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An exercise-inducible metabolite that suppresses feeding and obesity

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Exercise confers protection against obesity, type 2 diabetes and other cardiometabolic diseases[1–5]. However, the molecular and cellular mechanisms that mediate the metabolic benefits of physical activity remain unclear[6]. Here we show that exercise stimulates the production of *N*-lactoyl-phenylalanine (Lac-Phe), a blood-borne signalling metabolite that suppresses feeding and obesity. The biosynthesis of Lac-Phe from lactate and phenylalanine occurs in CNDP2^{+} cells, including macrophages, monocytes and other immune and epithelial cells localized to diverse organs. In diet-induced obese mice, pharmacological-mediated increases in Lac-Phe reduces food intake without affecting movement or energy expenditure. Chronic administration of Lac-Phe decreases adiposity and body weight and improves glucose homeostasis. Conversely, genetic ablation of Lac-Phe biosynthesis in mice increases food intake and obesity following exercise training. Last, large activity-inducible increases in circulating Lac-Phe are also observed in humans and racehorses, establishing this metabolite as a molecular effector associated with physical activity across multiple activity modalities and mammalian species. These data define a conserved exercise-inducible metabolite that controls food intake and influences systemic energy balance.

Exercise is a powerful physiological intervention that protects against obesity and obesity-associated cardiometabolic diseases[1–5]. By contrast, physical inactivity increases the risk of obesity, metabolic disease and all-cause mortality[4,5]. There has been a growing interest in identifying 'molecular transducers' that might mediate the cardiometabolic benefits of exercise[6]. Although large-scale multi-omics efforts (for example, by the Molecular Transducers of Physical Activity Consortium) have begun to generate molecular maps of the biological molecules regulated by physical activity[6–9], the functional relationships between these molecular changes and physiological outcomes remain poorly defined. In parallel, candidate approaches have uncovered specific functional exercise-regulated signalling molecules[10–14], but the extent to which these candidate molecules represent the most important mediators of physical activity remains unclear. We reasoned that a hybrid approach might be valuable in both providing a global view of the most relevant molecular changes associated with physical activity and designating high-priority, candidate molecular transducers of exercise.

Plasma metabolomics of exercise

To measure exercise-induced circulating metabolites in a global and unbiased manner, we performed both targeted and untargeted metabolomics of blood plasma from mice following an acute bout of treadmill running until exhaustion (Extended Data Fig. 1a). Our targeted metabolomics analysis detected increases in several metabolites, including lactate, fumarate and succinate, which were previously established to be regulated by physical activity[6–8,12,15,16] (Fig. 1a, Supplementary Table 1 and Methods). The metabolite most significantly induced by exercise, however, was found by untargeted metabolomics (Fig. 1a,b). This metabolite had a mass-to-charge ratio (*m/z*) of 236.0928 that was

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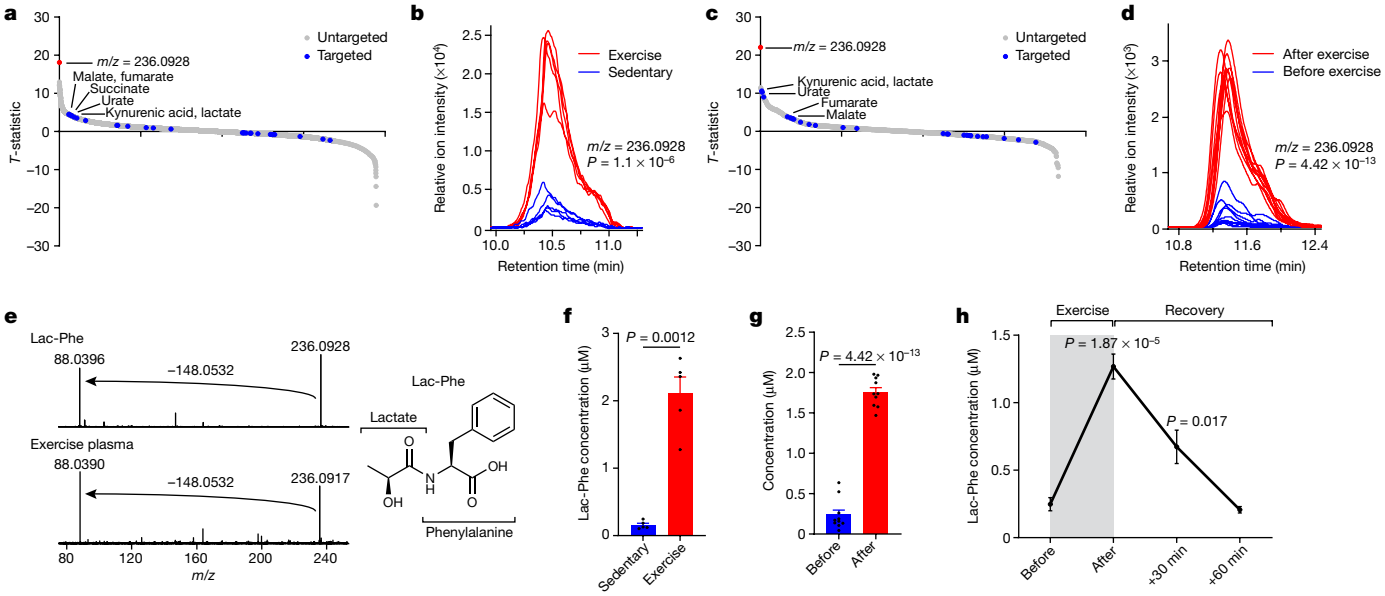

Fig. 1 | Lac-Phe is strongly induced in blood plasma after a single bout of running in mice and racehorses. a–**d**, *T*-statistic of all blood plasma peaks detected by targeted or untargeted metabolomics after a run in mice (**a**) and racehorses (**c**), and extracted ion chromatograms of the top *m/z* = 236.0928 mass in mice (**b**) and racehorses (**d**). **e**, Tandem mass spectrometry fragmentation (left) and structural assignment (right) of an authentic Lac-Phe standard (top) and endogenous *m/z* = 236.0928 mass (bottom) from mouse plasma. **f**,**g**, Absolute quantitation of Lac-Phe in mouse (**f**) and racehorse (**g**) plasma. **h**, Time course of Lac-Phe in blood plasma after a single bout of running to exhaustion in mice; the grey area indicates the time period of the exercise bout. For **a**, **b** and **f**, $N = 5$ per group. For **c**, **d** and **g**, $N = 10$ per group. Data are shown as the mean ± s.e.m. The experiments in **a**–**e** were performed once, the experiments in **f** and **g** were performed three times, and the experiments in **h** were performed two times. *P* values were calculated by Student's two-sided *t*-test.

consistent with a chemical formula of $C_{12}H_{14}NO_4^-$, but such a molecule did not match a metabolite in our original targeted list.

We also performed targeted and untargeted metabolomics of plasma from thoroughbred racehorses taken before and after a race. Racehorses exhibit the highest fractional increase in maximal oxygen consumption (VO_2) reported for any mammal (about 45-fold increase) and have long been studied for their superior athletic performance[17]. Notably, the same *m/z* = 236.0928 molecule was detected by untargeted metabolomics as the most significant exercise-inducible metabolite change (Fig. 1c,d and Supplementary Table 1).

Fragmentation of the *m/z* = 236.0928 metabolite revealed a prominent daughter ion of *m/z* = 88.040, which matched $C_3H_6NO_2^-$ (Fig. 1e). On the basis of these spectra, the parent metabolite was tentatively assigned to Lac-Phe, an amidated conjugate of lactate and phenylalanine (Fig. 1e). An authentic Lac-Phe standard produced by chemical synthesis exhibited identical fragmentation spectra (Fig. 1e) and retention times (Extended Data Fig. 1b–d) as the endogenous *m/z* = 236.0928 peak. Absolute quantitation of circulating Lac-Phe levels in mice and racehorses revealed peak concentrations of about 2 μM after exercise (Fig. 1f,g). Plasma Lac-Phe levels in mice were substantially increased immediately after running and returned to baseline by 1 h after exercise (Fig. 1h). Lac-Phe was also detected in multiple mouse tissues, but tissue Lac-Phe levels were unaltered by acute running (Extended Data Fig. 1e). Several additional lactoyl amino acids were increased in plasma after acute running in both mice and racehorses, with conjugates of lactate and hydrophobic amino acids exhibiting the most dramatic exercise-inducible changes (Extended Data Fig. 1f,g). We conclude that Lac-Phe is the most significantly induced circulating metabolite in two animal models of exercise.

Lac-Phe biosynthesis from lactate by CNDP2

Lac-Phe is a poorly studied metabolite of unknown function[18–22]. Increases in Lac-Phe levels through exercise has been previously reported[21]. Our metabolomics data further contextualize this increase as one of the most notable metabolite changes in acute exercise. The previous study[21] also provided in vitro evidence that the cytosolic enzyme CNDP2 can catalyse Lac-Phe synthesis through the condensation of lactate and phenylalanine (Extended Data Fig. 2a), but the physiological relevance of this biochemical reaction remained unclear. From the single-cell *Tabula Muris* database[23], *Cndp2* mRNA exhibited widespread expression across diverse cell types (Fig. 2a and Extended Data Fig. 2b), including macrophages and monocytes, other immune cells and epithelial cells. We used the mouse RAW264.7 macrophage cell line to model Lac-Phe production in vitro. Even though CNDP2 is an intracellular enzyme, nearly all the Lac-Phe from RAW264.7 cells was secreted in the conditioned medium (Fig. 2b). In a macrophage cell line in which *Cndp2* was knocked out using the CRISPR–Cas9 technique (CNDP2-KO cells), extracellular Lac-Phe levels were reduced by >75% (Fig. 2b and Extended Data Fig. 2c). Addition of lactate (25 mM) to the conditioned medium further increased extracellular levels of Lac-Phe by 85% (Fig. 2c). Similar lactate-stimulated, CNDP2-dependent Lac-Phe production was also observed in two additional epithelial cell lines (bladder RT4 cells and kidney TKPTS cells; Fig. 2d–g and Extended Data Fig. 2d,e). CNDP2 protein levels were unchanged in multiple mouse tissues after a single bout of running to exhaustion (Extended Data Fig. 2f–h). Lac-Phe is therefore a secreted metabolite produced by CNDP2 and regulated by extracellular lactate. The high levels of blood lactate achieved during exercise and the high level of secretion of Lac-Phe are two factors that probably drive an otherwise thermodynamically unfavourable intracellular condensation reaction.

Lac-Phe suppresses feeding and obesity

In the Type 2 Diabetes Knowledge Portal[24], two missense single nucleotide polymorphisms in human *CNDP2* are associated with body mass index: rs373836366 D279N ($P = 2.54 \times 10^{-6}$, $\beta = +0.4857$) and rs780772968 K374T ($P = 8.48 \times 10^{-4}$, $\beta = +0.7144$) (Extended Data Fig. 3a,b). On the

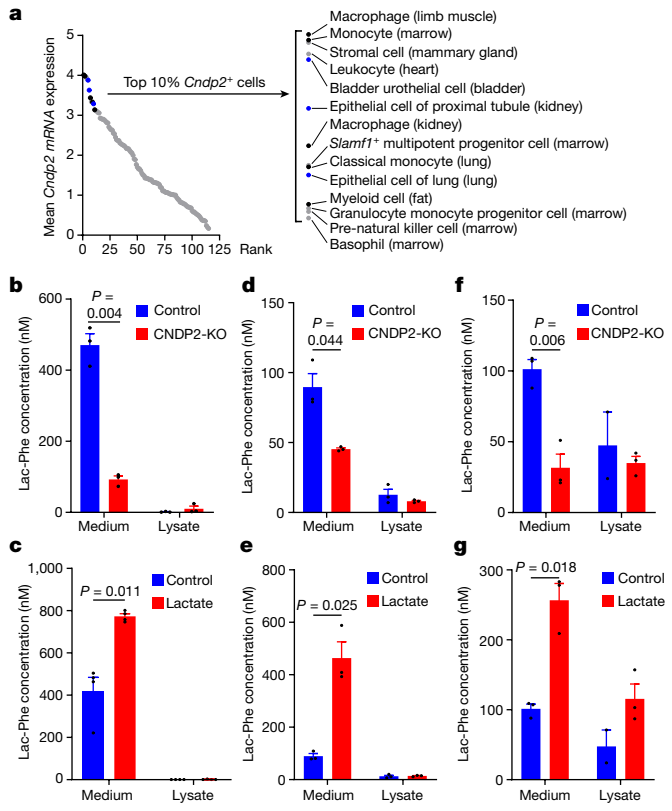

Fig. 2 | CNDP2-dependent and lactate-dependent production and secretion of Lac-Phe in vitro. a, Mean *Cndp2* mRNA expression across mouse cells and tissues from *Tabula Muris*. Highlighted black dots indicate monocytes and macrophages, whereas highlighted blue dots indicate epithelial cells. **b**–**g**, Lac-Phe levels in conditioned medium and cell lysate of WT and CNDP2-KO RAW264.7 macrophage cells (**b**,**c**), RT4 bladder epithelial cells (**d**,**e**) and TKPTS kidney epithelial cells (**f**,**g**) under the indicated condition. Lactate-treated cells were supplemented with 25 mM lactate for 24 h. For **b**–**g**, $N = 3$ per group. Data are shown as the mean ± s.e.m. The experiments shown in **b** were performed three times and the experiments in **c**–**g**, were performed once. *P* values were calculated by Student's two-sided *t*-test.

basis of these data, we hypothesized that exercise-inducible Lac-Phe might function as a molecular signal to regulate energy balance. We used metabolic chambers to determine the effect of acute Lac-Phe increases on whole-body energy parameters in diet-induced obese (DIO) mice. Lac-Phe was administered at a dose that increased circulating levels (50 mg kg^{-1}, intraperitoneal (i.p.) injection; Extended Data Fig. 3c). Blood lactate levels were unchanged under these conditions (Extended Data Fig. 3d). Acute Lac-Phe treatment suppressed food intake by approximately 50% compared with vehicle-treated mice over a period of 12 h (Fig. 3a). Of note, ambulatory activity was not different between groups (Fig. 3b), which demonstrates that the suppression of feeding behaviours was not simply due to reduced movement. Acute Lac-Phe treatment also did not alter oxygen consumption, carbon dioxide production or respiratory exchange ratios (Extended Data Fig. 3e–g). In a separate group of DIO mice, Lac-Phe administration did not alter kaolin or water intake, which demonstrates that a component of nausea is not involved in its hypophagic effects (Extended Data Fig. 3h–j). Lac-Phe also did not alter circulating levels of other appetite-regulating

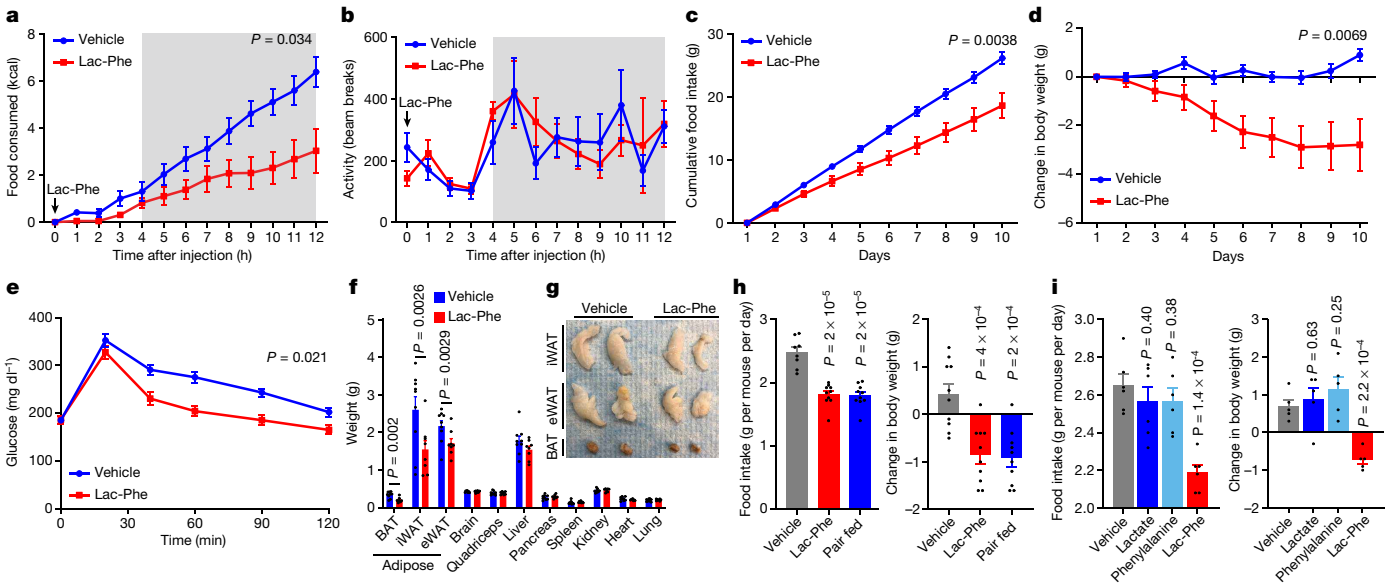

Fig. 3 | Lac-Phe suppresses food intake and obesity and improves glucose homeostasis. a,**b**, Cumulative food intake (**a**) and ambulatory activity (**b**) of 22-week-old male DIO mice following injection of either vehicle or Lac-Phe (50 mg kg^{-1}, i.p.). Grey areas indicate the time period of the night cycle. **c**,**d**, Cumulative food intake (**c**) and change in body weight (**d**) of 22-week-old male DIO mice treated every day with vehicle or Lac-Phe (50 mg kg^{-1} day^{-1}, i.p.). **e**, Glucose tolerance test (1 g kg^{-1} glucose) results of vehicle-treated mice and Lac-Phe treated mice. This assay was performed after a 6-h fast 1 day following the last Lac-Phe dose on day 10. **f**,**g**, Tissue weights (**f**) and representative images of adipose tissues (**g**) from mice after 10 days of vehicle or Lac-Phe treatment. BAT, brown adipose tissue; eWAT, epididymal white adipose tissue; iWAT, inguinal white adipose tissue. **h**, Average daily food intake (left) and change in body weight (right) of 15-week-old male DIO mice after 5 days of treatment with vehicle, Lac-Phe (50 mg kg^{-1} day^{-1}, i.p.) or vehicle-treated pair-fed mice. **i**, Average daily food intake (left) and change in body weight (right) of 13-week-old male DIO mice after 7 days of treatment with vehicle, lactate (50 mg kg^{-1} day^{-1}, i.p.), phenylalanine (50 mg kg^{-1} day^{-1}, i.p.) or Lac-Phe (50 mg kg^{-1} day^{-1}, i.p.). For **a** and **b**, $N = 6$ per group. For **c**–**g**, $N = 9$ for vehicle and $N = 8$ for Lac-Phe groups. For **h**, $N = 8$ for vehicle, $N = 10$ for Lac-Phe and $N = 10$ for pair-fed groups. For **i**, $N = 5$ per group. Data are shown as the mean ± s.e.m. The experiments shown in **a** and **b** were performed once, the experiments shown in **c** and **d** were performed three times, and the experiments shown in **e**–**i** were performed once. *P* values were calculated by two-way analysis of variance (ANOVA) with repeated measures (**a**–**e**) or Student's two-sided *t*-test (**f**–**i**).

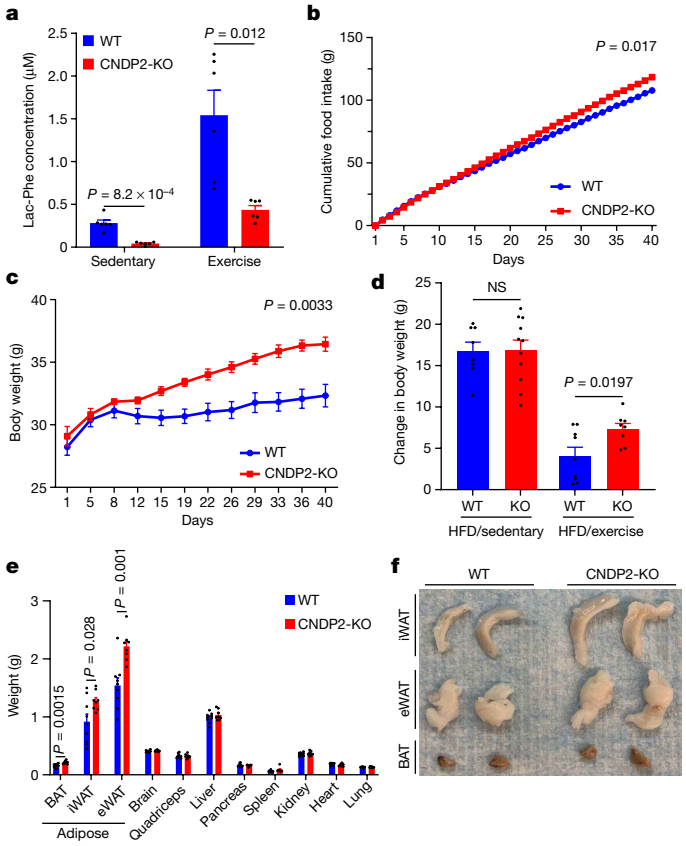

Fig. 4 | Increased food intake and obesity in mice genetically deficient in Lac-Phe production. a, Plasma Lac-Phe levels in male WT and CNDP2-KO mice under sedentary conditions or after a single bout of exhaustive treadmill running. **b**,**c**, Cumulative daily food intake (**b**) and body weight (**c**) of male WT and CNDP2-KO mice under an obesogenic diet and exercise training regimen in which mice were fed high fat diet (60% kcal from fat) and exercised by treadmill running 5 days per week (Methods). **d**, Change in body weight of WT and CNDP2-KO (KO) mice after 40 days of obesogenic diet alone (HFD/sedentary) or obesogenic diet with treadmill running (HFD/exercise). **e**,**f**, Tissue weights (**e**) and representative images of adipose tissues (**f**) of WT and CNDP2-KO mice following a combined obesogenic diet and treadmill running protocol. Tissue weights and images were taken on day 41. For **a**, $N = 6$ per group. For **b**, **c**, **e** and **f**, $N = 9$ for WT and $N = 8$ for CNDP2-KO. For **d**, $N = 8$ for WT HFD/sedentary, $N = 11$ for CNDP2-KO HFD/sedentary, $N = 9$ for WT HFD/exercise and $N = 8$ for CNDP2-KO HFD/exercise. Data are shown as the mean ± s.e.m. The experiments shown in **a**–**c** were performed two times, and the experiments shown in **d**–**f** were performed once. *P* values were calculated by two-way ANOVA with repeated measures (**b** and **c**) or Student's two-sided *t*-test (**a**, **d** and **e**).

hormones, including leptin and ghrelin (Extended Data Fig. 3k,l). In chow-fed, lean mice, Lac-Phe did not suppress food intake, even at up to threefold higher doses (150 mg kg^{-1}, i.p. injection; Extended Data Fig. 4). These data demonstrate that pharmacological administration of Lac-Phe to obese but not lean mice specifically suppresses energy intake without altering energy expenditure pathways.

Chronic administration of Lac-Phe to DIO mice (50 mg kg^{-1} day^{-1} i.p. for 10 days) resulted in reduced cumulative food intake and reduced body weight compared with control mice (Fig. 3c,d). After 10 days, mice treated with Lac-Phe also exhibited improved glucose homeostasis (Fig. 3e) and reduced adiposity (Fig. 3f,g) without any changes in the weights of the other organs (Fig. 3f). Oral dosing of Lac-Phe in obese mice did not suppress food intake or body weight (Extended Data Fig. 5), which is probably due to the chemical lability of Lac-Phe peptide bond in the digestive system. Control pair-fed mice exhibited similar changes to body weight as mice treated with Lac-Phe (Fig. 3h), which provides confirmation that the effects of Lac-Phe on adiposity and obesity are solely due to its effects on energy intake. Last, although Lac-Phe substantially suppressed food intake and body weight, lactate or phenylalanine alone did not produce any changes (Fig. 3i), which demonstrates that the intact peptide conjugate is required for the effects of Lac-Phe on energy balance. We conclude that chronic Lac-Phe treatment reduces adiposity, obesity and improves glucose tolerance.

To determine the physiological contribution of Lac-Phe to the anti-obesity effects of exercise, we used mice in which *Cndp2* was knocked out globally as a genetic model of Lac-Phe deficiency. In CNDP2-KO mice, circulating Lac-Phe was substantially reduced compared with wild-type (WT) mice in both the sedentary state and after a single bout of treadmill running, respectively (Fig. 4a). Acute run times to exhaustion were not different between genotypes (Extended Data Fig. 6a). Plasma levels of other lactoyl amino acids were also reduced in CNDP2-KO mice under sedentary but not exercised conditions (Extended Data Fig. 6b–d). By contrast, plasma carnosine levels were unchanged (Extended Data Fig. 6e). CNDP2 is therefore a principal biosynthetic enzyme responsible for basal and exercise-inducible Lac-Phe production in vivo.

When given a high-fat diet (60% kcal from fat), WT and CNDP2-KO mice exhibited similar food intake and body weight (Extended Data Fig. 6f,g), which demonstrates that reductions in basal Lac-Phe are not sufficient to alter energy balance. We therefore considered the possibility that exercise training would be required to uncover a metabolic phenotype of CNDP2-KO mice, similar to what has been reported for other exercise-inducible molecules[25]. In a new group of WT and CNDP2-KO mice fed a high-fat diet, we concurrently implemented a moderate and chronic treadmill running exercise protocol that had previously shown anti-obesity effects in mice[26] (40 min day^{-1} starting at 6 m min^{-1} and increasing by 2 m min^{-1} every 5 min, 5 days per week). Both genotypes ran for equivalent times with this chronic exercise protocol (WT mice, 41 ± 1.2 min day^{-1}; CNDP2-KO mice, 41 ± 1.7 min day^{-1} (mean ± s.e.m.), $P > 0.05$). Starting from day 10, CNDP2-KO mice exhibited increased food intake compared with control mice (Fig. 4b). At this same time point, CNDP2-KO mice also increasingly diverged in their body weight compared with control mice (Fig. 4c). The chronic running protocol suppressed body weight by 75% in WT mice over the entire period of the experiment, whereas only 57% body weight suppression was observed in CNDP2-KO mice ($P < 0.05$; Fig. 4d). Last, dissection of tissues revealed increased adipose tissue mass in CNDP2-KO mice, with no changes in lean mass (Fig. 4e,f). The 10-day delay of onset for the feeding phenotype may in part reflect the hypophagic action of Lac-Phe selectively in obese but not lean animals (Fig. 3 and Extended Data Fig. 4). We therefore conclude that genetic ablation of Lac-Phe biosynthesis leads to increased energy intake and weight gain following exercise training, but not in the sedentary state.

The ATP transporter ABCC5 has been shown to mediate low-affinity Lac-Phe efflux from cells in vitro[21]. However, Lac-Phe levels did not differ between WT mice and mice with global ABCC5 KO in either the sedentary state or after a single bout of running (Extended Data Fig. 7). These data demonstrate that genetic ablation of ABCC5 is not sufficient to alter circulating levels of Lac-Phe.

Lac-Phe levels in human exercise

Lac-Phe levels were next determined in humans in two independent exercise cohorts. First, we re-analysed untargeted plasma mass spectrometry data from a previously published deeply phenotyped cohort of volunteers that underwent an acute treadmill running exercise[7] ($N = 36$; Fig. 5a). A yet unassigned peak corresponding to a metabolite with a chemical formula matching Lac-Phe ($C_{12}H_{14}NO_4^-$) was ranked

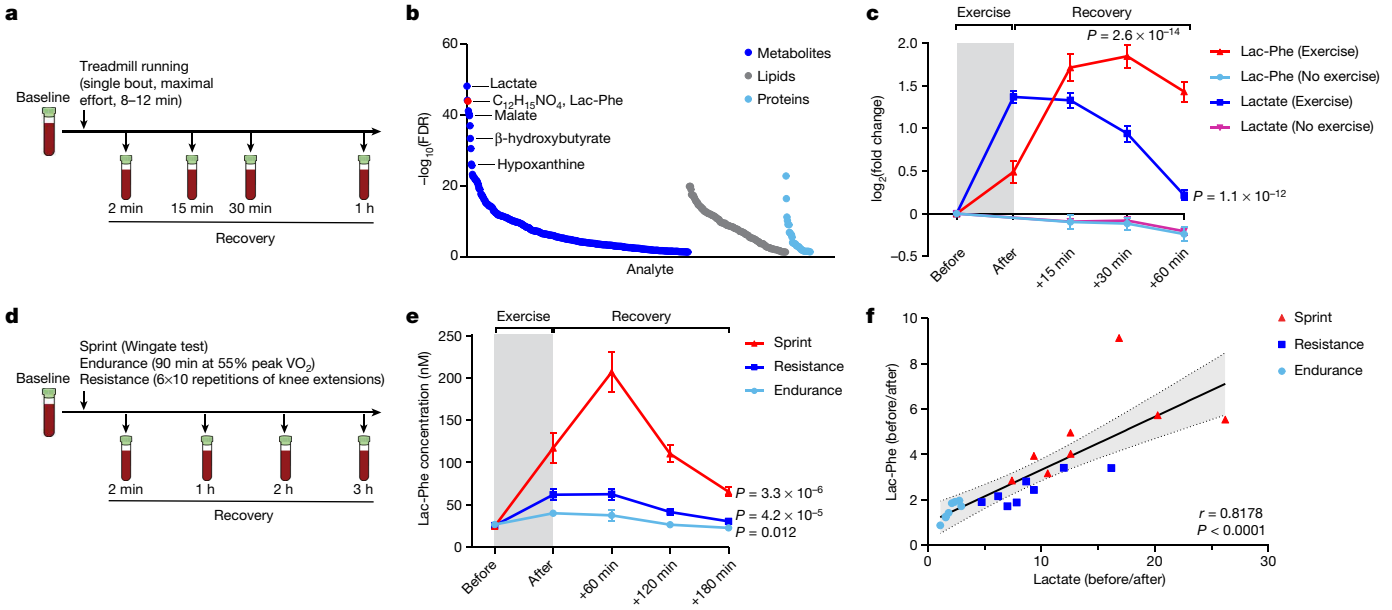

Fig. 5 | Sustained increases in Lac-Phe following human exercise. **a**, Schematic of the human acute treadmill exercise study design (cohort 1). $N = 36$. **b**, Exercise-regulated metabolites, lipids and proteins from blood plasma in cohort 1. A previously unassigned metabolite with a chemical formula matching that of Lac-Phe (red) was ranked the third most exercise-regulated molecule in the entire dataset. **c**, Time course of Lac-Phe and lactate in the blood in cohort 1 after exercise in the non-exercised control group. **d**, Schematic of the human crossover acute exercise study design (cohort 2). $N = 8$. **e**, Time course of Lac-Phe levels before and after exercise in cohort 2. **f**, Correlation of plasma Lac-Phe and lactate levels immediately before and after exercise across the three exercise modalities in cohort 2. A simple linear regression is shown in the dark black line with 95% confidence intervals shown in the grey areas. Data are shown as the mean ± s.e.m. In **c** and **e**, grey areas indicate the time period of the exercise bout. The experiments were performed once. *P* values were calculated by two-way ANOVA with repeated measures.

the third most significantly induced by exercise in the entire dataset (Fig. 5b). Tandem mass spectrometry experiments confirmed that this previously unassigned metabolite is Lac-Phe (Extended Data Fig. 8a,b). Plasma Lac-Phe levels in humans showed a sustained increase following exercise (Fig. 5c). By comparison, lactate levels peaked at the cession of exercise and rapidly returned to baseline after 1 h (Fig. 5c) and phenylalanine levels were unchanged (Extended Data Fig. 8c).

We also measured plasma Lac-Phe levels in a second group of volunteers who had each undergone three distinct exercise trials ($N = 8$ for endurance, sprint and resistance trials; Methods and Fig. 5d). Lac-Phe exhibited strong and sustained increases across all three exercise modalities (Fig. 5e). The sprint exercise induced the most substantial increase in plasma Lac-Phe, followed by resistance training and then endurance training. Even at 3 h after exercise, individuals in the sprint intervention group still showed plasma Lac-Phe levels that were increased above the baseline (Fig. 5e). Across all three exercise modalities, the before compared with immediately after exercise fold-change in Lac-Phe and lactate exhibited a strong correlation (Pearson's $r = 0.82$, $P < 0.0001$; Fig. 5f and Extended Data Fig. 8d). These data establish Lac-Phe as one of the top exercise-regulated metabolites in humans. Distinct physical activity modalities in humans lead to different quantitative changes in circulating Lac-Phe levels that correlate with blood lactate concentration.

Discussion

Our metabolomics analyses and functional characterization of Lac-Phe provide several new insights about the molecular responses to physical activity. The generation of a signalling metabolite (Lac-Phe) from a metabolic fuel (lactate) establishes a functional and biochemical coupling between the metabolic state during exercise and long-lasting endocrine signalling. That macrophages and monocytes, immune cells, epithelial cells and other CNDP2$^+$ cell types secrete Lac-Phe suggests that many additional cell types beyond muscle can sense and respond to physical activity. Finally, although Lac-Phe inhibits feeding and obesity similar to other metabolic hormones, including GLP-1 and GDF15, we suspect the as yet unidentified receptors and the neural circuits activated by Lac-Phe are likely to be distinct. Future work uncovering the downstream molecular and cellular mediators of Lac-Phe action in the brain may provide new therapeutic opportunities to capture the cardiometabolic benefits of physical activity for human health.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-022-04828-5.

1. Eriksson, K. F. & Lindgärde, F. Prevention of type 2 (non-insulin-dependent) diabetes mellitus by diet and physical exercise. *Diabetologia* **34**, 891–898 (1991).
2. Rejeski, W. J. et al. Lifestyle change and mobility in obese adults with type 2 diabetes. *N. Engl. J. Med.* **366**, 1209–1217 (2012).
3. Stampfer, M. J., Hu, F. B., Manson, J. E., Rimm, E. B. & Willett, W. C. Primary prevention of coronary heart disease in women through diet and lifestyle. *N. Engl. J. Med.* **343**, 16–22 (2000).
4. Helmrich, S. P., Ragland, D. R., Leung, R. W. & Paffenbarger, R. S. Physical activity and reduced occurrence of non-insulin-dependent diabetes mellitus. *N. Engl. J. Med.* **325**, 147–152 (1991).
5. Rawshani, A. et al. Risk factors, mortality, and cardiovascular outcomes in patients with type 2 diabetes. *N. Engl. J. Med.* **379**, 633–644 (2018).
6. Sanford, J. A. et al. Molecular Transducers of Physical Activity Consortium (MoTrPAC): mapping the dynamic responses to exercise. *Cell* **181**, 1464–1474 (2020).
7. Contrepois, K. et al. Molecular choreography of acute exercise. *Cell* **181**, 1112–1130.e16 (2020).
8. Morville, T., Sahl, R. E., Moritz, T., Helge, J. W. & Clemmensen, C. Plasma metabolome profiling of resistance exercise and endurance exercise in humans. *Cell Rep.* **33**, 108554 (2020).

9. Lewis, G. D. et al. Metabolic signatures of exercise in human plasma. *Sci. Transl. Med.* **2**, 33ra37 (2010).
10. Roberts, L. D. et al. β-Aminoisobutyric acid induces browning of white fat and hepatic β-oxidation and is inversely correlated with cardiometabolic risk factors. *Cell Metab.* **19**, 96–108 (2014).
11. Stanford, K. I. et al. 12,13-diHOME: an exercise-induced lipokine that increases skeletal muscle fatty acid uptake. *Cell Metab.* **27**, 1111–1120.e3 (2018).
12. Reddy, A. et al. pH-gated succinate secretion regulates muscle remodeling in response to exercise. *Cell* **183**, 62–75.e17 (2020).
13. Yuan, Y. et al. Exercise-induced α-ketoglutaric acid stimulates muscle hypertrophy and fat loss through OXGR1-dependent adrenal activation. *EMBO J.* **39**, e103304 (2020).
14. Klein, A. B. et al. Pharmacological but not physiological GDF15 suppresses feeding and the motivation to exercise. *Nat. Commun.* **12**, 1041 (2021).
15. Green, H. J. & Fraser, I. G. Differential effects of exercise intensity on serum uric acid concentration. *Med. Sci. Sports Exerc.* **20**, 55–59 (1988).
16. Schranner, D., Kastenmüller, G., Schönfelder, M., Römisch-Margl, W. & Wackerhage, H. Metabolite concentration changes in humans after a bout of exercise: a systematic review of exercise metabolomics studies. *Sport. Med. Open* **6**, 11 (2020).
17. Gaffney, B. & Cunningham, E. P. Estimation of genetic trend in racing performance of thoroughbred horses. *Nature* **332**, 722–724 (1988).
18. Hagenfeldt, L. & Naglo, A. S. New conjugated urinary metabolites in intermediate type maple syrup urine disease. *Clin. Chim. Acta* **169**, 77–83 (1987).
19. Bottesini, C., Tedeschi, T., Dossena, A. & Sforza, S. Enzymatic production and degradation of cheese-derived non-proteolytic aminoacyl derivatives. *Amino Acids* **46**, 441–447 (2014).
20. Sgarbi, E. et al. Microbial origin of non proteolytic aminoacyl derivatives in long ripened cheeses. *Food Microbiol.* **35**, 116–120 (2013).
21. Jansen, R. S. et al. *N*-lactoyl-amino acids are ubiquitous metabolites that originate from CNDP2-mediated reverse proteolysis of lactate and amino acids. *Proc. Natl Acad. Sci. USA* **112**, 6601–6606 (2015).
22. Sharma, R. et al. Circulating markers of NADH-reductive stress correlate with mitochondrial disease severity. *J. Clin. Invest.* **131**, e136055 (2021).
23. The Tabula Muris Consortium. Single-cell transcriptomics of 20 mouse organs creates a *Tabula Muris*. *Nature* **562**, 367–372 (2018).
24. Locke, A. E. et al. Genetic studies of body mass index yield new insights for obesity biology. *Nature* **518**, 197–206 (2015).
25. Ringholm, S. et al. PGC-1α is required for exercise- and exercise training-induced UCP1 up-regulation in mouse white adipose tissue. *PLoS ONE* **8**, e64123 (2013).
26. Kim, Y. J., Kim, H. J., Lee, W. J. & Seong, J. K. A comparison of the metabolic effects of treadmill and wheel running exercise in mouse model. *Lab. Anim. Res.* **36**, 1–8 (2020).

Methods

Cell line cultures

All cell lines were obtained from the American Type Culture Collection (ATCC) and grown at 37 °C with 5% CO_2. RAW264.7 and HEK293T cells were grown in DMEM with 10% FBS and penicillin–streptomycin (pen–strep). RT4 cells were grown in McCoy's 5a medium with 10% FBS and pen–strep. TKPTS cells were grown in DMEM:F12 medium with 7% FBS, 0.006 mg ml^{-1} insulin and pen–strep. All cell lines were used directly from ATCC vials and were not specifically authenticated for this study. Cell lines were negative following testing for mycoplasma contamination.

General animal information

Animal experiments were performed according to a procedure approved by the Stanford University Administrative Panel on Laboratory Animal Care. Studies using ABCC5-KO mice were approved by the Institute Animal Care and use Committee of the Netherlands Cancer Institute. Mice were maintained in 12-h light–dark cycles at 22 °C and about 50% relative humidity and fed a standard irradiated rodent chow diet. Where indicated, a high-fat diet (D12492, Research Diets 60% kcal from fat) was used. Male C57BL/6J (stock number 000664) and male C57BL/6J DIO mice (stock number 380050) were purchased from the Jackson Laboratory. Male C57BL/6NCrl mice (stock number 027) were purchased from the Charles River Laboratory. Whole-body CNDP2-KO mice (catalogue number C57BL/6NCrl-Cndp2em1(IMPC) Mbp/Mmucd, RRID: MMRRC_043492-UCD) were obtained from the Mutant Mouse Regional Resource Center, a NCRR-NIH funded strain repository. ABCC5-KO mice were obtained from the De Wolf group[27]. For in vivo injection of mice with compounds, including Lac-Phe, lactate and phenylalanine, compounds were dissolved in 18:1:1 (by volume) of saline:Kolliphor EL (Sigma Aldrich):DMSO. Compounds were administered to mice every day by i.p. injections at 5 µl g^{-1} body weight at the indicated doses. For all injection experiments, mice were mock injected with the vehicle for 3–5 days until body weights were stabilized. For the glucose tolerance tests, mice were fasted for 6 h then injected with glucose at 10 µl g^{-1} body weight. A dose of 1 g kg^{-1} was used in the glucose tolerance tests for obese mice treated with vehicle or Lac-Phe. Sample sizes were determined on the basis of previous experiments using similar methodologies. For the metabolite administration experiments, mice were randomly assigned to treatment groups. Experimenters were not blinded to groups.

Racehorse information and plasma collection

All racehorse plasma samples were obtained from anonymized diagnostic submissions and were therefore not classified as research samples. Racehorse blood (unmatched) was taken before and immediately after a race. The before-race samples were taken while the horses were resting in their stables. The after-race samples were taken immediately after a race lasting approximately 1–2 min at speeds of 9–11 m s^{-1} (>95% maximum VO_2). Plasma samples were collected by individual venipuncture into lavender-top tubes containing EDTA. Blood samples were then centrifuged at 4 °C to isolate the plasma then stored at −80 °C. Samples were shipped frozen to Stanford University where subsequent liquid chromatography and mass spectrometry (LC–MS) analyses took place.

Chemicals

L-Phenylalanine (AAA-1323814) was purchased from Fisher Scientific, sodium L-lactate (L7022) was purchased from Sigma. The synthesis of non-commercially available Lac-Phe is described below.

Synthesis of Lac-Phe

Sodium L-lactate (1.2 eq.) was dissolved in dichloromethane (0.2 M) and treated with 3-[bis(dimethylamino)methyliumyl]−3*H*-benzotriazol-1-oxide hexafluorophosphate (HBTU, 1.2 eq.) at 0 °C under argon. After 15 min, phenylalanine methyl ester hydrochloride (1.0 eq.) and *N*,*N*-diisopropylethylamine (3.0 eq.) in dichloromethane (0.2 M) was added to the mixture. The reaction was stirred for 16 h under argon at ambient temperature. One-third of the solvent was removed and the dichloromethane solution washed with 5% HCl, 5% $NaHCO_3$ and saturated NaCl solutions. The organic layer was dried ($MgSO_4$), filtered and concentrated. The resulting crude product was purified by column chromatography, eluting with ethyl acetate/hexane to afford the *N*-lactoyl phenylalanine methyl ester. The above ester (1.0 eq.) was dissolved in tetrahydrofuran (THF, 0.5 M) and treated with lithium hydroxide monohydrate (2.0 eq.) in water (0.5 M). The solution was stirred at ambient temperature for 2 h, and the solvent removed. The resulting residue was dissolved in dichloromethane and acidified by 5% HCl to pH 3. The resulting mixture was extracted with ethyl acetate three times, and the combined organic layers were washed with saturated NaCl solution. The organic layer was dried ($MgSO_4$), filtered, and concentrated. The resulting crude product was purified to recrystallization with ethyl acetate/hexane to give the *N*-lactoyl phenylalanine as a white powder. ^{1}H-NMR (400 MHz, D_2O): δ = 7.3–7.2 (m, 5H), 4.13 [dd, 1H], 4.10 [q, 1H], 3.21 [dd, 1H], 2.98 [dd, 1H], 1.10 [d, 3H]. LC–MS (*m/z*): 236.093 $[M-H]^-$.

Mouse running protocols

A six-lane Columbus Instruments animal treadmill (product 1055-SRM-D65) was used. Before treadmill running, mice were acclimated to the treadmill for 5 min. For acute exercise to exhaustion studies, treadmill running began at a speed of 7.5 m min^{-1} and a 4° incline. Every 3 min, the speed and incline were increased by 2.5 m min^{-1} and 2°, respectively. Once a speed of 40 m min^{-1} and incline of 30° was reached, both parameters were kept constant until mice reached exhaustion. Exhaustion was defined as when the mice remained on the shocker at the back of the treadmill for longer than 5 s. For chronic running experiments with WT and CNDP2-KO mice, mice were exercised 5 days per week, Monday to Friday, while on a high-fat diet (60% kcal from fat). Mice were allowed to rest over the weekend. Treadmill running was performed at a constant 5° incline and began at a speed of 6 m min^{-1}. Speed was increased by 2 m min^{-1} every 5 min until a maximum speed of 30 m min^{-1}. Mice were stopped after reaching exhaustion as described above, and run times were normalized between the two groups of mice. Running was performed in the mid-afternoon for all experiments (between 13:00 and 16:00).

Preparation of plasma samples for LC–MS analysis

Plasma was collected from mice through a submandibular bleed into lithium heparin tubes (BD, 365985) and immediately transferred onto ice. The blood was centrifuged at 4 °C at 5,000 r.p.m. for 5 min, and the top layer of plasma was aliquoted and frozen at −80 °C. To extract polar metabolites from plasma for LC–MS analysis, 150 µl of a 2:1 mixture of acetonitrile:methanol was added to 50 µl of plasma. The mixture was centrifuged at 4 °C for 10 min at 15,000 r.p.m. and the supernatant was transferred to a LC–MS vial.

Untargeted measurements of metabolites by LC–MS

Untargeted metabolomics measurements were performed using an Agilent 6520 Quadrupole time-of-flight LC–MS instrument. MS analysis was performed using electrospray ionization (ESI) in negative mode. The dual ESI source parameters were set as follows: the gas temperature was set at 250 °C with a drying gas flow of 12 l min^{-1} and the nebulizer pressure at 20 psi; the capillary voltage was set to 3,500 V; and the fragmentor voltage set to 100 V. Separation of polar metabolites was conducted using a Luna 5 µm NH2 100 Å LC column (Phenomenex 00B-4378-E0) with normal phase chromatography. Mobile phases were as follows: buffer A, 95:5 water:acetonitrile with 0.2% ammonium hydroxide and 10 mM ammonium acetate; buffer B, acetonitrile. The LC gradient started at 100% B with a flow rate of 0.2 ml min^{-1} from

0 to 2 min. The gradient was then linearly increased to 50% A/50% B at a flow rate of 0.7 ml min^{-1} from 2 to 20 min. From 20 to 25 min, the gradient was maintained at 50% A/50% B at a flow rate of 0.7 ml min^{-1}. Differential peak identification was performed with XCMS[28].

Targeted metabolomics

Targeted measurements was performed using an Agilent 6470 triple quadrupole LC–MS instrument. MS analysis was performed using ESI in negative mode. The AJS ESI source parameters were set as follows: the gas temperature was set at 250 °C with a gas flow of 12 l min^{-1} and the nebulizer pressure at 25 psi; the sheath gas temperature was set to 300 °C with the sheath gas flow set at 12 l min^{-1}; and the capillary voltage was set to 3,500 V. Separation of polar metabolites was performed as described above in the 'Untargeted measurements of metabolites by LC–MS' section. Multiple reaction monitoring was performed for the indicated metabolites with the listed dwell times, fragmentor voltage, collision energies, cell accelerator voltages and polarities. For targeted measurements of exercise-inducible metabolites, we selected previously reported metabolites[10–12,16,29,30]. The MS ionization parameters for the targeted metabolomics are presented in Supplementary Table 1. Quantification of the endogenous metabolite concentrations were performed by generating a standard curve with known concentrations of each metabolite. Metabolite standards were analysed alongside the plasma samples using the same targeted triple quadrupole method. A standard curve generated from the metabolite concentrations and total ion intensities were used to calculate the endogenous concentrations of each metabolite. The dilution of the plasma during the sample preparation was also taken into account for the quantification.

Generation of CNDP2-KO cells

The pLentiCRISPRv2 system[32] developed by the Zhang group was used to generate all CNDP2-KO cells. For the mouse cell lines, the single guide RNA (sgRNA) used was 5′-CAGTGAAATGAGATCCGTCA-3′. For human cell lines, the sgRNA used was 5′-ACAGAAACTCGCAAAATGGG-3′. As per the protocols described by the Zhang group, oligonucleotides for the sgRNA and reverse complement sequences were synthesized and cloned into the plentiCRISPRv2 vector. For the mouse cell lines, the following oligonucleotides were used: forward, 5′-CACCGCAGTGAAATGAGATCCGTCA-3′; reverse, 5′-AAACTGACGGATCTCATTTCACTGC-3′. For the human cell lines, the following oligonucleotides were used: forward, 5′-CACCGAC AGAAACTCGCAAAATGGG-3′; reverse, 5′-AAACCCCATTTTGCGA GTTTCTGTC-3′. Lentivirus particles were generated in the HEK293T cell line using Polyfect for the co-transfection of the cloned plentiCRISPRv2 plasmid with the viral packing psPAX2 plasmid and the viral envelope pMD2.G plasmid. A parental plentiCRISPRv2 plasmid was used as a control. Lentiviral supernatants were collected after 24 h and filtered through a 0.45-μM filter. The supernatant was then mixed in a 1:1 ratio with polybrene to a final concentration of 8 μg ml^{-1} polybrene. This mixture was added to cells at 40–50% confluence in 6-well plates. Transduced cells were transferred to a 10-cm plate followed by selection by puromycin for 3–6 days.

In vitro Lac-Phe production assay

Cells were plated in 12-well plates at 70–80% confluence. The next day, cells were washed two times with PBS and incubated in 0.5 ml serum-free medium. After overnight incubation, 400 μl of medium was removed and 20 μl of 1 M hydrochloride added to acidify the medium and to protonate Lac-Phe. Ethyl acetate (400 μl) was added into each sample and vortexed for 30 s to extract Lac-Phe into the organic layer. A total of 300 μl from the top layer was transferred to a new Eppendorf tube and dried down under a stream of nitrogen. The residue was re-suspended in 100 μl of an 80:20 mixture of acetonitrile:water. Cells were kept on ice to collect the lysate. PBS (150 μl) was added into each well and the cells scraped into an Eppendorf tube. This step was repeated again to ensure all cells were collected. Cells were then centrifuged at 4 °C for 10 min at 2,000*g* and the supernatant removed to obtain the cell pellet. A volume of 100 μl of a 2:1:1 mixture of acetonitrile:methanol:water mixture was used to lyse the cells and precipitate large proteins. The mixture was centrifuged at 4 °C for 10 min at 15,000 r.p.m. and the supernatant was transferred to a LC–MS vial.

Western blot analysis

Cells were collected and lysed by sonication in RIPA buffer containing 1:100 HALT protease inhibitor. Cell lysates were centrifuged at 4 °C for 10 min at 13,000 r.p.m. to remove residual cell debris. Protein concentrations of the supernatant were normalized using a Pierce BCA protein assay kit and combined with 4× NuPAGE LDS sample buffer with 10 mM DTT. Samples were then boiled for 10 min at 95 °C. Prepared samples were run on a NuPAGE 4–12% Bis-Tris gel then transferred to nitrocellulose membranes. Blots were blocked for 30 min at room temperature in Odyssey blocking buffer. Primary antibodies (rabbit anti-CNDP2 and rabbit anti-β-tubulin) were added to the Odyssey blocking buffer at a ratio of 1:1,000. Blots were incubated in the indicated primary antibodies overnight while shaking at 4 °C. The following day, blots were washed three times with PBS-T, 10 min each time, before staining with the secondary antibody for 1 h at room temperature. The secondary antibody used was a goat anti-rabbit antibody diluted in blocking buffer to a ratio of 1:10,000. Following secondary antibody staining, the blot was washed three times with PBS-T before being imaged with an Odyssey CLx Imaging System.

Breeding and genotyping of CNDP2-KO mice

CNDP2-KO and WT animals were obtained from the International Mouse Phenotyping Consortium (IMPC) and generated by heterozygous breeding crosses. Genotyping was performed as follows: tail clippings were obtained from littermates and boiled for 30 min at 95 °C in 100 μl of 50 mM NaOH to extract genomic DNA. The solution was neutralized by adding 21 μl of 0.5 M Tris (pH 7.2). PCRs were performed by using primers for either the *CNDP2* WT allele (forward, 5′-CAGATGGCTCGGAGATACCAC-3′; reverse, 5′-TTCCCGCTCCACCAAGGTGAAG-3′) or *CNDP2* KO allele (forward, 5′-GCTCTGTAAGGGAAAGAGATGACCC-3′; reverse, 5′-AATA GGACATACCCAGTTCTGTGAGG-3′). Promega GoTaq master mix was used for the PCR reaction. Each 25 μl reaction consisted of 12.5 μl of the Promega master mix (M7122), 2.5 μl of a 10 μM mixture of forward and reverse primers, 2 μl of genomic DNA and 8 μl of ultrapure water. The thermocycling program on a Bio-Rad C1000 Touch Thermo Cycler began with an initial 30 s at 95 °C, followed by cycles of 30 s at 98 °C, 30 s at 58 °C and 45 s at 72 °C, followed by 5 min at 72 °C and finally held at 4 °C. PCRs for WT primers consisted of 30 cycles, whereas PCRs for KO primers consisted of 48 cycles. Samples were run on a 2% agarose gel with 0.2 mg ml^{-1} ethidium bromide. WT alleles are expected to yield a PCR product of 160 base pairs in size where KO alleles are expected to yield PCR products that are 440 base pairs in size.

Kaolin and water intake assays

Mice were individually housed and allowed ad libitum access to kaolin pellets (research diets K50001) for 5 days before the start of the experiment. Mice were also fed ad libitum with a high-fat diet and had ad libitum access to water. On the day of the experiment, kaolin and food pellets were replaced with fresh pellets. Mice were then injected with either vehicle or Lac-Phe and kaolin, given a high-fat diet, and water intake was measured after 24 h.

Leptin and acylated ghrelin measurements

Plasma from obese mice at time 0 or 30 min after injection with 50 mg kg^{-1} Lac-Phe was used to measure leptin and acylated ghrelin levels. A mouse leptin ELISA kit (Crystal Chem, 90030) and a mouse acylated ghrelin EIA kit (Cayman, 10006307) were used according to the manufacturer's instructions.

Human exercise study of a single bout of acute treadmill running

This study (cohort 1) was conducted by researchers at the Snyder Lab at Stanford University, and a detailed description of the experiment model and details of the participants are provided in ref. [7]. In brief, 36 healthy individuals were enrolled and consented to participate in the exercise study approved by the Stanford University Institutional Review Board (IRB 23602). Participants arrived at the test facility having fasted overnight (10–12 h) and underwent symptom-limited cardiopulmonary exercise (CPX) testing on ramp treadmills. Protocols were individualized to the fitness level of each participant, and participants were encouraged to exercise until full maximal exercise capacity, which lasted between 8 and 12 min. Blood was collected intravenously from the upper forearm before exercise and at 2 min, 15 min, 30 min and 1 h after exercise. The blood plasma was isolated and analysed using an untargeted metabolomics platform.

Research design and methods for human endurance, sprint and resistance training

Eight healthy males were recruited to the study (cohort 2). The participants were 26.5 ± 3.7 years old, with body–mass index values of 23.5 ± 2.1 kg m^2. They were non-smokers and not participating in regular physical activity (≤1 session per week) with a fitness level at 42.6 ± 4.2 ml min^{-1} kg^{-1}. Before participation in any experimental practice, the volunteers were given written and oral information regarding potential risk of participation. Informed content was obtained from all volunteers in accordance with the Declaration of Helsinki II. The study was approved by the Ethics Committee of the Capital Region of Denmark (journal number H-18051389). The participants arrived at the test facilities in the morning (8:00), after an overnight fast (10 h fast). Body composition was measured by dual-energy X-ray absorptiometry (Lunar DPX-IQ DEXA Scanner, Lunar). Maximal oxygen uptake (VO_2 peak) was measured with an incremental ramp test on a Monark Ergomedic 893E bicycle (Monark) to evaluate training status. The test comprised 5 min at 100 W and 5 min at 150 W, followed by a 25 W increase per min until exhaustion. Expired air was collected during the test, using an online gas analyser (CareFusion, MasterScreen-CPX). Before each trial, participants were asked to refrain from strenuous physical activity for a minimum of 48 h. The day before each trial, a standardized diet (60 E% carbohydrate, 25 E% fat, 15 E% protein) was given in weighted portions. Calculation of energy requirement was based on the World Health Organization formula[31] and daily physical activity level. On the morning of the experimental days, the participants arrived in the fasted state at 8:00 by car or public transportation to avoid redundant physical activity. A venflon catheter (BD VenflonTM Pro Safety) was inserted in an antecubital vein for blood sampling at rest, acutely after exercise (0 min) and during recovery from exercise (15, 60, 120 and 180 min). After centrifugation, plasma was pipetted into aliquots (200 µl) and stored at −80 °C until further analysis. All participants underwent the following three identical experimental trials, separated only by the exercise modalities performed: (1) an endurance exercise trial; (2) a sprint exercise trial; and (3) a resistance exercise trial. The trials were performed in a randomized order, and each trial was separated by at least 10 days. The endurance exercise trial consisted of 90 min of continuous cycling at 55% VO_2 peak. The load was established during preliminary testing, although VO_2 measurements were conducted and evaluated during the trial to ensure that the estimated load elicited 55% VO_2 peak and to account for a potential drift in VO_2 during exercise. In case of insufficient or excessive loading, adjustments were implemented. The sprint exercise trial consisted of a 5-min warm-up at 50 W, followed by three bouts of 30-s all-out sprint (Wingate tests) on an ergometer bike. Each Wingate test was interspersed by 4 min of active recovery on 5 W. The resistance exercise trial was based on bilateral knee extension exercise. The trial was initiated by a warm-up consisting of 3 sets of 10 repetitions with a load corresponding to 50% of the 10 repetition maximum load. Each of the warm-up sets were interspersed by 2 min of rest. Following the warm-up, 6 sets of 10 repetitions were performed at a load corresponding to 10 repetition maximum, with each set interspersed by 2 min of rest.

Statistics

All data are expressed as the mean ± s.e.m. unless otherwise specified. Student's two-sided *t*-test was used for pair-wise comparisons. Two-way analysis of variance with repeated measures in one factor was used for time course data of repeated measurements, with post hoc Sidak's multiple comparisons test. Statistical methods were not used to predetermine sample size. Unless otherwise specified, statistical significance was set at $P < 0.05$.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

All data generated or analysed during this study are included in this published article and its supplementary information files. Source data are provided with this paper.

27. De Wolf, C. J. F. et al. cGMP transport by vesicles from human and mouse erythrocytes. *FEBS J.* **274**, 439–450 (2007).
28. Smith, C. A. et al. XCMS: processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching, and identification. *Anal. Chem.* **78**, 779–787 (2006).
29. Agudelo, L. Z. et al. Skeletal muscle PGC-1α1 modulates kynurenine metabolism and mediates resilience to stress-induced depression. *Cell* **159**, 33–45 (2014).
30. Coxon, J. P. et al. GABA concentration in sensorimotor cortex following high-intensity exercise and relationship to lactate levels. *J. Physiol.* **596**, 691–702 (2018).
31. *Human Energy Requirements: Report of a Joint FAO/WHO/UNU Expert Consultation* (FAO, WHO, UNU, 2005).
32. Sanjana, N. E., Shalem, O. & Zhang, F. Improved vectors and genome-wide libraries for CRISPR screening. *Nat. Methods* **11**, 783–784 (2014).

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Author contributions V.L.L. performed the exercise studies, gain and loss of function studies, in vitro studies and MS analyses. Y.H. performed the acute feeding experiments (with the help of H.L.). K.C. re-analysed the MS data from the previously published human exercise experiment. J.T.K., A.L.W., J.T.T. and A.S.-H.T. assisted in tissue collection and exercising mice for the chronic running experiments. X.L. assisted in preparing plasma samples for MS analyses. P.-J.H.Z. performed the metabolic chamber studies. R.S.J. performed studies using ABCC5-KO mice. B.M. assisted in MS analyses. K.Y.L. and A.C.Y. assisted in collecting brain tissue. C.S.C., C.T.V., B.K. and E.A.R. performed the human exercise studies. W.W. and S.M.T. assisted in the mouse running experiments. B.C.M. and R.M.A. assisted in the horse metabolomics studies. S.M.B. synthesized Lac-Phe. J.Z.L., Y.X., G.A.W., A.S., B.K., E.A.R., M.P.S. and K.v.d.W. supervised the work. V.L.L., J.Z.L., Y.H. and Y.X. conceived the experiments and wrote the manuscript with contribution from the other authors.

Competing interests Stanford University is in the process of applying for a patent application (US2022027261) covering lactoyl amino acids for the treatment of metabolic diseases that lists J.Z.L., V.L.L., S.M.B., Y.X. and Y.H. as inventors.

Additional information
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Correspondence and requests for materials should be addressed to Yong Xu or Jonathan Z. Long.
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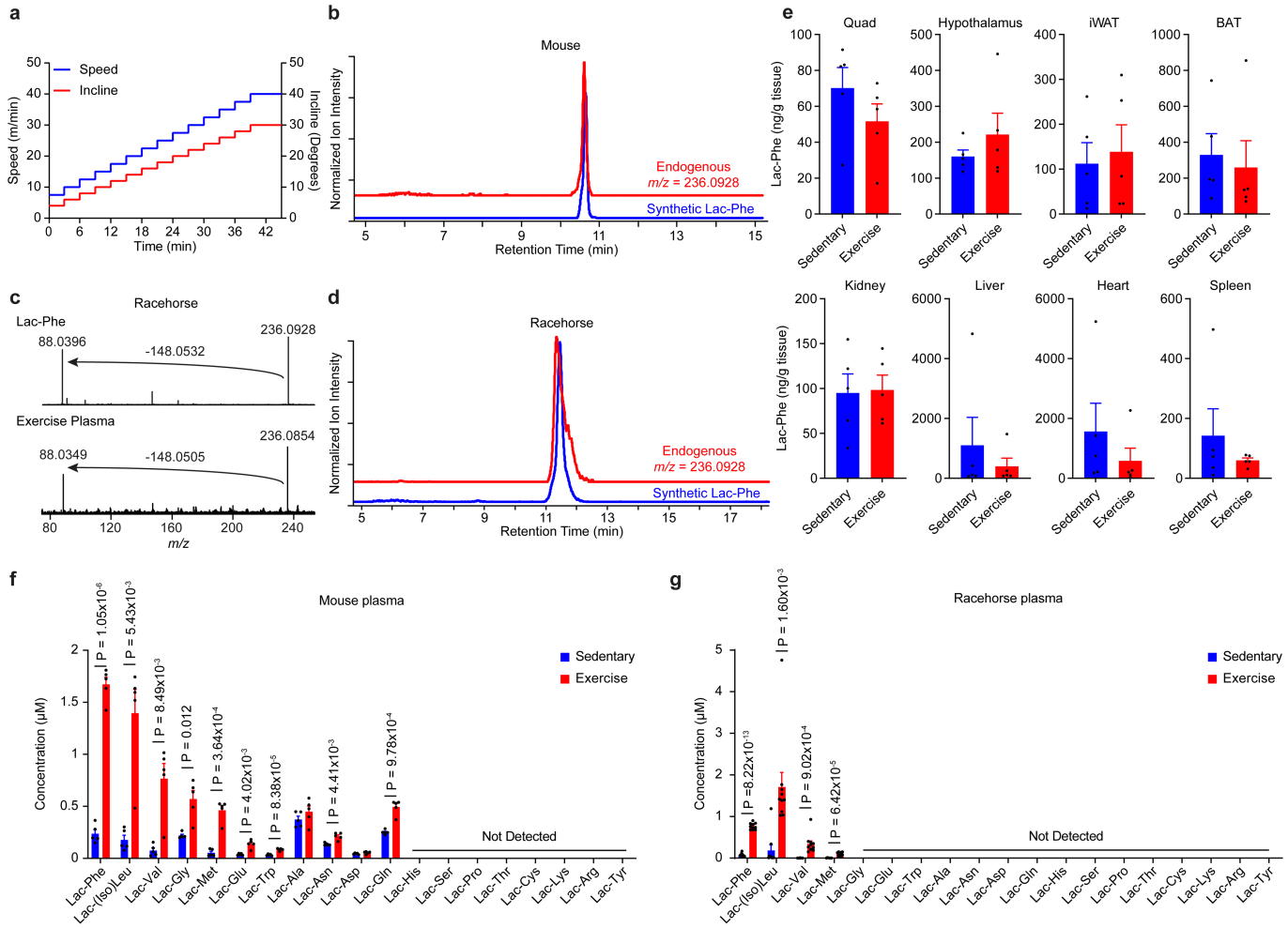
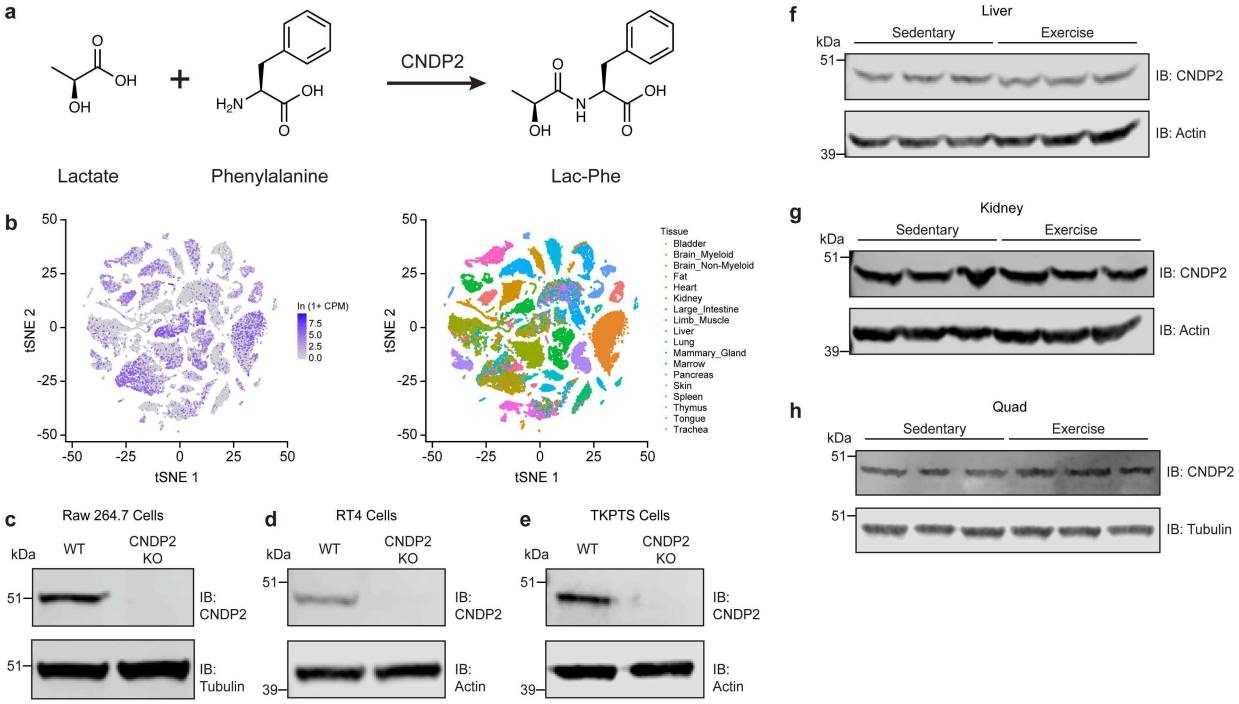
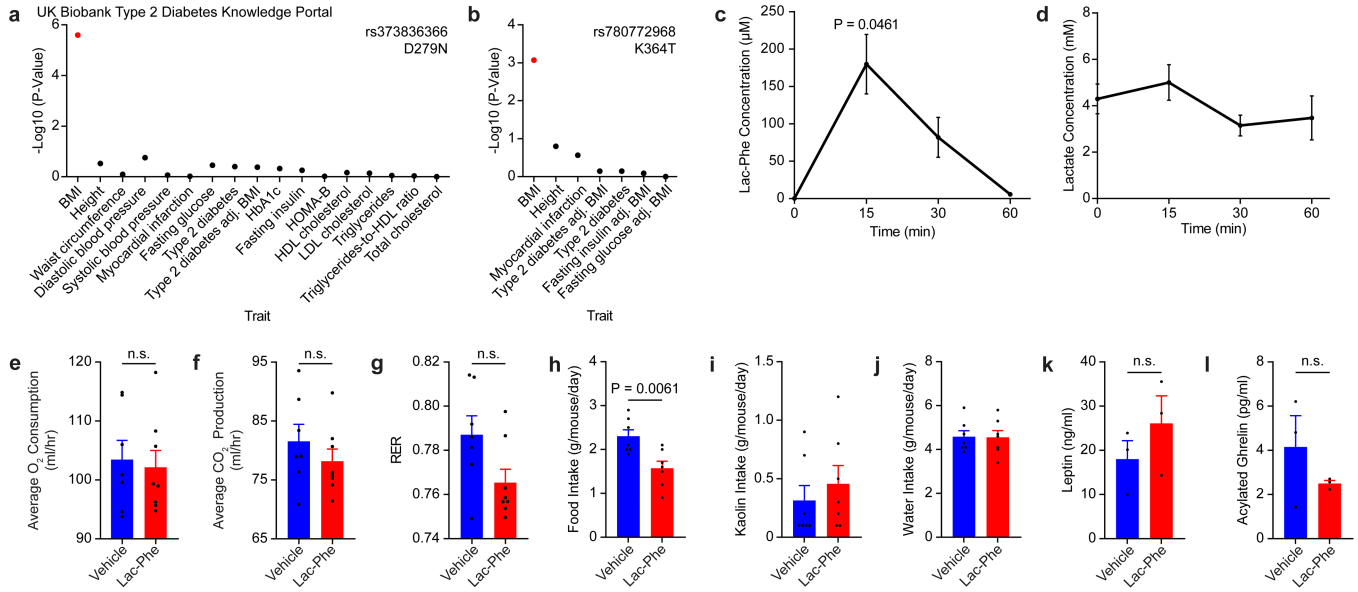


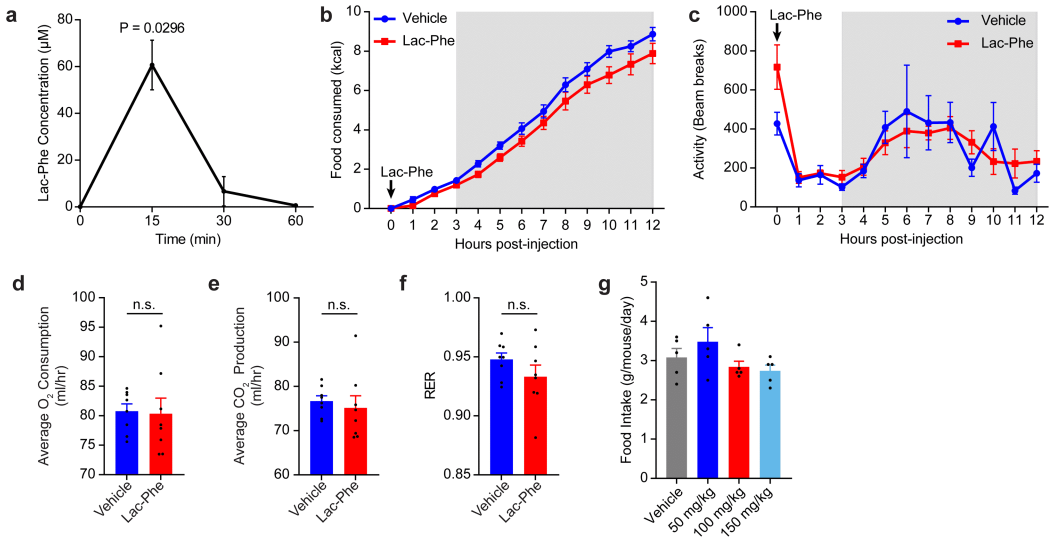
Extended Data Fig. 1 | Additional metabolomic characterization of Lac-Phe and lactoyl amino acid dynamics in mouse and thoroughbred racehorse exercise. (**a**) Schematic of speed and incline in the acute running protocol for mice. (**b**) Extracted ion chromatograms the endogenous m/z = 236.0928 peak in mouse plasma in comparison with a synthetic Lac-Phe standard. (**c, d**) Fragmentation spectra (**c**) extracted ion chromatogram (**d**) of the the endogenous m/z = 236.0928 peak in horse plasma in comparison with a synthetic Lac-Phe standard. (**e**) Quantitation of Lac-Phe levels in the indicated tissue from either sedentary mice (blue) or after a single bout of exhaustive running (red). (**f, g**) Quantitation of the indicated lactoyl amino acid level in blood plasma from mouse (**f**) or racehorse (**g**). For (**e**) and (**f**), N = 5/group; for (**g**), N = 10/group. Data are shown as mean ± SEM. All experiments were performed once. P-values were calculated by Student's two-sided t-test.



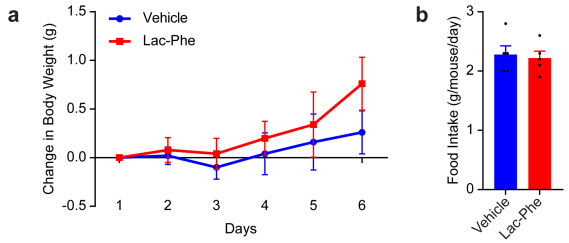
Extended Data Fig. 2 | Additional characterization and validation of CNDP2 protein in cells and in mice. (**a**) Schematic of the chemical reaction catalyzed by CNDP2 to produce Lac-Phe. (**b**) *Cndp2* mRNA expression across tissues from *Tabula Muris*. (**c-e**) Anti-CNDP2 (top) and anti-tubulin (bottom) Western blots in WT and CNDP2-KO RAW264.7 (**c**), RT4 (**d**), or TKPTS (**e**) cells. (**f-h**) Anti-CNDP2 (top) and anti-actin (bottom) Western blots of liver (**f**), kidney (**g**), or quadraceps (**h**) harvested from either sedentary mice (left three lanes) or mice after a single bout of exhaustive exercise (right three lanes). For gel source data, see Supplementary Fig. 1. For (**c**), experiments were performed twice. For (**d-h**), experiments were performed once.



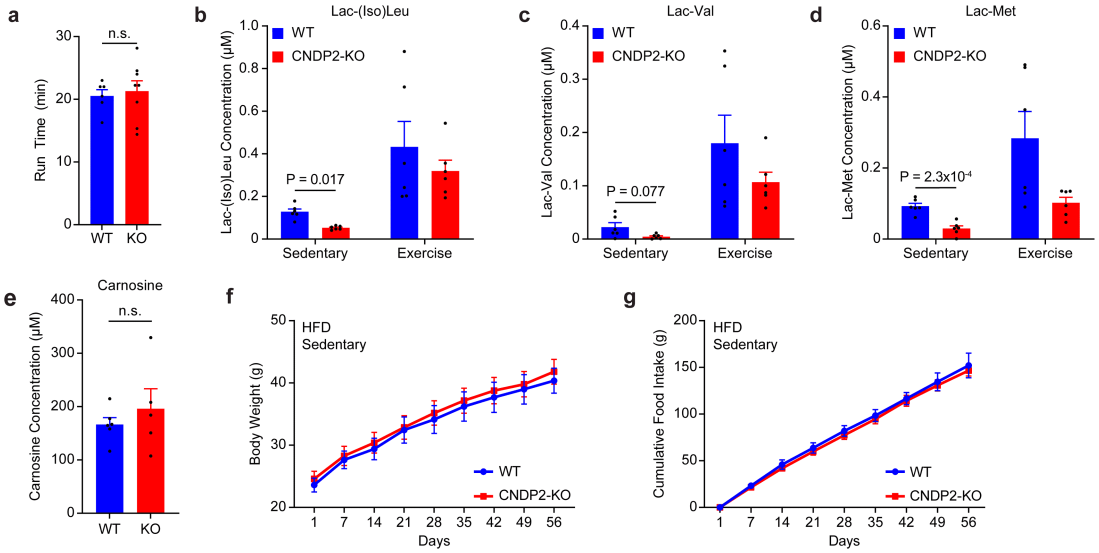
Extended Data Fig. 3 | Additional characterization of the effects of Lac-Phe administration to diet-induced obese mice. (**a, b**) Phenotype associations of the single nucleotide polymorphisms rs373836366 (**a**) and rs780772968 (**b**) from the Type 2 Diabetes Knowledge Portal. (**c, d**) Plasma Lac-Phe levels (**c**) and blood lactate levels (**d**) in plasma of mice following a single injection of Lac-Phe (50 mg/kg, IP). (**e-g**) 12 h oxygen consumption VO_2 (**e**), carbon dioxide production VCO_2 (**f**), and respiratory exchange ratio RER (**g**) of 22-week old DIO mice following a single injection of vehicle or Lac-Phe (50 mg/kg, IP). (**h-j**) Food intake (**h**), kaolin intake (**i**), and water intake (**j**) in 21-week old DIO mice following a single injection of vehicle or Lac-Phe (50 mg/kg, IP). (**k, l**) Plasma leptin (**k**) and acyl-ghrelin (**l**) levels in 17-week old DIO mice 30 min after a single injection of vehicle or Lac-Phe (50 mg/kg, IP). For (**c**), (**d**), (**k**), and (**l**), N = 3/group. For (**e-j**), N = 7/group. Data are shown as means ± SEM. All experiments were performed once. P-values were calculated by Student's two-sided t-test.



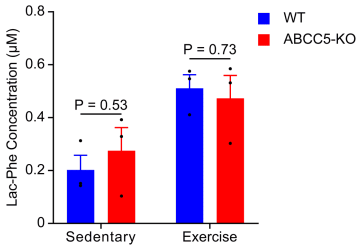
Extended Data Fig. 4 | Metabolic effects of Lac-Phe administration to chow-fed, lean mice. (**a**) Lac-Phe levels in plasma of male lean mice (22–27 g) following a single injection of Lac-Phe (50 mg/kg, IP). (**b-f**) 12 h food consumption (**b**), ambulatory activity (**c**), oxygen consumption VO_2 (**d**), carbon dioxide production VCO_2 (**e**), and respiratory exchange ratio RER (**f**) of chow fed lean mice following a single injection of Lac-Phe (50 mg/kg, IP). (**g**) 24 h food intake in lean mice after a single injection of Lac-Phe at the indicated dose. For (**a**), N = 3/group. For (**b-f**), N = 8/group. For (**g**), N = 5/group. Data are shown as means ± SEM. For (**a-f**), experiments were performed once. For (**g**), experiments were performed two times. P-values were calculated by Student's two-sided t-test.



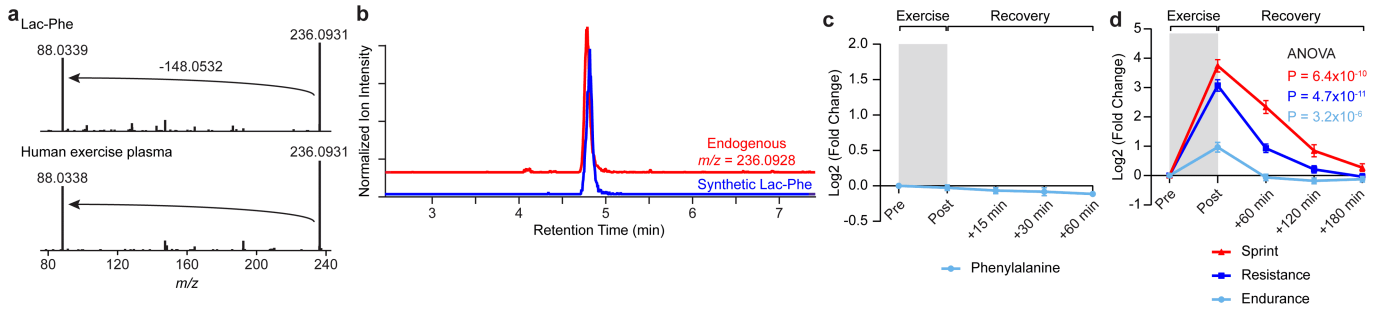
Extended Data Fig. 5 | Metabolic effects of oral Lac-Phe administration to diet-induced obese mice. (**a, b**) Change in body weight (**a**) and daily food intake (**b**) of 16-week diet-induced obese mice treated with Lac-Phe (50 mg/kg/day, PO). N = 5/group. Data are shown as means ± SEM. Experiments were performed once.



Extended Data Fig. 6 | Additional characterization of CNDP2-KO mice. (**a**) Treadmill time until exhaustion for WT and CNDP2-KO ("KO") mice. (**b-d**) Plasma levels of the indicated lactoyl amino acid in WT or CNDP2-KO mice in the sedentary state or after a single bout of acute exhaustive running ("exercise"). (**e**) Plasma levels of carnosine in sedentary WT or CNDP2-KO mice. (**f, g**) Body weight (**f**) and cumulative daily food intake (**g**) of WT (blue) or CNDP2-KO (red) mice under high fat diet, sedentary conditions. For (**a**), N = 6 for WT and N = 8 for CNDP2-KO; for (**b-d**), N = 6/group; for (**e**), N = 6 for WT and N = 5 for CNDP2-KO; for (**f,g**), N = 8 for WT and N = 11 for CNDP2-KO. Data are shown as means ± SEM. For (**a-g**), experiments were performed once. P-values for (**a-e**) were calculated by Student's two-sided t-test.



Extended Data Fig. 7 | Plasma Lac-Phe levels in WT and ABCC5-KO mice sedentary mice or after a single bout of treadmill running to exhaustion. N = 3/group. Data are shown as means ± SEM. Experiments were performed once. P-values were calculated by Student's two-sided t-test.



Extended Data Fig. 8 | Additional characterization of plasma Lac-Phe levels in humans. (**a, b**) Tandem MS fragmentation (**a**) and co-elution (**b**) of an authentic Lac-Phe standard and the endogenous *m/z* = 236.0928 mass from human plasma run on the Snyder laboratory untargeted metabolomics platform (see Methods). (**c**) Time course of phenylalanine levels in blood before and after a single acute bout of treadmill running from the human acute treadmill exercise study (Cohort 1, N = 36). (**d**) Time course of lactate levels before and after sprint (red), resistance (blue), and endurance (light blue) exercise from the human crossover acute exercise study (Cohort 2, N = 8). For (**c, d**), data are shown as mean ± SEM, **p < 0.01, ***p < 0.001. Experiments were performed once. P-values were calculated by two-way ANOVA with repeated measures.

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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	Untargeted metabolomics data were collected on Agilent QTOF 6520 using Agilent's LC/MS Data Acquision software (version B.08.00). Targeted metabolomics data were collected on Agilent QQQ 6470 using Agilent's LC/MS Data Acquision software (version B.08.02).
Data analysis	Both targeted and untargeted data were analyzed using Agilent Qualitative Analysis (version B.07.00). XCMS analysis was performed by XCMS online (https://xcmsonline.scripps.edu). Data was analyzed on Prism version 8.0 and RStudio.

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

All of the data supporting the findings of this paper can be found within the paper and in the supplementary files, including data from the untargeted metabolomics XCMS analysis. Publicly available datasets include Tabula Muris (https://tabula-muris.ds.czbiohub.org/).

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 tests were used to predetermine sample size. Sample sizes were chosen based on previously published work and literature in the field that have conducted similar experiments as well as the number of replicates sufficient to detect outlier samples. For in vitro experiments, sample sizes of 3-5 (biological replicates) were used. For in vivo experiments sample sizes of greater than 5 were used. Exact numbers were pre-determined based on the experimental design, number of animals available, and the animal housing conditions.
Data exclusions	Mice were excluded from in vivo experiments based on body weight changes that were greater than three standard deviations from the mean.
Replication	The number of replicates is indicated in each of the figure legends.
Randomization	For animal experiments where the same genotype of mice was used (acute exercise metabolomics experiments, injection experiments), mice were randomly assigned in the study groups. In experiments involving WT and KO animals, mice were assigned to each group based on genotype. Mice were age and sex matched for all experiments.
Blinding	For all experiments, the investigators were not blinded because all measurement modalities were quantitative. All samples and experiments were performed in the same way regardless of group or treatment.

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
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	CNDP2 Antibody (1:1000, 14925-I-AP, Protein Tech). Beta-Tubulin Antibody (1:1000, ab6046, Abcam). Goat anti-rabbit secondary (1:10,000, A21429, Life Technology).
Validation	All antibodies listed are commercially available and used for western blotting. Information on antibody validation are on the manufacturer's websites. Links for each antibody are listed below. 1. CNDP2 Antibody (https://www.ptglab.com/products/CNDP2-Antibody-14925-1-AP.htm#product-information) 2. Beta-Tubulin Antibody (https://www.abcam.com/beta-tubulin-antibody-loading-control-ab6046.html?productWallTab=ShowAll) 3. Goat anti-rabbit secondary Antibody (https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21429)

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	RAW 264.7 (TIB-71) from American Type Culture Collection (ATCC); RT4 (HTB-2) from American Type Culture Collection (ATCC); TKPTS (CRL-3361) from American Type Culture Collection (ATCC)

Authentication	All cell lines used were acquired from ATCC.
Mycoplasma contamination	These cell lines were negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Experiments were performed on the following mouse strains: 1. WT male mice from Jackson C57BL/6J (stock no. 000664) at 6-8 weeks old. 2. Male diet induced obese mice from Jackson C57BL/6J DIO mice (stock no. 380050) from 13-25 weeks old. 3. WT male mice from Charles River C57BL/6NCrl (stock no. 027) from 16-22 weeks old. 4. Male whole body CNDP2 knockout mice (catalog number, C57BL/6NCrl-Cndp2em1(IMPC)Mbp/Mmucd, RRID: MMRRC_043492-UCD) from Mutant Mouse Regional Resource Center. Mice were 16-22 weeks old. 5. Male whole body ABCC5 knockout mice from De Wolf et al., FEBS J (2007) were 6-20 weeks old.
Wild animals	Wild animals were not used in this study.
Field-collected samples	Field-collected samples were not used for this study.
Ethics oversight	Mice studies were approved by the Stanford University Administrative Panel on Laboratory Animal Care (APLAC) and the Institute Animal Care and use Committee of the Netherlands Cancer Institute. All horse plasma samples were obtained from anonymized diagnostic submissions and were therefore not classified as research samples.

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	Eight young healthy men were recruited to the study. The participants were 26.5±3.7 years, normal weight (BMI 23.5±2.1 kg/m2), non-smokers and not participating in regular physical activity (≤ 1 session/week) with a fitness level at 42.6±4.2 ml/min/kg.
Recruitment	Recruitment occurred at the University of Copenhagen. Prior to participation in any experimental practice, subjects were given written and oral information regarding potential risk of participation. Informed content was obtained from all subjects, in accordance with the Declaration of Helsinki II.
Ethics oversight	The study was approved by The Ethics Committee of the Capital Region of Denmark (Journal number: H-18051389).

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