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	Atherosclerotic aortic root images were obtained with EVOS XL Imaging System. Western blot images were obtained with Bio-Rad ChemiDocMP Imaging system. Flow cytometry data were obtained with BD FACSDiva software v 6.0. Immunofluorescence images were obtained with ZEN Black Edition software (2011).
Data analysis	Atherosclerotic aortic root quantification and immunofluorescence image quantification was performed by using ZEN Blue Edition software (2011). Western blot densitometry was performed by using ImageJ bundled with 64-bit Java 1.8.0. Flow cytometry data was analyzed with FlowJo Version 10.8.1. All other data were analyzed by using Graphpad Prism version 9.

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 raw data that support the findings of this study are available from the corresponding author 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	For Study #1, fourteen participants (8 men, 6 women) were studied. For Study #2, nine participants (3 men, 6 women) were studied.
Reporting on race, ethnicity, or other socially relevant groupings	We studied both men and women, but sex and/or gender was not considered in the data analysis because we had no reason to test sex- or gender-based hypotheses. Participants' sex was determined by self-report and participants' sex is clearly noted in Table 1 and relevant figure legends.
Population characteristics	For Study #1, the participants are healthy with average age 41 ± 3 years; and average BMI 27.9 ± 1.3 kg/m2. For Study #2, the participants are healthy with average age 41 ± 5 years and average BMI 28.4 ± 1.2 kg/m2.
Recruitment	Study participants (both men and women) were recruited through word-by-mouth, by using the Washington University Research Participant Registry, and by advertisement (posters and flyers in the local community). Participants were eligible to participate in the study if they met the following inclusion criteria: i) age ≥21 years and ≤70 years and ii) body mass index ≥25.0 and ≤35.0 kg/m2. Potential participants were excluded if they: i) were vegetarian or vegan or had intolerance to any ingredients in the study meals, ii) had a history of intestinal resection or rerouting; iii) had a disease or used medications that could affect the study outcome measures; iv) had significant (± 3%) body weight changes during three months before the study, v) consumed more than one alcoholic drink per day; or vi) engaged in structured exercise for more than 90 minutes per week.
Ethics oversight	The study received approval from institutional Review Board, Human Research Protection Office at Washington University, School of Medicine.

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	Atherosclerosis experiments on ApoE-null mice produce lesions with a standard deviation of ~20%. Assuming two-tailed testing, α=0.05, and power=80%, detecting differences in lesion area of 25%-30% between 2 groups of ApoE-null mice requires n=9-12 mice. All atherosclerosis studies in this manuscript met or exceeded the minimum number of mice required by these sample size calculations and are denoted in the figures/figure legends. For the human subjects studies, we initially chose the sample size based on the robustness of mTOR activation following exposure to amino acids and high- and low-protein oral gavage in our mouse models. A post-hoc power analysis we conducted with the data we obtained from our study determined that the sample size was sufficient to detect a statistically significant difference in mTOR activation (assessed as phospho-S6 content) after ingesting the very high protein and standard protein liquid meals. The postprandial phospho-S6 content in monocytes 3 h after ingesting the very high protein liquid meal containing 50% of total energy as protein was 2.3 ± 2.2 (mean ± SD) times greater than after ingesting the corresponding control meal containing 10% of total energy as protein. Using these data and assuming <0.05 probability of Type I error, 90% power, and two-sided testing with a paired study design, we estimate that 13 participants are needed to detect a 2.3 fold difference. Ten participants would be sufficient to detect the same effect size assuming 80% power.
Data exclusions	No data were excluded from the analyses.
Replication	All primary macrophage experiments were replicated at least 3 times by independent experiments and for data to be included in the

Replication	manuscript, all attempts at replication were successful. All studies involving human subjects and the mouse cohorts were performed once after determination of the appropriate sample size using power calculations (see sample size section above). Additional details regarding replication and sample size can be found in figure legends.
Randomization	Atherosclerosis experiments in mice were conducted on littermate mice housed in the same facility born within 2-3 weeks of each other. Allocation of mice to each treatment group was random. For in vitro experiments, cells were randomly allocated into different groups. In the human subjects studies, the order of the different test meals was randomized.
Blinding	Operators for data collection were blinded. Investigators were blinded to group allocation during data 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

Antibodies

Antibodies used

Fluorescence Activated Cell Sorting (FACS)
Pacific Blue-conjugated anti-human CD45 (BioLengend, 304029, HI30, B304029, 1:200)
PE-conjugated anti-human CD14 (BioLengend, 301806, M5E2, B356127, 1:200)
APC-conjugated anti-human CD16 (BioLengend, 302012, 3G8, B300870 1:200)
Pacific Blue-conjugated anti-mouse CD45 (BioLengend, 103126, 30-F11, B272423, 1:200)
PerCP-Cy5.5-conjugated anti-mouse CD11b (BioLengend, 101228, M1/70, B276002, 1:200)
Alexa Fluor®700-conjugated anti-mouse Ly-6C (BioLengend 128024, HK1.4, B243043, 1:200)
p-S6 (Ser235/236) (Cell Signaling Technology 4856S, 2F9, 9, 1:200)
Alexa Fluor® 488 Goat anti-Rabbit IgG (H+L) (Invitrogen, A11008, 2051237, 1:500).

Immunofluorescence Microscopy
MOMA2 (Biorad, MCA519C, 1:250)
LC3 (MBL, PM036, 034, 1:250)
p-S6 (Ser235/236) (Cell Signaling Technology 4856S, 2F9, 9, 1:100)
mTOR (Cell Signaling Technology, 2983, 7C10, 16, 1:250)
Lamp2 (Abcam, ab13524, GL2A7, GR324599-14, 1:250)
COX IV (Cell Signaling Technology, 11967, 4D11-B3-E8, 4, 1:200)
CD68 (Biorad, MCA1957, FA-11, 1807, 1:250).
Alexa Fluor® 488 Goat anti-Rabbit IgG (H+L) (Invitrogen, A11008, 2051237, 1:250)
Alexa Fluor® 488 Goat anti-Rat IgG (H+L) (Invitrogen, A11006, 2105157, 1:250).
Alexa Fluor® 594 Goat anti-Rat IgG (H+L) (Invitrogen, A11007, 2026149, 1:250)
Alexa Fluor® 594 Goat anti-Mouse IgG (H+L) (Invitrogen, A11005, 2110496, 1:250)

Western Blotting
ULK1 (Sigma, A7481, 037M4812V, 1:2000)
p-ULK1 (Ser757) (Cell Signaling Technology,14202, D706U, 4, 1:2000)
p-S6K (Thr389) (Cell Signaling Technology, 9234, 108D2, 12, 1:1000)
S6K (Cell Signaling Technology, 2708, 49D7, 8, 1:2000)
p-S6 (Ser240/244) (Cell Signaling Technology, 2215, 18, 1:1000)
S6 (Cell Signaling Technology, 2217, 5G10, 7, 1:2000)
p-AMPKα(Thr172) (Cell Signaling Technology, 2535, 40H9, 27, 1:1000)
AMPKα (Cell Signaling Technology, 2532, 21, 1:2000)
HRP-linked anti-rabbit IgG (Cell Signaling Technology, 7074, 28, 1:2000)

Validation

Fluorescence Activated Cell Sorting (FACS)
Pacific Blue-conjugated anti-human CD45: https://www.biolegend.com/nl-be/products/pacific-blue-anti-human-cd45-antibody-3331
PE-conjugated anti-human CD14: https://www.biolegend.com/nl-be/products/pe-anti-human-cd14-antibody-796
APC-conjugated anti-human CD16: https://www.biolegend.com/nl-be/products/apc-anti-human-cd16-antibody-565
Pacific Blue-conjugated anti-mouse CD45: https://www.biolegend.com/en-us/products/pacific-blue-anti-mouse-cd45-antibody-3102.
PerCP-Cy5.5-conjugated anti-mouse CD11b: https://www.biolegend.com/en-us/products/percpcyanine55-anti-mouse-human-cd11b-antibody-4257

Alexa Fluor®700-conjugated anti-mouse Ly-6C: https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-mouse-ly-6c-antibody-6757
p-S6 (Ser235/236): https://www.cellsignal.com/products/primary-antibodies/phospho-s6-ribosomal-protein-ser235-236-2f9-rabbit-mab/4856
Alexa Fluor® 488 Goat anti-Rabbit IgG (H+L): https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11008

Immunofluorescence Microscopy
MOMA2: https://www.bio-rad-antibodies.com/monoclonal/mouse-macrophages-monocytes-antibody-moma-2-mca519
LC3: https://www.mblintl.com/products/pm036
p-S6 (Ser235/236): https://www.cellsignal.com/products/primary-antibodies/phospho-s6-ribosomal-protein-ser235-236-2f9-rabbit-mab/4856
mTOR: https://www.cellsignal.com/products/primary-antibodies/mtor-7c10-rabbit-mab/2983
Lamp2: https://www.abcam.com/lamp2-antibody-gl2a7-ab13524
COX IV: https://www.cellsignal.com/products/primary-antibodies/cox-iv-4d11-b3-e8-mouse-mab/11967
CD68: https://www.bio-rad-antibodies.com/monoclonal/mouse-cd68-antibody-fa-11-mca1957
Alexa Fluor® 488 Goat anti-Rabbit IgG (H+L): https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11008
Alexa Fluor® 488 Goat anti-Rat IgG (H+L): https://www.thermofisher.com/antibody/product/Goat-anti-Rat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11006
Alexa Fluor® 594 Goat anti-Rat IgG (H+L): https://www.thermofisher.com/antibody/product/Goat-anti-Rat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11007
Alexa Fluor® 594 Goat anti-Mouse IgG (H+L): https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11005

Western Blotting
ULK1: https://www.sigmaaldrich.com/catalog/product/sigma/a7481
p-ULK1 (Ser757): https://www.cellsignal.com/products/primary-antibodies/phospho-ulk1-ser757-d7o6u-rabbit-mab/14202
p-S6K (Thr389): https://www.cellsignal.com/products/primary-antibodies/phospho-p70-s6-kinase-thr389-108d2-rabbit-mab/9234
S6K: https://www.cellsignal.com/products/primary-antibodies/p70-s6-kinase-49d7-rabbit-mab/2708
p-S6 (Ser240/244): https://www.cellsignal.com/products/primary-antibodies/phospho-s6-ribosomal-protein-ser240-244-antibody/2215
S6: https://www.cellsignal.com/products/primary-antibodies/s6-ribosomal-protein-5g10-rabbit-mab/2217
p-AMPKα(Thr172): https://www.cellsignal.com/products/primary-antibodies/phospho-ampka-thr172-40h9-rabbit-mab/2535
AMPKα: https://www.cellsignal.com/products/primary-antibodies/ampka-antibody/2532
HRP-linked anti-rabbit IgG: https://www.cellsignal.com/products/secondary-antibodies/anti-rabbit-igg-hrp-linked-antibody/7074

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	All mice used in this study were on C57BL/6J background and ordered from Jackson Lab. ApoE genotyping was performed using standard PCR techniques. Mice housed in a specific pathogen–free barrier facility at 22-24 °C and 30% humidity with a standard light-dark cycle (12:12) and routine checks of each animal's health status. Mice were weaned at 3 weeks of age to a standard mouse chow diet (Labdiet, 5053). For in vivo atherosclerosis-related-experiments, male mice of the stated genotype were started at ~8 weeks of age with low-protein (TD140106: 0.15% cholesterol, 7% energy protein, 42% energy fat, 51% energy carbohydrate), moderate-protein (TD140105: 0.15% cholesterol, 21% energy protein, 42% energy fat, 36% energy carbohydrate) or high protein (TD04524: 0.15% cholesterol, 46% energy protein, 43% energy fat, 11% energy carbohydrate) Western-type diets (all from Harlan). For the evaluation of both the sufficiency and the requirement of a high amount of leucine in the pathogenesis of atherosclerosis associated with a high protein Western Diet (HP WD), mice were randomized to 6 different diets, including ApoE KO mice were randomized to 6 different diets, including: 1) moderate-protein Western Diet [called MP-WD], 2) high-protein Western Diet [called HP-WD], 3) MP-WD to which leucine, but no other amino acids, was added to match the leucine content of the HP-WD [called MP-WD+Leu], 4) MP-WD to which amino acids were added to match the total AA content of the HP-WD [called MP-WD+AA], 5) MP-WD to which additional amino acids, except for leucine, were added to match the contents of all amino acids, except leucine, in the HP-WD [called MP-WD+AA (Normal Leu)], and 6) nitrogen-adjusted version of MP-WD+AA (normal Leu), which consisted of the MP-WD with additional amino acids, except leucine, to make it isonitrogenous with the HP-WD [called MP-WD+AA (Normal Leu (IsoN))]. The detailed content of each diet was shown in Supplement Table 1. For the evaluation of plasma amino acid and TG concentrations and mTORC1 signaling in blood monocytes after gavage with different diets, both male and female C57BL/6J mice were used. Mice were fasted for 4 hours and then given by gavage a solution that contained either 0.8 g protein per kg or 1.6 g protein per kg or a solution that contained 0.8 g protein per kg plus leucine to match the total leucine content of the solution with 1.6 g protein per kg. Blood was collected for isolation of PBMCs and to determine plasma amino acid, cholesterol, and TG concentrations at time points indicated. The dosage of protein for gavage was chosen based on results we obtained previously with the goal to mimic postprandial circulating amino acid concentrations observed in our human studies. For in vitro experiments, macrophages derived from male and female mice aged between 2 to 6 months were used and within each experiment only mice with the same sex and similar age were compared.
Wild animals	The study did not involve wild animals.
Reporting on sex	For the evaluation of plasma amino acid and triglyceride concentrations and mTORC1 signaling in blood monocytes after gavage with different diets, both male and female C57BL/6J mice were used. For in vitro experiments, macrophages derived from male and female mice aged between 2 to 6 months were used. Within each experiment only mice with the same sex and similar age were compared.

Field-collected samples	The study did not involve field-collected samples.
Ethics oversight	All research on animals were approved by the Washington University Animal Studies Committee.

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	NCT03946774 and NCT03994367
Study protocol	The study protocol could be found on clinicaltrials.gov (NCT03946774 and NCT03994367)
Data collection	For Study #1, participants were enrolled between October 1, 2019 and December 17, 2020. For Study #2, participants were enrolled between November 15, 2019 and December 2, 2020. The study site was in the Clinical and Translational Research Unit (CTRU) at Washington University School of Medicine in St. Louis, MO. Blood samples were processed immediately after collection to isolate PBMCs and plasma. Plasma samples for the evaluation of plasma amino acid concentration were stored at -80 degrees celsius until further analysis.
Outcomes	The primary outcome was the evaluation of mTORC1 signaling in circulating monocytes. The secondary outcome was the quantification of serum amino acids from participants. We chose mTORC1 signaling in circulating monocytes as the primary outcome because we found in animal models that mTORC1 activation drives atherosclerosis. Serum amino acid concentrations were the secondary outcome to determine the relationship between specific amino acids and monocyte mTORC1 activation. For evaluation of mTORC1 signaling in circulating monocytes, whole blood was collected in chilled tubes containing EDTA. For analysis with Western Blotting, whole blood was collected in chilled tubes containing EDTA. Peripheral blood mononuclear cells (PBMCs) were isolated via centrifugation with Ficoll-Paque PLUS. CD14+ monocytes were isolated using the EasySep Human Monocyte Enrichment Kit. The analysis followed a standard Western Blotting protocol while primary antibodies for pS6 and S6 were utilized. For FACS-based analysis, PBMCs were isolated via centrifugation with Ficoll-Paque PLUS and stained with fluorochrome-conjugated macrophage markers CD45, CD14, and CD16. Cells were then fixed with 4% paraformaldehyde, permeabilized with 0.3% saponin, and incubated with p-S6 primary antibody followed by Alexa Fluor® 488 secondary antibody . All samples were analyzed using the BD Biosciences Canto II or LSR II flow cytometer and quantified using FlowJo software. The evaluation of mTORC1 signaling was based on the ratio of pS6/S6 intensity in analysis with Western Blotting and MFI of pS6 in FACS analysis. To determine amino acid concentrations, plasma was isolated from each sample by centrifugation and was then stored at -80 C until analysis. Gas chromatography-mass spectrometry (GC-MS) was used to quantify amino acid (except arginine) concentrations in human plasma. Arginine concentration in plasma was quantified by using liquid chromatography/tandem mass spectrometry.

Plants

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

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	FACS analysis for mitochondria dysfunction and ROS levels was performed on cultured HMDMs. Cultured macrophages were plated (Greiner 665102), treated with the indicated reagents, and incubated with MitoTracker Red CMXRos (250 nM; Life Technologies, M7512), MitoTracker Green FM (200 nM; Life Technologies, M7514) at 37°C for 30min. Cells were collected

	and resuspended in FACS buffer (2% FBS in PBS) for subsequent flow cytometry. FACS analysis for phosphorylated S6 ribosomal protein (phospho-S6) was performed on human blood monocytes and mouse blood monocytes. For human blood monocytes, PBMCs were isolated via centrifugation with Ficoll-Paque PLUS and stained with fluorochrome-conjugated macrophage markers CD45, CD14, and CD16 (Biolegend). Mouse blood leukocytes were isolated and stained with fluorochrome-conjugated monocytes markers CD45, CD11b, and Ly-6C (Biolegend). Cells were then fixed with 4% paraformaldehyde, permeabilized with 0.3% saponin, and incubated with p-S6 (Cell Signaling Technology 4856S, 1:200) followed by Alexa Fluor® 488 secondary antibody (Invitrogen A11008; 1:500).
Instrument	All samples were analyzed using the BD Biosciences Canto II or LSR II flow cytometer.
Software	All samples were analyzed using the BD Biosciences Canto II or LSR II flow cytometer software and quantified using FlowJo software.
Cell population abundance	Appropriate gating as detailed below ascertained purity of macrophages for further analyses.
Gating strategy	Gating strategy for human circulating monocytes used sequential gating on SSC-A/FSC-A, FSC-H/FSC-W, SSC-H/SSC-W, CD45/FSC-A, CD14/CD16 followed by gating for the specific marker of interest (i.e. pS6). Gating strategy for mouse blood monocytes used sequential gating on SSC-A/FSC-A, FSC-H/FSC-W, SSC-H/SSC-W, CD45/FSC-A, Ly6C/CD11b followed by gating for the specific marker of interest (i.e. pS6). Gating strategy for cultured HMDMs used SSC-A/FSC-A followed by gating for the specific marker of interest (i.e.Mitotracker).

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.