscript entitled "**Loss of mitochondrial protease OMA1 alters processing of the GTPase OPA1 and causes obesity and defective thermogenesis in mice**" (EMBOJ-2011-79558) by Quirós *et al*.
We have extensively revised the previous manuscript, incorporating new experiments to directly address the criticisms raised by the reviewers. We would take this opportunity to thank our reviewers for both their positive comments and insightful criticisms that have allowed us to greatly improve our work demonstrating the importance of mitochondrial quality control in metabolic homeostasis. Likewise, we are grateful for your positive comments on our work as well as for your suggestions related to the recently published paper by Pidoux *et al* in The EMBO Journal, defining the functional relevance of Opa1 in lipid droplets. We were very excited by this work and had

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immediately sought to confirm whether our observed deregulation of Opa1 processing in *Oma1*-deficient mice had a mechanistic link to perilipin activation in brown adipose tissue. As shown in Editorial Figure 1 at the end of this letter, we did not observe any change in perilipin phosphorylation in *Oma1*-deficient BAT in comparison to normal tissue. Although it cannot be completely excluded without a more exhaustive and definitive analysis, this observation does suggests that perilipin functions normally in our mice and likely does not contribute to the numerous BAT defects we have presented in the manuscript. However, in light of the novelty of the findings by Pidoux *et al*, we have cited this paper and discussed the relevance of this work in our revised manuscript's discussion.

Our specific response to each of the reviewers' comments is outlined below.

Reviewer #1:

The manuscript is very interesting and describes an unexpected link between a mitochondrial protease and lipid metabolism; the main problem that shall be addressed before publication is that an experimental proof that the phenotype depends on changes in Opa1 processing is missing. While this reviewer fully appreciates the difficulty in obtaining such a proof in vivo (it requires the generation of a mouse model expressing a mutant Opa1 that can't be cleaved by Oma1), it is conversely important to show that for example the reduced oxidation of palmitate in Oma1-/- BAT is directly dependent on Opa1. In fig. 5H authors show that the pattern of basal Opa1 cleavage is very much different in Oma1-/- BAT also in basal conditions; this appears to be the only change in mito dynamics proteins (Drp1 and Mfn2 whose levels are altered by qPCR in HFD seem conversely to be normal in control diet at the protein level). Thus, the authors should capitalize on this exact experiment to provide a direct link between Oma1 and Opa1 in the control of palmitate oxidation. I suggest that BAT cells should be transfected with a siRNA for Opa1 and a mutant of Opa1 not cleavable by Oma1 (an S1 mutant) re-expressed and palmitate oxidation analyzed. This experiment will allow to establish a direct link between Oma1, Opa1 and the metabolic phenotype observed.

We are in agreement that building upon our demonstrations of deregulated Opa1 processing in *Oma1*-deficient BAT is essential in providing a more definitive link to the metabolic alterations we have presented. We also appreciate the reviewer's understanding of the inherent difficulties in providing such information *in vivo*. In accordance with the reviewer's suggested experimental approach, we have analyzed the specific relation of Oma1 mediated processing of Opa1 in the context of b-oxidation in isolated brown adipocytes. Our initial experiment measured palmitate oxidation in primary brown adipocytes from *Oma1*$^{+/+}$ and *Oma* $^{-/-}$ mice. As we shown in Figure 6A, *Oma1*-deficient cells exhibited a decrease in palmitate oxidation compared with control cells, confirming those observations made in tissue (Figure 5D). Next, we down-regulated Opa1 in the wild-type (*Oma1*$^{+/+}$) brown adipocytes using siRNA and demonstrated that palmitate oxidation was decreased compared with the scrambled siRNA control (Figure 6B), confirming that Opa1 is required for normal levels of palmitate oxidation. Then, to verify that Oma1-proteolytic processing of Opa1 is essential for efficient palmitate oxidation, we transfected in combination with the Opa1

siRNA, two different plasmids containing either the human wild-type Opa1 cDNA (Opa1-S1) or a mutated Opa1 cDNA in which the S1 site that is processed by Oma1 had been deleted (Opa1-ΔS1). Both Opa1 constructions were resistant to the siRNAs targeting mouse Opa1 due to their human origin and lack of homology in the targeted region. As expected, re-expression of the Opa1-S1 isoform partially restored palmitate oxidation rates, whereas re-expression of Opa1-ΔS1 maintained lower oxidation levels similar to those observed in Opa1 knockdown cells. These results corroborate that the inability to oxidize lipids in *Oma1*-deficient brown adipose tissue, and more specifically brown adipocytes, is derived from absent Opa1 proteolysis by Oma1.

Reviewer #2:

Some questions/points

1) Review the oil red O data in figure 2, there appears to be an increase in oil red O staining in the livers of OMA1 CD- not on a high fat diet. Is this true and if so should be noted in text?

The reviewer is correct and this has now been clarified in the text of the results section.

2) I am not sure why adiponectin increases with high fat feeding. This is somewhat surprising as the literature often demonstrates a reduction in adiponectin with obesity. Not sure of reason here unless it relates to genetic background.

The reviewer is again correct as adiponectin has been demonstrated to decrease due to obesity. Our inconsistency in adiponectin levels between normal and high fat diets appears to stem from the samples from the two diets being split across separate Elisa plates of differing batch number (this was not the case for any of the other hormones). While the comparative differences between the genotypes are accurate, comparison between samples from the two diets are not. As we find this discrepancy unsatisfactory, and the experiment itself not essential to the revised manuscript, we have removed this figure and replaced it with a serum analysis of free fatty acid levels that were requested by reviewer #3.

3) on p8 the authors refer to PPAR genes Are these PPAR alpha or beta or gamma genes?

The genes we refer to are targets of PPAR alpha and gamma, as we show in the network interaction of microarray data using Ingenuity Pathway Analysis in Supplementary Figure 4.

4) The authors note a granuloma in mesenteric fat tissues- Does this relate to the observations of Saverio Cinti in Journal of Lipid Research that obesity is associated with adipocyte death and macrophage infitration?

This is a noteworthy point, the granulomatous lesions we observed in *Oma1*$^{-/-}$ mice showed an

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increase in necrosis and macrophage infiltration that are consistent with the crown-like structures described in the article mentioned by the reviewer. To ensure that our observations are able to be contextualized against the literature, we have referenced the Journal of Lipid Research article describing these similar structures.

5) The observed reduction in fat oxidation coupled with reduced energy expenditure argue for an alteration in oxidative metabolism as the authors suggest. The loss of heat and inability to maintain heat may relate primarly to brown fat dysfunction as confirmed by the cold studies. C Ronald Kahn with Dr. Saverio Cinti have demonstrated that the 129 mice is relatively protected against obesity due to expression of ectopic fat (I believe PNAS article). Since these mice were generated on C57BL6J and then crossed to C57BL6J only once they have a significant complement of 129sJ genes and therefore ectopic brown fat. The complement of brown fat might even enhance the potential for the increase in obesity although of course skeletal muscle and liver are affected as well. This might be worthwhile mentioning in the text briefly

We believe the reviewer meant that our mice due to their mixed C57BL6J and 129sJ background may potentially exhibit some level of genetic obesity resistance due to ectopic brown adipose tissue that is characteristic of the 129 strain, rather than the suggested susceptibility to obesity. As the *Oma1*$^{-/-}$ mice are a model of obesity rather than lipodystrophy, we believe that any potential contribution from the 129 complement of genes and the potential accompanying obesity resistance due to ectopic brown fat are not a factor in our model.

Reviewer #3:

This is a very interesting study on a topic of interest. However, some of the conclusions are not justified and some of the approaches need revisions.

1. It is hard to conclude that the absence of the isoform c of OPA1 is the direct cause for decreased beta oxidation and that this is the mechanism for impaired metabolism.

As discussed in our response to reviewer #1 on a point very similar to that raised by the reviewer, we are in agreement and have provided in the revised manuscript a direct connection between the b-oxidation defects and Opa1 processing by Oma1. In summary of those experiments, we measured palmitate oxidation *in vitro* using primary brown adipocytes obtained from *Oma1*$^{+/+}$ and *Oma1*$^{-/-}$ mice. As shown in Figure 6A, *Oma1*-deficient cells showed a decrease in palmitate oxidation rate compared with control cell lines. Furthermore, down-regulation of Opa1 in the wild-type brown adipocytes using siRNA showed a decrease in palmitate oxidation rates, indicating that Opa1 is necessary for normal palmitate oxidation rates (Figure 6B). Further, we transfected in combination with the Opa1 siRNA, two different plasmids containing either the human wild-type Opa1 cDNA (Opa1-S1) or a mutated Opa1 cDNA in which the S1 site that is processed by Oma1 had been deleted (Opa1-ΔS1). As anticipated, the expression of Opa1-S1 partially restored normal palmitate oxidation, while Opa1-ΔS1 maintained low b-oxidation levels. These results indicate that Opa1 processing by Oma1 is necessary for normal b-oxidation function.

2. The conclusion that there are no age-related effects is not justified since the authors do not show mice at 15 and 24 months. Effects on aging can also be acceleration of age-related diseases or more degeneration.

To ensure that our conclusions regarding age-related defects in *Oma1*$^{-/-}$ mice are more clearly justified, we have analyzed older mice as per the reviewer's suggestion. In our histological analysis of 18-month-old, the oldest mice we currently have available, we again did not find any neurodegeneration that was not consistent with normal aging and was present in the wild-type comparison (Supplementary Figure 2B). Although somewhat out of the scope of the present manuscript, we also performed an exhaustive histological examination of multiple organs looking for overt signs of age or disease-related pathologies, finding that *Oma1*-deficient mice presented histopathology in line with normal aging and comparable to the wild-type mice. These results indicate that *Oma1*-deficient mice do not display any clear sign of age-related disease nor early degeneration.

3. At 8 weeks there is a marked increase in body weight in KO with HFD. Are the different effects in brown fat and liver also seen at 8 weeks? This is important as the defects in liver and BAT could be a consequence of the inflammatory response caused by obesity.

This is a very valid point, which we will discuss in the following paragraph. On a normal chow diet, where the obesity phenotype is not induced, we found significant differences in BAT function when lipolysis due to temperature exposure and b-oxidation were assessed in *Oma1*-deficient mice. These results were similarly found when we had induced obesity with the high-fat diet and allow us to conclude that the primary defect we have described in BAT is not a consequence of inflammation due to obesity. The analysis of normal chow diet liver b-oxidation did not show any apparent differences, however under high-fat diet *Oma1*$^{-/-}$ displayed a decrease in oxidation compared with wild-type controls. In this case, we cannot exclude that an inflammatory response is potentially modulating oxidation in this circumstance. To reflect this, we have mentioned in the text this possibility, and clarified that the BAT results are not due to inflammation.

4. How do the authors explain the early increase in WAT by HFD in the KO? Do the authors suggest that it is related to decreased beta oxidation (although it is thought that does not play any important role in WAT)?

We do not suggest that the increase in WAT is due to defects in b-oxidation because, as the reviewer has rightly pointed out, b-oxidation in WAT does not play an important role. We do propose that the increase in WAT by HFD in the *Oma1*-deficient mice is due to an increase in lipid storage in WAT due to defects in lipid metabolism (as we showed in Supplementary Figure 4), hormone and metabolic deregulation (Figure 2), energy deregulation (Figure 3) and potentially other unknown defects that we have not anticipated.

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5. Measurements of insulin and ITT at 8 weeks of high fat diet would be informative. Is the obesity triggered by higher insulin sensitivity or by a primary defect in the white adipocyte?

Thank you for this suggestion; it was not something we had initially considered. During the high fat diet we did assess insulin tolerance at both 8 and 12 weeks, finding no difference at either time point. In the revised manuscript, we have mentioned both experiments, explaining that as we did not find differences in insulin sensitivity, the onset of the obesity phenotype is not due to higher insulin sensitivity and is more likely triggered by defects in white adipocytes or other metabolic alterations.

6. The authors should perform respiration analysis in isolated mitochondria with palmitoyl carnitine? This is an important experiment in order to understand if the defect in beta oxidation is in the mitochondria per se (cpt translocation and ETF). It would also be valuable to do the same experiments in MEFS or primary cultures, with labeled palmitate or inhibition of respiration by etomoxir in the presence of palmitate and/or oleate.

As we explain in the legend of Figure 5C and 5D, we did the experiments in mitochondrial-enriched fractions from liver and brown adipose tissue. Thus, the differences in b-oxidation are due to defects in mitochondria. We have also repeated the palmitate oxidation assays using primary brown adipocytes isolated from *Oma1*$^{+/+}$ and *Oma1*$^{-/-}$ mice to further confirm those defects that were demonstrated in the tissue oxidation assays. As shown in Figure 6A, analysis of b-oxidation in brown adipocytes from *Oma1*-deficient cells compared with controls corroborated with our results from tissue and delineated that BAT harbors a mitochondrial defect due to absent Oma1.

7. Authors did not show if the body composition was the same in KO and control mice before HFD. This is especially important since the analysis of insulin, leptin and adiponectin plasma levels were different even under standard diet.

As we mentioned in the text, we began the HFD experiment with 4-week-old mice, and these mice did not display differences in body weight. For the revised manuscript, we have sacrificed 4-week-old mice and this time specifically weighed individual fat deposits to more carefully ensure that body composition was equal between the genotypes. As shown in Supplementary Figure 2D, *Oma1*-deficient mice showed the same composition of fat deposits as their wild-type littermates, excluding any prior difference in this parameter before being introduced to a HFD.

8. The molecular weights of the OPA1 isoforms should be labeled in each of the figures where they appear.

We added the molecular weights of Opa1 in all western-blots.

9. In multiple figures the n is not clear and the age of the mice is not stated. The number of mice, number of litters and age should be mentioned in each experiment.

We have added and clarified the number and age of the mice used in each experiment in the legend of each figure.

10. Authors normalized the food intake to the total weight of mice, which is not appropriate; Authors should have normalized it to their lean body mass.

We have changed the normalization of the food intake to the lean body weight.

11. Authors should address the possibility that reduced heat production in the KO mice is due to reduced fuel availability rather than uncoupling of the BAT.

Our histological examination of BAT from both wild-type mice and *Oma1*-deficient mice did not show an appreciable difference in the quantity of lipid droplets between the two genotypes. Similarly, analysis of FFA in the blood was not different between *Oma1*$^{+/+}$ and *Oma1*$^{-/-}$ mice (Figure 2F), which collectively provide suitable evidence that there are no gross differences in fuel availability that could underlie the reduced heat production in the *Oma1*$^{-/-}$ mice. Further, our comprehensive palmitate oxidation analysis in both BAT tissue and primary brown adipocytes clearly shows the presence of a mitochondrial defect.

12. For the heat production experiment, the Authors exposed the mice to cold for 3 hours, and in the first hours thermo genesis is for the most due to muscle shivering which is ignored here. Authors should have exposed the mice for a day or more to the cold to see the mere BAT thermogenesis effect.

In line with the reviewer's suggestion, we have repeated the cold exposure experiment, this time exposing the mice for a longer time period. As we shown in Figure 3H, we exposed the mice to cold for 12 hours. Time points beyond this were not consistent with our facility's animal ethics as the *Oma1*-deficient mice had at this time point begun to show severe hypothermia- with *Oma1*$^{-/-}$ mice displaying a clear cold intolerance in comparison to wild-type controls.

13. The RQ measurement is not clear, Authors did not mention what diet the mice were on. Authors should also provide the RQ for both conditions before and after HFD.

The analysis of energy balance was made in mice maintained on a normal chow diet. As we have shown in Figure 3B-C, we found a significant reduction in both oxygen consumption and carbon dioxide production in *Oma1*-deficient mice. However, despite these differences, the

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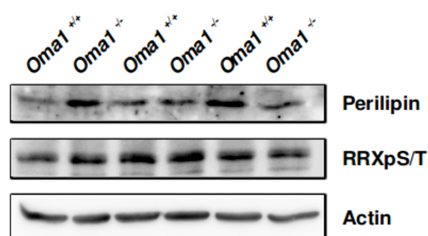
respiration quotient value (RQ) did not show differences because the reduction in carbon dioxide production was similar to the reduction in oxygen consumption (Figure 3E). To repeat this analysis for mice on a high fat diet is not feasible within the relatively limited time allocated for a resubmission. The mice we have presented in this work were maintained on a high fat diet for 6 months prior to experiments. Further, the whole body metabolic analysis requires transport and clearance of the mice between Institutes in Oviedo and Barcelona. Nevertheless, even though repetition might yield interesting results, we are satisfied with the results obtained under a chow diet.

In conclusion, we want to thank again the reviewers for their constructive comments and criticisms that have facilitated the substantial improvement of our work. In the hope that this revised work can be now accepted for publication in The EMBO Journal.

Editor Figure 1



Editor Figure 1. Western-blot analysis of perilipin, phosphoperilipin (PKA substrate antibody (anti-RRXpS/T)) and actin levels in differentiated brown adipocytes extracted from *Oma1*$^{+/+}$ and *Oma1*$^{-/-}$ mice.

2nd Editorial Decision 07 February 2012

Thank you for sending us your revised manuscript. Our original referee 1 has now seen it again, and you will be pleased to learn that in his/her view you have addressed all criticisms in a satisfactory manner. The paper will now be publishable in The EMBO Journal.

Prior to formal acceptance, there are a number of editorial issues that need further attention:

* Please add scale bars together with explanations to figures 3I, 4C, S3, S5D and an explanation for the scale bar in figure S2C.

* Please add the number of independent repeats to the legends of figures 6C, S6, S7

Please let us have a suitable amended manuscript as soon as possible. Thank you for your kind cooperation in this matter.

Yours sincerely,

Editor
The EMBO Journal

REFEREE COMMENTS

Referee #1

The authors exhaustively addressed my previous concerns. The experiment of fig. 6 in which they measure the effect of different variants of Opa1 on b-oxidation is very important and tells us that Oma1 works in this pathway via Opa1. The paper will be a nice addition to The EMBO Journal

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