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Insulin-stimulated adipocytes secrete lactate to promote endothelial fatty acid uptake and transport

Ayon Ibrahim, Michael Neinast, Kristina Li, Michael Noji, Boa Kim, Marc Bornstein, Katy Wellen, Raffiu Mohammed and Zoltan Arany
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MS TITLE: Insulin-stimulated adipocytes secrete lactate to promote endothelial fatty acid uptake and transport

AUTHORS: Ayon Ibrahim, michael neinast, kristina li, michael noji, boa kim, marc bornstein, katy wellen, raffiu mohammed, and Zoltan Arany
ARTICLE TYPE: Short Report

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, while both reviewers are positive about the discoveries, they also raise several valid criticisms that prevent me from accepting the paper at this stage. They suggest, however, that a revised version might prove acceptable, if you can address their concerns. If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript. We would then return it to the reviewers.

We are aware that you may be experiencing disruption to the normal running of your lab that makes experimental revisions challenging. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

The authors discovered that adipose derived lactate stimulates the uptake of fatty acids into endothelial cells. THis is interesting and novel and describes a new mechanism of crosstalk between adipose and endothelium.

Comments for the author

In this manuscript, Ibrahim A. and colleagues explore the role of insulin-induced adipocytes-derived metabolites such as lactate in endothelial fatty acid transport. Authors previously reported that the role of muscle-derived 3-HIB as an important regulator of endothelial FA partitioning. The authors nicely demonstrated by using series of in vitro experiments that adipocyte-derived media/lactate in response to insulin promotes endothelial fatty acid uptake. Fatty acids play a major role in type 2 diabetes and atherosclerosis therefore the current study gained by in vitro system can bridge the knowledge of metabolic diseases. The experiments are very well designed and described.
However, the mechanism is likely more complicated than proposed by the authors and there are some key concerns about the interpretations and presentation of the data as reported.

Specific concerns are:
1) 3-HIB increases the FA uptake in endothelial cells (Current and previous studies). In the current study, authors observe that adipocytes secretes 3-HIB and its level is considerably elevated in response to insulin. In contrast inhibition of 3-HIB in adipocytes does not abolish the FA uptake in ECs.
However, inhibition of lactate leads to a reduction of endothelial FA uptake.
Interestingly, there appears to be a cell-specific derived metabolite difference between myocytes and adipocytes in their ability to regulates endothelial FA trafficking. How do authors explain this discrepancy?

2) In the circulation, the major portion of fatty acid (esterified) is carried by Triglyceride-rich lipoproteins (TRLs) such as chylomicrons and VLDL particles. TRL distributes lipids to various tissue such as adipose, heart, and muscle by interacting with an enzyme lipoprotein lipase on the capillary bed of endothelial cells, which catalyzes TAG into fatty acids and glycerol. As a result, FA and glycerol are taken by these tissues by crossing the endothelial cell barrier. Did the authors measure LPL-mediated TAG lipolysis and FA uptake in ECs after treatment of Lactate/ adipocytes derived insulin-conditioned media (ICM)?

3) It is difficult to interpret how the adipocyte-derived lactate regulates endothelial FA trafficking based on an in-vitro setting because, under physiological conditions, endothelial cells also produce lactate (endothelial cells are highly glycolytic). Please discuss.

4) The authors state that “As shown in figure 1C and 3B, the kinetics of ICM mediated FA uptake in ECs and lactate production into ICM are overlapping to each other". Lactate production from adipocyte in response to insulin is increasing with time (saturation is not achieved) however, the saturation time point for ICM stimulated FA uptake in ECs is 24 hr. Thus, the authors should change their conclusion.

5) Another important point to be considered is that the authors showed that ICM or lactic acid promotes FA uptake in ECs. How lactic acid/ICM increases the FA uptake? Is the effect direct or indirect? Whether it regulates the expression or the activity of fatty acid transporters (FATP3,4 or CD36)? What could be the possible mechanism?

7)The authors did not use C2C12 cells in this article, so they should remove the description of the C2C12 muscle cell line from the method section

8) In Figure 1C, the 30 h time point of CM (#) does not seem significant compared to DMEM+I at the 0-time point.

Reviewer 2

Advance summary and potential significance to field

This is an interesting and well performed study by Arany group, which identifies lactate as an active metabolite produced by adipocytes in response to insulin that mediates endothelial FA transport. Overall the study is relevant and the main conclusion supported by the data.

Comments for the author

This study will be strengthened by adding additional mechanistic data.
Specific points:
1- How lactate enhances EC FA uptake?. Is lactate increasing the expression of FA transporters (e.g. CD36 FABP?) in ECs?.
2- It will be interesting to assess how lactate influences FA homeostasis in EC. Immunofluorescence analysis to assess lipid droplet accumulation (TAG synthesis or LD lipolysis) that might influence the flux of FA across the endothelium in response to lactate at different time points will provide novel insights of how lactate promotes EC FA transport.

First revision

Author response to reviewers' comments

We thank the reviewers for their interest in our work and their constructive input. We have addressed below in detail each point raised by the reviewers. We include 6 panels of new data, and we provide a tracked version of the manuscript to facilitate review.

Reviewer 1
Advance Summary and Potential Significance to Field: The authors discovered that adipose derived lactate stimulates the uptake of fatty acids into endothelial cells. THis is interesting and novel and describes a new mechanism of crosstalk between adipose and endothelium.

Thank you

Reviewer 1 Comments for the Author:
In this manuscript, Ibrahim A. and colleagues explore the role of insulin-induced adipocytes-derived metabolites such as lactate in endothelial fatty acid transport. Authors previously reported that the role of muscle-derived 3-HIB as an important regulator of endothelial FA partitioning. The authors nicely demonstrated by using series of in vitro experiments that adipocyte-derived media/lactate in response to insulin promotes endothelial fatty acid uptake. Fatty acids play a major role in type 2 diabetes and atherosclerosis therefore, the current study gained by in vitro system can bridge the knowledge of metabolic diseases. The experiments are very well designed and described. However, the mechanism is likely more complicated than proposed by the authors and there are some key concerns about the interpretations and presentation of the data as reported.

Specific concerns are:

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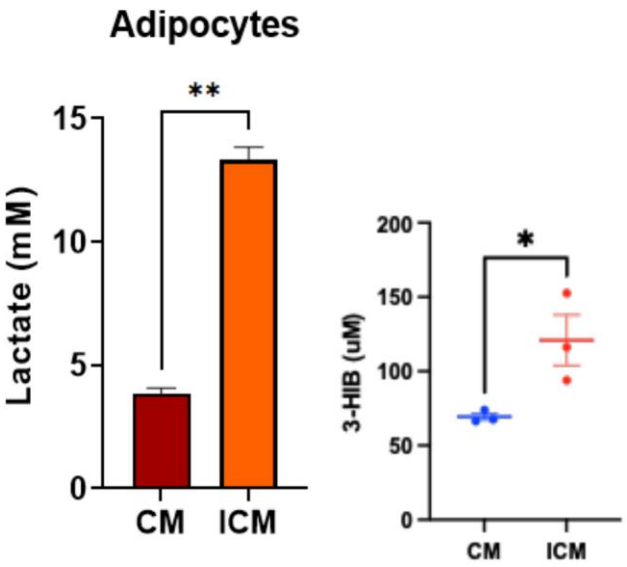
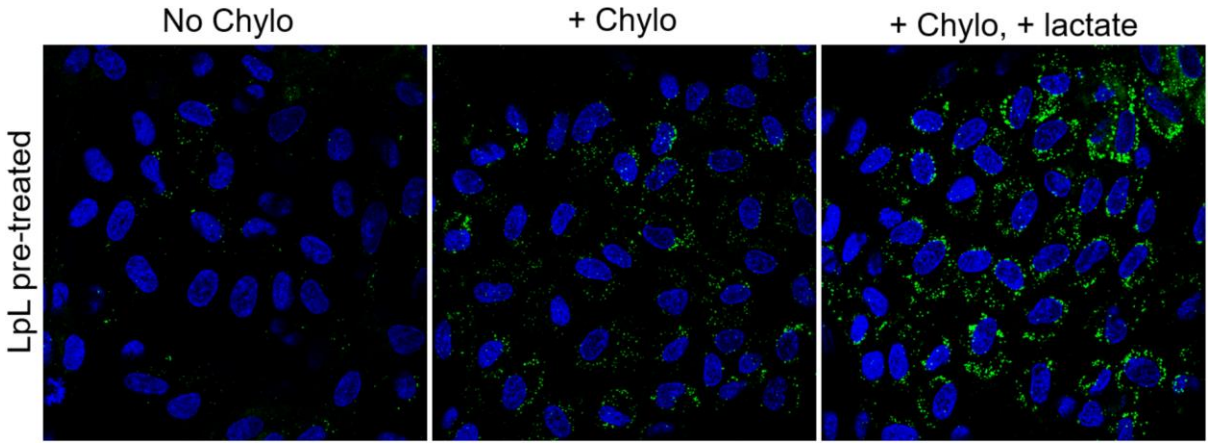
in response to insulin. In contrast, inhibition of 3-HIB in adipocytes does not abolish the FA uptake in ECs. However, inhibition of lactate leads to a reduction of endothelial FA uptake. Interestingly, there appears to be a cell-specific derived metabolite difference between myocytes and adipocytes in their ability to regulates endothelial FA trafficking. How do authors explain this discrepancy?

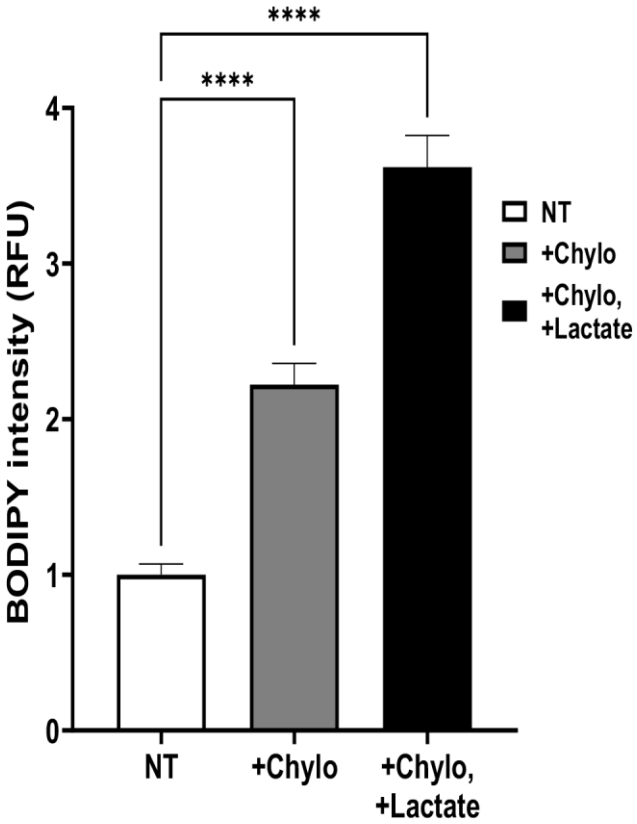
This is a good question. There are at least two possibilities: different ECs in different tissues may respond differently to, for example, lactate vs 3HIB. This is difficult to address in cell culture, because ECs do not retain most attributes of their tissue of origin when culture.
Alternatively – and we favor this hypothesis – the interstitial concentrations of metabolites, e.g. 3HIB and lactate, to which the endothelium is exposed differ between tissues. Consistent with this notion, we provide new data to show that the absolute concentration of lactate secreted by adipocytes is nearly two orders of magnitude larger than 3-HIB, likely explaining why signaling by lactate dominates the paracrine activity.



2) In the circulation, the major portion of fatty acid (esterified) is carried by Triglyceride-rich lipoproteins (TRLs) such as chylomicrons and VLDL particles. TRL distributes lipids to various tissue such as adipose, heart, and muscle by interacting with an enzyme lipoprotein lipase on the capillary bed of endothelial cells, which catalyzes TAG into fatty acids and glycerol. As a result, FA and glycerol are taken by these tissues by crossing the endothelial cell barrier. Did the authors measure LPL-mediated TAG lipolysis and FA uptake in ECs after treatment of Lactate/ adipocytes derived insulin-conditioned media (ICM)?

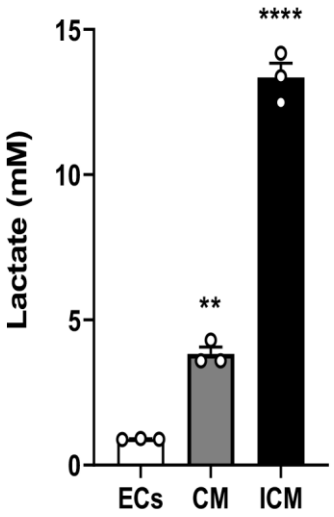
This is an excellent suggestion. We conducted an experiment (in quadruplicate) in which endothelial cells were pretreated with exogenous Lipoprotein lipase (LpL) at 10 U/mL for 1 hour, and then incubated with chylomicrons +/- lactate (at 25 µg/mL and 10 mM, respectively) for 1 hour, the timepoint at which we have found lactate to maximally induce maximal free fatty acid uptake (Figure 4B), followed by staining with free BODIPY to analyze lipid droplet content. As shown by the representative images and quantification below (new Figure 4D), we observed that endothelial cells in culture readily take up fat from chylomicrons, and that this uptake is robustly promoted by co-treatment of lactate, analogous to the results with lactate and free fatty acid uptake.





3) It is difficult to interpret how the adipocyte-derived lactate regulates endothelial FA trafficking based on an in-vitro setting because, under physiological conditions, endothelial cells also produce lactate (endothelial cells are highly glycolytic). Please discuss.

It is true that endothelial cells produce most of their energy through glycolysis with, as we have shown (Kim et. al. EMBO J 2018), a high glycolytic index. Importantly, however, the absolute metabolic activity of ECs, and thus the absolute levels of lactate secreted, are far below that of differentiated adipocytes. To illustrate this point, we show in new Supplemental Figure 1B that, as measured by YSI metabolomics, an equivalent number of adipocytes secrete ~5x more lactate than ECs, and ~13x more when the adipocytes are further stimulated with insulin. In addition: (1) the absolute mass of adipocytes in adipose tissue is significantly larger than that of ECs; and (2) ECs likely secrete a substantial portion of lactate into the plasma, where it can be diluted into a large volume. Thus it is highly likely that the vast majority of lactate in adipose interstitial tissue originates from adipocytes rather than ECs. We have added these points to the discussion.



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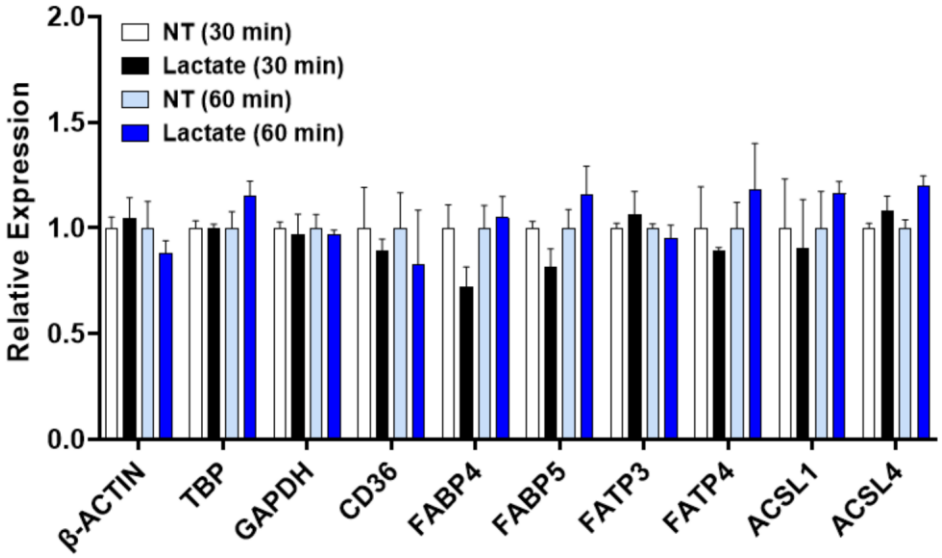
We have revised our text to reflect the connection more accurately:
“…the kinetics of lactate appearance into ICM matched closely the kinetics of ICM activity to promote FA uptake in ECs, approaching a maximal level at around 24 hours (Figures 1C and 3B).”

5) Another important point to be considered is that the authors showed that ICM or lactic acid promotes FA uptake in ECs. How lactic acid/ICM increases the FA uptake? Is the effect direct or indirect? Whether it regulates the expression or the activity of fatty acid transporters (FATP3,4 or CD36)? What could be the possible mechanism?

Based on the time-scale at which lactate carries out its effect, with significant induction of fatty acid uptake within just 5 minutes (Figure 4B), we doubt that lactate mediates its effect via gene expression to any appreciable degree; indeed, we do not observe changes in the expression of fatty acid transporter and acyl CoA synthetase genes 60 minutes after lactate treatmen, as shown in the figure below. The mechanism by which lactate affects FA uptake remains unclear and is under active investigation (beyond the scope of the current report), and may be effecting rapid changes in protein signal transduction or membrane fluidity/permeability. We have added this speculation to the discussion.



7) The authors did not use C2C12 cells in this article, so they should remove the description of the C2C12 muscle cell line from the method section

Thank you, we have removed the description.

8) In Figure 1C, the 30 h time point of CM (#) does not seem significant compared to DMEM+I at the 0-time point.

While the delta is indeed small, the 30h CM time-point did achieve statistical significance with p=0.027, as show below in the screenshot of the ANOVA carried out of the data in GraphPad Prism.

Alpha	0.05							
Dunnett's multiple comparisons test	**Mean Diff.**	**95.00% CI of diff.**	**Below threshold?**	**Summary**	**Adjusted P Value**	**C-?**		
DMEM+I vs. 1	-200.3	-1878 to 1478	No	ns	0.9995	E	1	
DMEM+I vs. 2	81.67	-1596 to 1760	No	ns	0.9998	F	2	
DMEM+I vs. 4	-646.0	-2324 to 1032	No	ns	0.8060	G	4	
DMEM+I vs. 6	-1363	-3041 to 314.9	No	ns	0.1434	H	6	
DMEM+I vs. 8	-1278	-2956 to 399.5	No	ns	0.1863	I	8	
DMEM+I vs. 12	-1640	-3318 to 37.87	No	ns	0.0570	J	12	
DMEM+I vs. 24	-2610	-4288 to -931.8	Yes	**	0.0016	L	24	
DMEM+I vs. 30	-1851	-3529 to -173.1	Yes	*	0.0270	M	30	
Test details	**Mean 1**	**Mean 2**	**Mean Diff.**	**SE of diff.**	**n1**	**n2**	**q**	**DF**
DMEM+I vs. 1	10027	10227	-200.3	571.6	3	3	0.3505	18
DMEM+I vs. 2	10027	9945	81.67	571.6	3	3	0.1429	18
DMEM+I vs. 4	10027	10673	-646.0	571.6	3	3	1.130	18
DMEM+I vs. 6	10027	11390	-1363	571.6	3	3	2.384	18
DMEM+I vs. 8	10027	11305	-1278	571.6	3	3	2.236	18
DMEM+I vs. 12	10027	11667	-1640	571.6	3	3	2.869	18
DMEM+I vs. 24	10027	12637	-2610	571.6	3	3	4.565	18
DMEM+I vs. 30	10027	11878	-1851	571.6	3	3	3.238	18

Reviewer 2:

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Thank you.

Reviewer 2 Comments for the Author:
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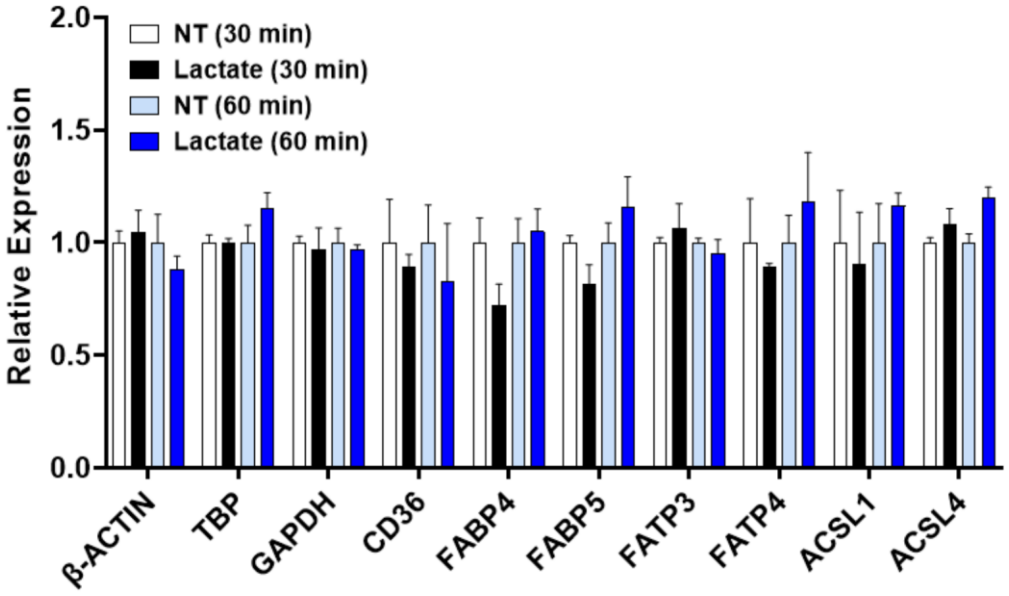
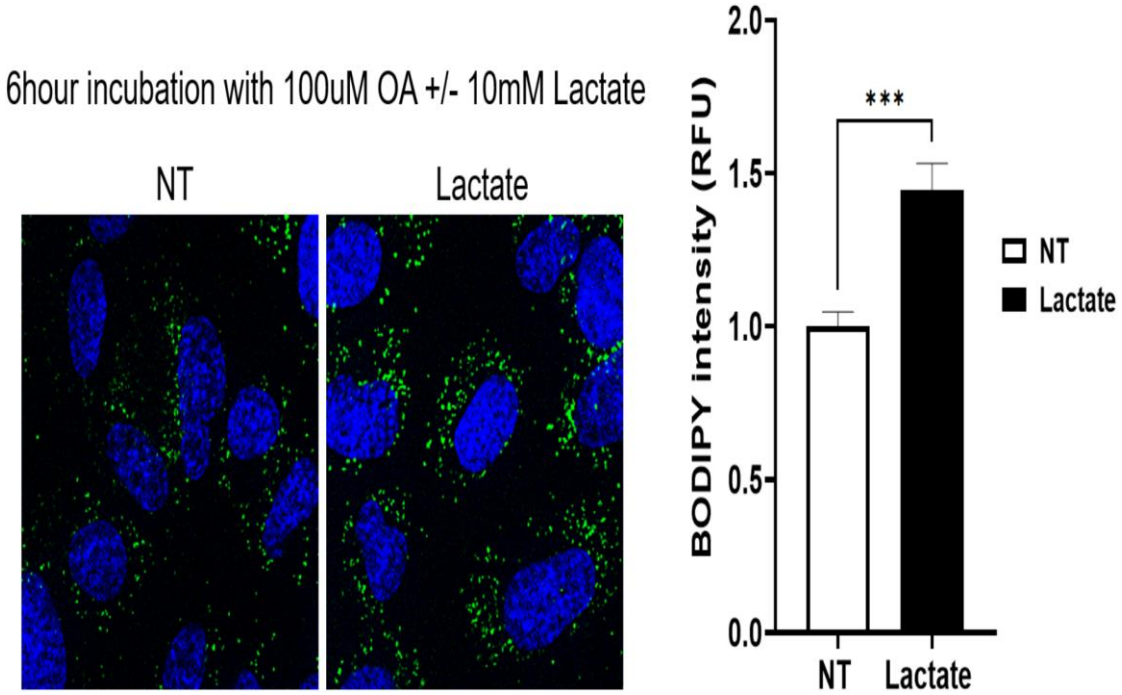


2- It will be interesting to assess how lactate influences FA homeostasis in EC. Immunofluorescence analysis to assess lipid droplet accumulation (TAG synthesis or LD lipolysis) that might influence the flux of FA across the endothelium in response to lactate at different time points will provide novel insights of how lactate promotes EC FA transport.

Thank you for this suggestion. As shown new Figure 4C, cells treated with 10 mM lactate for 6 hours (as well as 100 μM oleic acid for lipid droplet loading) displayed about 45% more neutral lipid signal, detected by fluorescent staining using free BODIPY (which binds neutral lipids) as compared to cells given only the oleic acid (labeled NT for 'no treatment'). Thus lactate promotes the formation of lipid droplets in ECs, a likely consequence of the increased FA uptake.



Second decision letter

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AUTHORS: Ayon Ibrahim, Michael Neinast, Kristina Li, Michael Noji, Boa Kim, Marc Bornstein, Katy Wellen, Raffiu Mohammed, and Zoltan Arany
ARTICLE TYPE: Short Report

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks. Thank you for submitting this interesting work to JCS and for your thoughtful responses to the referee comments.