



Royal Netherlands Academy of Arts and Sciences (KNAW) KONINKLIJKE NEDERLANDSE AKADEMIE VAN WETENSCHAPPEN

Missing the rhythm in skeletal muscle mitochondrial respiration

Harmsen, Jan-Frieder; Kalsbeek, Andries

published in

American Journal of Physiology - Endocrinology and Metabolism
2026

DOI (link to publisher)

[10.1152/ajpendo.00469.2025](https://doi.org/10.1152/ajpendo.00469.2025)

document version

Publisher's PDF, also known as Version of record

[Link to publication in KNAW Research Portal](#)

citation for published version (APA)

Harmsen, J.-F., & Kalsbeek, A. (2026). Missing the rhythm in skeletal muscle mitochondrial respiration. *American Journal of Physiology - Endocrinology and Metabolism*, 330(2), E265-E266.
<https://doi.org/10.1152/ajpendo.00469.2025>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the KNAW public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying the publication in the KNAW public portal.

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

E-mail address:

pure@knaw.nl

LETTER TO THE EDITOR

Missing the rhythm in skeletal muscle mitochondrial respiration

Jan-Frieder Harmsen^{1,2,3} and Andries Kalsbeek^{4,5,6,7}

¹Department of Nutrition and Movement Sciences, NUTRIM Institute of Nutrition and Translational Research in Metabolism, Maastricht University Medical Center, Maastricht, The Netherlands; ²Healthy Living Spaces Lab, Institute for Occupational, Social and Environmental Medicine, Medical Faculty, RWTH Aachen University, Aachen, Germany; ³Faculty of Architecture, RWTH Aachen University, Aachen, Germany; ⁴Department of Endocrinology and Metabolism, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands; ⁵Netherlands Institute for Neuroscience, Royal Netherlands Academy of Arts and Sciences, Amsterdam, The Netherlands; ⁶Laboratory of Endocrinology, Department of Laboratory Medicine, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands; and ⁷Amsterdam Gastroenterology, Endocrinology and Metabolism, Amsterdam, The Netherlands

TO THE EDITOR: Fitzgerald et al. (1) recently reported that markers of mitochondrial function and oxidative metabolism do not exhibit intrinsic circadian regulation in female mouse skeletal muscle. Their careful sampling across multiple hind limb muscles and the inclusion of several mitochondrial indices represent a valuable contribution to the discussion of sex-specific chronobiology. Together with previous work, their null findings build a clear case that mitochondrial transcripts, proteins, and enzymatic activity do not display 24-h rhythmicity in female mice. However, several aspects of the article's interpretation of the circadian literature on mitochondrial respiration warrant clarification. In particular, some prior studies—including our own—were cited incorrectly or in ways that understate the presence of 24-h rhythms in mitochondrial respiration, whereas a foundational recent study central to this field was not referenced. Therefore, we think that the results of Fitzgerald et al. (1) suggest an interesting sex difference in circadian physiology, instead of a total absence of circadian rhythmicity in mitochondrial physiology.

Foremost, it is important to be strict on terminology. A physiological or metabolic process repeating in cycles of ~24 h is only then regulated by the circadian clock, if this process is generated endogenously, that is, self-sustained in the absence of external stimuli such as ambient lighting, temperature conditions, and the timing of physical activity and food availability (2, 3). Although the authors controlled for the influence of feeding time by regular recurring feeding across 24 h, they did not keep ambient lighting conditions constant (e.g., mice were kept under a 12-h light/12-h dark cycle). Therefore, we believe it would be more justified to instead conclude that mitochondrial markers did not display diurnal, daily, or 24-h rhythmicity, instead of circadian.

Although not our principal concern, two features of the setup could dampen or obscure rhythms in the current study. First, female mice were not staged according to the estrous cycle, potentially mixing phases with distinct hormonal milieus that influence mitochondrial biogenesis and oxidative metabolism; thus, a phase-specific rhythm could be

diluted in group averages. Second, liquid meals were administered before every sampling point, including during the animals' rest/fasting period by gavage, an inherently stressful procedure. Although this feeding design resembles a constant routine, other zeitgebers were not kept constant, so that the presence of endogenous circadian rhythmicity cannot be derived. Moreover, this feeding design should have been performed for a longer duration to install a condition of constant feeding. Instead, in the current setup, the feeding and acute stress occurring during the rest and fasting period might act as potent modulators of peripheral clocks and mitochondrial function, and together might phase-shift or flatten 24-h oscillations that otherwise could have been detected under light/dark, as shown in male rats fed only during the dark active period (4).

More substantively, the authors inconsistently refer to some prior work that could give the impression that skeletal muscle mitochondrial function is generally arrhythmic. Our own study in male rats is cited as “no effect of time-of-day” in ad libitum-fed animals, which is strictly correct for statistical significance. However, we observed a clear trend toward diurnal variation in mitochondrial respiration when animals were fed ad libitum, significant diurnal variation when animals were fed in the dark phase, and a markedly reduced amplitude when fed in the light phase. Thus, we interpret those data as diurnal rhythms in mitochondrial respiration, whose amplitude depends on feeding-activity alignment, and as evidence that time-restricted feeding can enhance the diurnal rhythm (4).

In addition, the authors miss an important study relevant to the article's discussion: in myotubes derived from healthy older male donors, Gabriel et al. (5) demonstrated robust cell-autonomous oscillations in gene expression and mitochondrial metabolism. These myotubes displayed a rhythmic oxygen consumption rate with an approximate 16-h period in vitro, accompanied by cycling expression of numerous genes involved in mitochondrial oxidative metabolism, inner-membrane structure, and lipid handling. These oscillations coincided with rhythmic activity of mitochondrial enzymes and



Correspondence: J.-F. Harmsen (janfrieder.harmsen@gmail.com).

Submitted 24 October 2025 / Revised 8 November 2025 / Accepted 8 November 2025



coordinated expression of clock-regulated transcription factors, suggesting that mitochondrial metabolism is intrinsically rhythmic in human skeletal muscle cells. In contrast, myotubes from age-matched male donors with type 2 diabetes (T2D) exhibited markedly blunted or absent oscillations, showing fewer rhythmic genes, reduced amplitude, and altered phase distribution. Importantly, T2D cells lacked rhythmicity in oxidative capacity. Collectively, these findings provide mechanistic evidence that skeletal muscle mitochondria are circadian-regulated under healthy conditions but that this temporal organization becomes disrupted upon metabolic disease.

In support, rhythmic skeletal muscle mitochondrial respiration in intact human muscle fibers has also been demonstrated using experimental designs that allowed entrainment through tightly controlled feeding/fasting and activity/rest cycles. First, van Moorsel et al. (6) observed a significant 24-h rhythm in human muscle oxidative capacity under standardized real-life conditions (e.g., 3 subsequent meals and physical activity during daytime and sleep during the night) in young men. In contrast, this 24-h rhythm in oxidative capacity was absent in older men with insulin resistance subjected to the same real-life conditions (7). Secondary analyses of these two cohorts, Gemmink et al. (8) reported 24-h oscillations in mitochondrial network connectivity only in young men but not in the older men. Noteworthy also, Harmsen et al. (9) found most skeletal muscle metabolites associated with the TCA cycle to be rhythmic in both cohorts. These studies were partly not correctly cited or omitted, yet are directly relevant to the question under investigation. However, as highlighted by Fitzgerald et al. (1), all the studies we refer to in this letter focused exclusively on males.

For the finding of absent rhythmicity in maximal respiration, only a predominantly fast-twitch glycolytic muscle was tested (e.g., tibialis anterior), which does not exclude the possibility that predominantly slow-twitch oxidative muscle would display rhythmicity also in female mice. The fact that the authors found much greater rhythm amplitude in *Bmal1* expression in the slow-twitch muscle (e.g., soleus)—a novel and important finding—compared with the two fast-twitch muscles seems supportive of this possibility.

We appreciate Fitzgerald et al. (1) for advancing sex-specific chronobiology and agree that rigorous control of behavioral zeitgebers is essential. For future studies we would suggest: 1) estrous staging or ovariectomy with hormone control; 2) behavior-congruent feeding also in females versus full constant-routine protocols; 3) higher-frequency sampling and constant-condition *ex vivo* assessments; and 4) inclusion of more posttranslational and functional endpoints sensitive to clock output. In conclusion, we commend the authors for stimulating renewed discussion of this topic and hope that integrating overlooked literature will refine future hypotheses about sex-specific and tissue-specific clock control of mitochondrial physiology.

GRANTS

J.-F.H. is funded by the project “Profilbildung Built and Lived Environment” (PB22-062). The project “Profilbildung Built and Lived Environment” is receiving funding from the program “Profilbildung

2022,” an initiative of the Ministry of Culture and Science of the State of North Rhine-Westphalia.

DISCLAIMERS

The content and any opinions expressed in this article are those of the authors and do not necessarily represent the views of the American Physiological Society.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.-F.H. and A.K. drafted manuscript; edited and revised manuscript; approved final version of manuscript.

REFERENCES

1. Fitzgerald LS, Reynoso Spurrier CS, Lau N, Melamed M, Burnett LA, Meyer GA, Gui C, Hevener AL, Sanford JA, Schenk S. Markers of mitochondrial function and oxidative metabolism in skeletal muscle do not display intrinsic circadian regulation in female mice. *Am J Physiol Endocrinol Metab* 329: E828–E838, 2025. doi:10.1152/ajpendo.00027.2025.
2. Deota S, Pendergast JS, Kolthur-Seetharam U, Esser KA, Gachon F, Asher G, Dibner C, Benitah SA, Escobar C, Muoio DM, Zhang EE, Hotamışligil GS, Bass J, Takahashi JS, Rabinowitz JD, Lamia KA, de Cabo R, Kajimura S, Longo VD, Xu Y, Lazar MA, Verdin E, Zierath JR, Auwerx J, Drucker DJ, Panda S. The time is now: accounting for time-of-day effects to improve reproducibility and translation of metabolism research. *Nat Metab* 7: 454–468, 2025. doi:10.1038/s42255-025-01237-6.
3. Gutierrez-Monreal MA, Harmsen JF, Schrauwen P, Esser KA. Ticking for metabolic health: the skeletal-muscle clocks. *Obesity (Silver Spring)* 28, Suppl 1: S46–S54, 2020. doi:10.1002/oby.22826.
4. de Goede P, Wüst RCI, Schomakers BV, Denis S, Vaz FM, Pras-Raves ML, van Weeghel M, Yi C-X, Kalsbeek A, Houtkooper RH. Time-restricted feeding during the inactive phase abolishes the daily rhythm in mitochondrial respiration in rat skeletal muscle. *FASEB J* 36: e22133, 2022. doi:10.1096/fj.202100707R.
5. Gabriel BM, Altıntaş A, Smith JAB, Sardon-Puig L, Zhang X, Basse AL, Laker RC, Gao H, Liu Z, Dollet L, Trebak JT, Zorzano A, Huo Z, Rydén M, Lanner JT, Esser KA, Barrès R, Pilon NJ, Krook A, Zierath JR. Disrupted circadian oscillations in type 2 diabetes are linked to altered rhythmic mitochondrial metabolism in skeletal muscle. *Sci Adv* 7: eabi9654, 2021. doi:10.1126/sciadv.abi9654.
6. van Moorsel D, Hansen J, Havekes B, Scheer FAJL, Jörgensen JA, Hoeks J, Schrauwen-Hinderling VB, Duez H, Lefebvre P, Schaper NC, Hesselink MKC, Staels B, Schrauwen P. Demonstration of a day-night rhythm in human skeletal muscle oxidative capacity. *Mol Metab* 5: 635–645, 2016. doi:10.1016/j.molmet.2016.06.012.
7. Wefers J, Connell NJ, Fealy CE, Andriessen C, de Wit V, van Moorsel D, Moonen-Kornips E, Jörgensen JA, Hesselink MKC, Havekes B, Hoeks J, Schrauwen P. Day-night rhythm of skeletal muscle metabolism is disturbed in older, metabolically compromised individuals. *Mol Metab* 41: 101050, 2020. doi:10.1016/j.molmet.2020.101050.
8. Gemmink A, Daemen S, Wefers J, Hansen J, van Moorsel D, Astuti P, Jorgensen JA, Kornips E, Schaart G, Hoeks J, Schrauwen P, Hesselink MKC. Twenty-four hour rhythmicity in mitochondrial network connectivity and mitochondrial respiration: a study in human skeletal muscle biopsies of young lean and older individuals with obesity. *Mol Metab* 72: 101727, 2023. doi:10.1016/j.molmet.2023.101727.
9. Harmsen J-F, van Weeghel M, Parsons R, Janssens GE, Wefers J, van Moorsel D, Hansen J, Hoeks J, Hesselink MKC, Houtkooper RH, Schrauwen P. Divergent remodeling of the skeletal muscle metabolome over 24 h between young, healthy men and older, metabolically compromised men. *Cell Rep* 41: 111786, 2022. doi:10.1016/j.celrep.2022.111786.