nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Computer software for diet registration: Vitakost V3, SENS motion system: SENS V5.2, Fitnesstracker: Polar Loop-watch or Garmin Vivofit V3.3-watch V4.
Data analysis	Statistical software: Graphpad Prism V8-10, Statistical software: Perseus V1.6.14.0, Statistical Software: R Studio (V. 4.3.3), Graphical illustrations: Biorender V(N.A.).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier

PXD047177. Username: reviewer_pxd047177@ebi.ac.uk, Password: tTnMejzF. All source data to figures and extended data figures are uploaded and available. All other data are available from the lead contact upon reasonable request.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Only male participants were recruited, and the findings therefore only apply to men. The sex was based on self-reporting. Only men were studied in order to be able to compare with existing literature that has primarily been investigating low dietary protein intake in male individuals and male rodents. Also, since we know that substantial sex differences exist in the regulation of energy - and specifically lipid - metabolism, we chose in this first proof-of-concept study including three very demanding, comprehensive, and invasive human studies of ethical and practical reasons to limit to study to one sex. Thereby, we avoid sex as confounder that would increase the metabolic heterogeneity of the study groups, which would have demanded much greater numbers of volunteers to test our hypothesis. We do recognize and encourage that future studies should investigate whether the observed findings apply to women as well.
Reporting on race, ethnicity, or other socially relevant groupings	All participants were of self-identified white North European ancestry.
Population characteristics	23 young, healthy, lean men participated in the study. The participants characteristics can be found in Table 1.
Recruitment	Participants were recruited via posters around Copenhagen and at the University, via posts at the website "Forsøgsperson.dk" and the website of the Department of Biomedical Sciences, University of Copenhagen. No specific self-selection biases appears relevant for this study.
Ethics oversight	All study participants were given oral and written study information. Written informed consent to take part in the experiment was obtained from all participants before entering the study. The study was approved by the Copenhagen Ethics Committee (H-18005023) and conformed to the Declaration of Helsinki.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to pre-determine sample sizes, but our sample sizes are similar to our previous dietary studies investigating metabolic effects of changes in dietary macronutrient composition (PMID: 38914224; 33122393; 30707625; 30269983; 28768703; 27548521)
Data exclusions	No data were excluded from the analyses, except what is defined in the figure legends.
Replication	Replication of the study results were not performed due to the strained logistics of recruiting and testing the study participants. However, there is consistency in the protocol of study 1 and the LPHC group in study 2, where all main findings are replicated. This strengthens the conclusions of that part of the study. All other experiments were not replicated.
Randomization	All study participants underwent the same experimental procedures, so there was no randomization in the human studies. In the mouse experiments, mice were block randomised to groups according to body weight.
Blinding	Participants underwent the same interventions, so it was not applicable to blind any group allocation. It was not possible to blind which dietary intervention, the individuals were on at experimental test days. For mouse experiments, diets were visually differently looking, so it was not possible to blind dietary interventions. Parts of the data collection were performed blinded including blood analysis of hormones, metabolites, proteomics analysis. Investigators were blinded during data analysis - but not during the statistical analyses.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Wildtype C57BL/6 male mice were obtained from Charles River Laboratories. Germline FGF21 KO male mice were generated on a C57BL/6 background. FGF21+/- mice were crossed to generate FGF21 KO (-/-) and WT (+/+) littermates. The mice were 8 weeks old, when initiating the 12-week dietary intervention, and hence 20 weeks when terminated.
Wild animals	The study did not involve wild animals.
Reporting on sex	Only male mice were used primarily in order to secure homology to the use of male participants in the corresponding human study in the present paper.
Field-collected samples	The study did not involve samples collected in the field.
Ethics oversight	Animal experiments were conducted according to local, national, and EU ethical guidelines (Regierungspräsidium Karlsruhe), and adhered to ARRIVE guidelines.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A