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Dietary protein restriction elevates FGF21 levels and energy requirements to maintain body weight in lean men

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Dietary protein restriction increases energy expenditure and enhances insulin sensitivity in mice. However, the effects of a eucaloric protein-restricted diet in healthy humans remain unexplored. Here, we show in lean, healthy men that a protein-restricted diet meeting the minimum protein requirements for 5 weeks necessitates an increase in energy intake to uphold body weight, regardless of whether proteins are replaced with fats or carbohydrates. Upon reverting to the customary higher protein intake in the following 5 weeks, energy requirements return to baseline levels, thus preventing weight gain. We also show that fasting plasma FGF21 levels increase during protein restriction. Proteomic analysis of human white adipose tissue and in FGF21-knockout mice reveal alterations in key components of the electron transport chain within white adipose tissue mitochondria. Notably, in male mice, these changes appear to be dependent on FGF21. In conclusion, we demonstrate that maintaining body weight during dietary protein restriction in healthy, lean men requires a higher energy intake, partially driven by FGF21-mediated mitochondrial adaptations in adipose tissue.

Dietary manipulations for health and body weight control have traditionally focused more on the quantity and quality of fat and carbohydrates, whereas the role of varying protein intake has been less studied[1]. High protein (HP) intake has traditionally been considered superior for health and body mass regulation, mainly based on studies investigating caloric restriction[2,3] or during maintenance of prior weight loss[4,5] as well as for counteraction of muscle wasting in the elderly[6]. Also, it has been proposed in the protein leverage hypothesis that eating diets with low protein content could lead to higher daily energy intake because of the potential that strong regulation of protein intake may cause overconsumption of fats and carbohydrates (hence total energy) in diets with a low proportion of energy from protein, while an under-consumption could be expected from diets with a high proportion of protein[7]. However, a cross-sectional study of 6,381 middle-aged adults indicated that low habitual protein intake (less than ten energy per cent (E%) protein) compared with moderate (10–19 E% protein) or HP intake (≥20 E% protein) reduced overall mortality and resulted in a lower incidence of type 2 diabetes-related mortality[8]. Furthermore, one study in which 858 mice were given ad libitum access to one of 25 diets varying in the content of protein, carbohydrate and fat suggested that calorically sufficient diets restricted in protein improved insulin sensitivity and extended life span[9].

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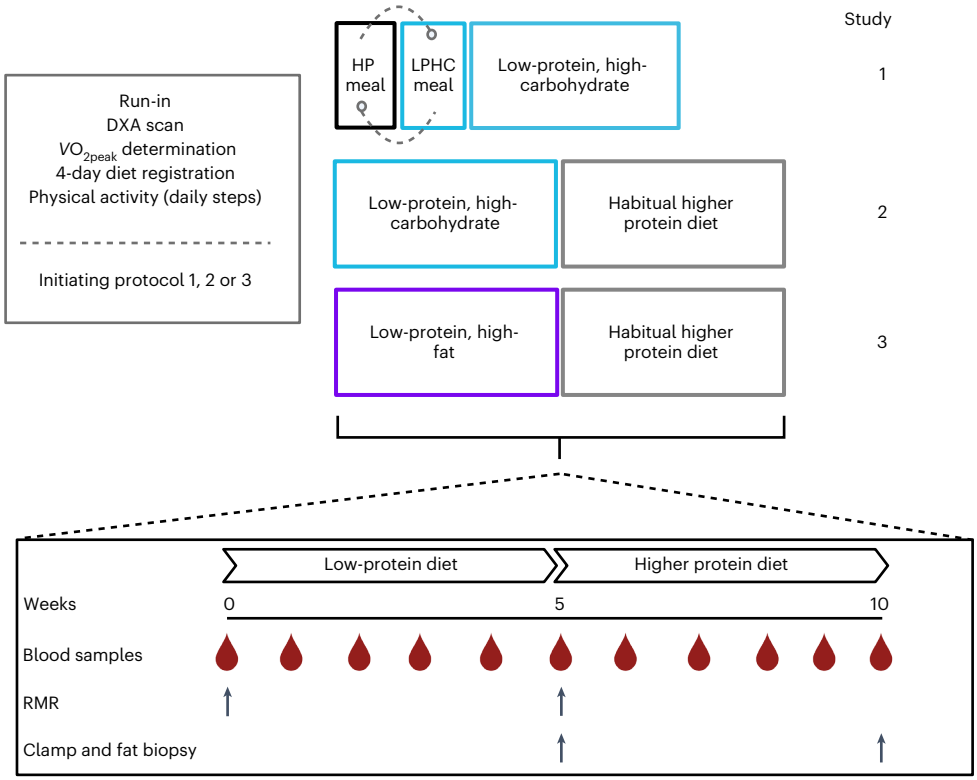

Fig. 1 | Study design. A three-arm study was performed. In study 1, healthy, lean men ingested either a LPHC meal or a habitual HP meal in a randomized order, separated by 72 h, followed by a 5-week LPHC diet. In studies 2 and 3, participants ingested either a LPHC diet or a LPHF diet for 5 weeks followed by a standard HPD for another 5 weeks. All diets were eucaloric. Resting metabolic rate (RMR) was measured before and after the protein-restricted interventions. A hyperinsulinemic-euglycemic clamp was performed after the protein-restricted and HPD interventions. Basal subcutaneous abdominal fat biopsy was obtained after the protein-restricted and HPD interventions. Graphical illustration created in BioRender.com.

Although HP diets have been proposed as superior for mitigating reductions in energy expenditure and lean body mass during caloric restriction[2,3], several lines of evidence from rodent studies during conditions of eucaloric or ad libitum intake of energy suggest that a prolonged intake of a protein-restricted diet enriched in carbohydrate increases energy expenditure[10–15]. Accordingly, in human volunteers with mild obesity, intake of protein-restricted diets (constituting 7–9% of energy) has been associated with decreased body weight[10,16]. Additionally, investigations in humans have suggested that consuming hypercaloric diets with low protein content (3–5% of energy) resulted in less weight gain than diets with higher protein content[17,18], although this also seems to include a component of changed body composition in terms of less lean body mass gain in response to the overfeeding.

These combined findings from studies in both mice and humans imply a connection between limiting protein intake and raising energy expenditure. It is worth noting that the protein levels in the restricted diets used in most of these human studies are below the recommended daily minimum required protein intake guidelines (>0.83 g kg^{-1} body weight per day) set forth by the Nordic Nutrition Recommendations and the World Health Organization (WHO).

Significant gaps in our understanding of the effects of prolonged protein-restricted diets on energy expenditure in healthy, lean men within the context of weight maintenance warrant further investigation. Furthermore, the potential effects of substituting protein with carbohydrates or fats in the protein-restricted diet have not yet undergone systematic examination, and the potential underlying molecular and metabolic mechanisms of protein restriction in humans have not been elucidated. These knowledge gaps hold paramount importance in terms of their applicability to the broader population.

Emerging findings from studies in mice have highlighted the activation of fibroblast growth factor 21 (FGF21) in response to dietary protein restriction, supported by research in previous publications[11–14,19,20]. FGF21 appears to have a pivotal role as a metabolic orchestrator in adapting to protein limitations; however, the underlying molecular mechanisms remain incompletely understood. Although the precise mechanisms connecting protein restriction, plasma FGF21 concentrations and human energy expenditure remain elusive, intriguingly, a correlation has been established between heightened energy expenditure, raised fasting plasma FGF21 levels and the ability of individuals to counteract weight gain in response to acute hypercaloric protein-restricted diets[21].

In the present study, we engaged healthy male volunteers in an investigation aimed at comprehending the impact of protein restriction on energy demands, metabolic indicators and glucose homeostasis. The study design encompassed an acute low-protein meal and a prolonged (10 week) dietary intervention. The initial 5 weeks involved adherence to a protein-restricted diet that fulfilled recommended protein requirements within a eucaloric framework that maintained body weight. Subsequently, the following 5 weeks saw a return to the participants' habitual higher protein consumption. Notably, the deficit in protein intake was substituted with either dietary carbohydrates or fats.

Our underlying hypothesis posited that regardless of whether carbohydrates or fats replaced the curtailed protein intake, an elevation in energy intake would be imperative during the protein-restricted phase to uphold body weight. This was expected to coincide with escalated plasma FGF21 levels. Additionally, to gain further mechanistic insight and to investigate whether FGF21 has a direct role in mediating the increased need for energy to maintain body weight during protein restriction, our investigation encompassed a proteomic analysis of white adipose tissue from the male participants as well as wild-type (WT) and FGF21-knockout (KO) mice consuming a similar low-protein

Table 1 | Characteristics of the healthy young men in studies 1, 2 and 3, ingesting either a LPHC diet or LPHF diet followed by a HPD

	Study 1, LPHC (n=8)		Study 2, LPHC (n=8)			Study 3, LPHF (n=6)		
	Week 0	LPHC week 5	Week 0	LPHC week 5	HPD week 5	Week 0	LPHF week 5	HPD week 5
Age (years)	27±2	–	26±2	–	–	25±1	–	–
Body mass (kg)	85.4±8.8	84.5±7.8	85.3±11.9	84.1±11.9[a]	84.2±11.7[a]	77.9±8.6	77.5±8.9	78.4±8.9
Height (m)	1.82±0.05	–	1.84±0.07	–	–	1.85±0.06	–	–
BMI (kg m^{-2})	25.6±2.2	25.2±1.8	25.1±2.7	24.8±2.7[a]	24.8±2.7[a]	22.7±1.8	22.5±1.9	22.8±1.8
Body fat (%)	23.5±5.1	23.3±4.8	22.0±4.0	21.6±3.7	21.2±4.1	19.2±5.1	19.6±4.8	19.3±5.2
Fat mass (kg)	20.1±4.7	19.9±4.5	19.5±5.6	18.8±5.3	18.6±5.6	15.0±3.4	15.0±3.4	15.1±3.2
Visceral fat (g)	544±307	547±265	473±345	503±323	400±312	295±161	354±132	300±189
LBM (kg)	61.8±8.3	61.3±7.7	64.1±6.1	63.7±6.2	64.0±6.2	59.6±9.2	59.3±9.7	59.9±9.9
VO_{2peak} (ml min^{-1} kg^{-1})	43.9±3.2	43.4±3.4	43.8±5.3	42.2±4.3	43.0±5.5	40.8±7.7	38.8±8.1	42.2±6.8
TBW (l)				56.0±3.0	55.9±3.1		49.4±6.8	49.7±7.5

Values are mean±s.d. Paired t-test (study 1) and repeated measures one-way ANOVA (studies 2 and 3) with a Bonferroni post hoc test were applied within each study. [a]Different from pre-intervention (week 0) within each intervention. One VO_{2peak} value is missing the LPHF diet (week 5) (n=5). In LPHC study 2, DXA scan and VO_{2peak} measurement are missing in one subject after HPD; body composition and VO_{2peak} data were therefore excluded. TBW measurement is missing for three subjects in LPHC study 2 (n=5). BMI, body mass index; LBM, lean body mass; VO_{2peak}, peak oxygen uptake; TBW, total body water.

diet (LPD) as the male participants. This exploration aimed to shed light on plausible mechanisms that might underpin the energy-expending effects associated with protein restriction.

Results

Protein-restricted meal increases FGF21 and metabolic rate

Initially (study 1, Fig. 1), we investigated the acute intake of a low-protein, high-carbohydrate (LPHC; 8 E% protein) meal and a habitual higher protein (16 E% protein) meal of 4.8 ± 02 MJ in a cross-over design (Extended Data Table 1). This study included eight lean, healthy men (Table 1). An increase in postprandial plasma FGF21 levels by 63% within 90 min was obtained after the LPHC meal, an effect that was not observed following the HP meal (Fig. 2a,b). Plasma FGF21 levels remained 57% elevated 4 h following the LPHC meal (Fig. 2b). The plasma glucose level following the LPHC meal was similar to the HP meal, and the meal-induced insulin response was also largely similar except for a significantly lower insulin concentration after the LPHC meal at 45 min (Fig. 2c,d), despite an intake of 71 E% carbohydrate in the LPHC compared with 57 E% in the HP meal (201 ± 7 g vs 161 ± 5 g carbohydrate, respectively). Indirect calorimetry revealed an increased whole-body metabolic rate 3 h following the LPHC meal compared with the HP meal (Fig. 2e). Postprandial respiratory exchange ratio (RER) was increased compared with the fasting state in both meal tests, indicating a higher relative glucose oxidation after both meals (Fig. 2f).

Protein restriction raises energy needs and FGF21

All participants subsequently followed the LPHC diet for 5 weeks. Their body weight, and hence daily energy provision, remained unchanged compared with baseline values during the first 7–10 days (Fig. 2g). Notably, to prevent weight loss, it was necessary to increase energy intake consecutively in the following weeks of the LPHC intervention, and at the end of week 5, energy provision was increased by 19% (2.4 ± 0.8 MJ) in the LPHC diet (Fig. 2g). This increase in energy intake was accompanied by a 270% elevation in fasting plasma FGF21 levels at week 5 compared with pre-intervention (Fig. 2h). The increased energy intake was not attributed to alterations in physical activity level, as this remained unchanged during the intervention (Fig. 2i). The continuous increase in energy intake was not enough to prevent a small but significant weight loss (−1.0 ± 0.9 kg) over time (Fig. 2j), whereas fat and lean body mass did not change significantly (Table 1).

We next investigated whether a return to the participants' habitual higher protein diet (HPD) following a LPD necessitates a reduction in energy intake (study 2, Fig. 1).

Accordingly, in study 2, a new cohort of healthy young male volunteers (Table 1) consumed a LPHC diet for 5 weeks (Table 2). Notably, the participants maintained their daily energy provision at baseline values during the first 7–10 days of the LPHC diet, like our findings in study 1 (Fig. 3a,b). Thereafter, energy intake had to be gradually increased to maintain body weight, resulting in a 20% higher energy intake at the end of week 5 compared with pre-intervention (Fig. 3b,d), in line with the 19% increase in study 1 (Fig. 2g). These findings were obtained concomitantly with the maintenance of physical activity level during the intervention (Fig. 3f).

We then asked whether replacement of the protein with fat rather than carbohydrates in a LPD also led to an increased energy intake. To this end, we performed a new study including healthy male volunteers (study 3, Fig. 1). When the reduced dietary protein content was replaced by fat (low-protein (9 E%), high-fat (50 E%) (LPHF) diet) instead of carbohydrates (Table 3), a similar increase of 21% in energy provision during the intervention was required to maintain body weight (Fig. 3c,e). Again, the physical activity level remained constant during the intervention (Fig. 3g). Thus, the increase in energy provision did not differ regardless of whether protein was replaced by carbohydrates or fat (Fig. 3b,c).

Interestingly, when the participants returned to their habitual higher protein intake (HPD intervention), a downregulation of the daily energy provision was necessary for both the LPHC and LPHF interventions to keep body weight stable (Fig. 3d,e) and energy intake was gradually reduced to the pre-intervention levels (Fig. 3b,c).

Plasma FGF21 varies with protein intake

In studies 2 and 3, fasting plasma FGF21 levels increased by 361% and 208% from pre-intervention to week 5 with the LPHC and LPHF diet, respectively (Fig. 3h,i). The increase in plasma FGF21 level appeared in parallel with the increased energy provision during both the LPHC and LPHF diet (Fig. 3b,c,h,i). When switching back to the HPD, fasting plasma FGF21 levels rapidly returned to pre-intervention levels (Fig. 3h,i and Extended Data Fig. 1).

Physical activity level remained constant during the HPD and was similar to the physical activity level during LPHC and LPHF diets (Fig. 3f,g). Repeated measures correlation analysis between the change in energy intake and the change in circulating FGF21 levels during

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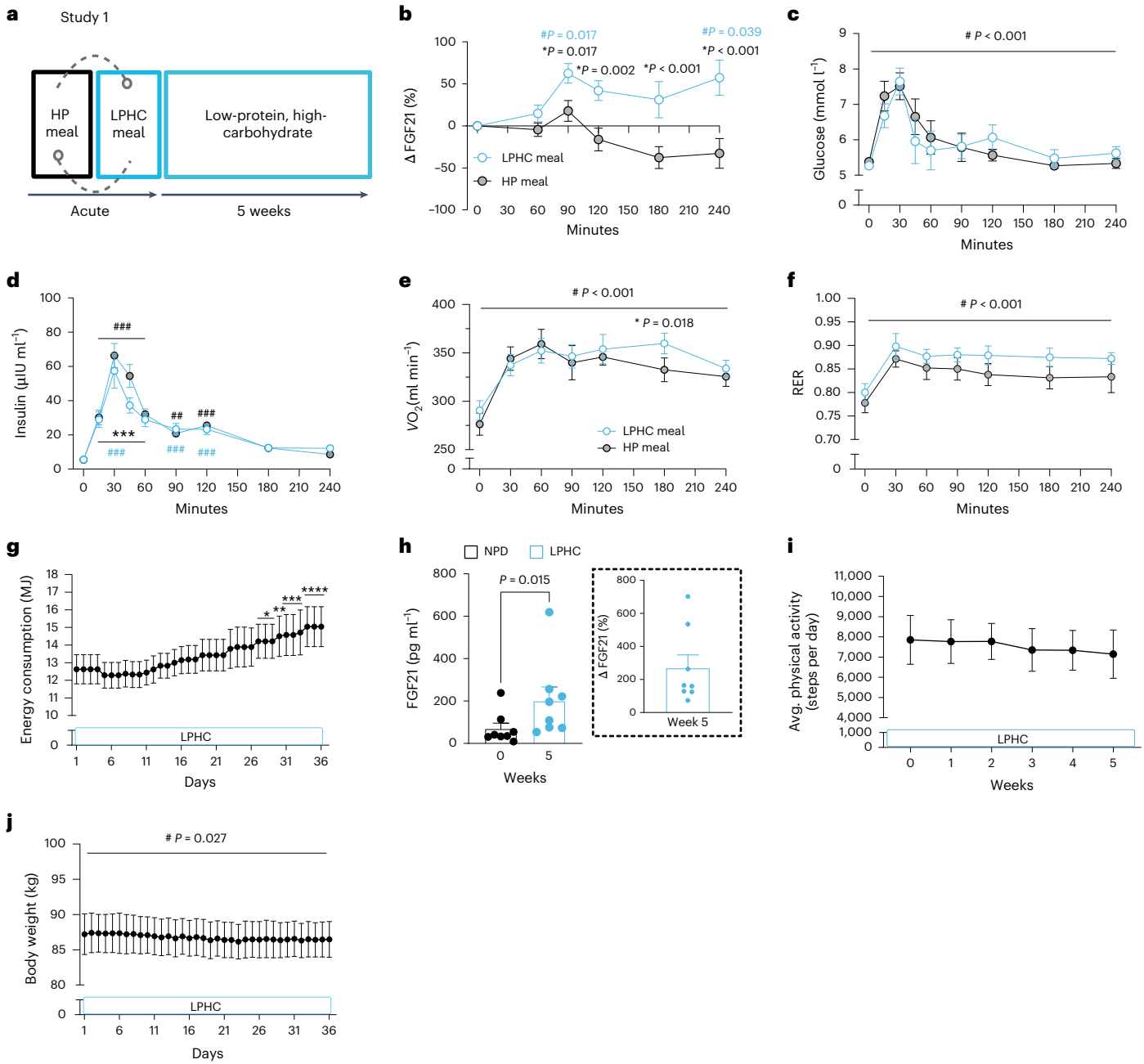

Fig. 2 | Effects of an acute low-protein meal and prolonged protein-restricted diet on circulating FGF21 levels and energy requirements for weight maintenance. a, Illustration of study 1, in which healthy, lean men ingested a LPHC meal or a habitual HP meal in randomized order followed by a eucaloric LPHC diet for 5 weeks. **b**, Fasting and postprandial plasma FGF21 level, **c**, glucose and **d**, insulin levels. **e**, Whole-body oxygen uptake. **f**, RER during the LPHC and HP meal. **g**, Daily energy provision during the LPHC intervention. **h**, Fasting plasma FGF21 levels before and after the LPHC intervention. **i**, Daily number of steps and **j**, daily measurement of body weight during the LPHC intervention. *P* values in **b**–**f** determined by repeated measures two-way ANOVA/mixed-effects model with Bonferroni multiple comparisons test; in **g**, **i** and **j**, repeated measures one-way ANOVA/mixed-effects model with a Bonferroni post hoc test; in **h**, two-tailed paired *t*-test. Asterisk (*) indicates difference between diets in **d** and **g** (different from day 0): $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; # indicates effect of time (or main effect of time in **c**, **e**, **f** and **j**) in **d**: $\#\#P < 0.01$, $\#\#\#P < 0.001$. All data are presented as mean ± s.e.m. Meal test data in **b**, **c**, **e**, **f**, $n = 9$; in **d**, $n = 8$ owing to insulin analysis issues for one participant. LPHC diet intervention data in **g**–**j**, $n = 8$, as one participant only completed the meal test.

the 5 week dietary intervention periods demonstrated a statistically significant association ($r = 0.70$; $P < 0.01$; Fig. 3j).

Other hormones with a potential impact on energy metabolism, such as plasma noradrenaline, triiodothyronine and glucagon, were unchanged in the fasting state during the LPHC and LPHF interventions (Extended Data Fig. 2a–f) as was resting metabolic rate (RMR) in the overnight-fasted state estimated from indirect calorimetry measurements expressed both in absolute terms and relative to lean body mass (Extended Data Fig. 3). This suggests that increased energy expenditure during the LPDs takes place mainly during meals (Fig. 2e). Fasting RER was unchanged after both the LPHC and LPHF interventions compared with the baseline levels (Extended Data Fig. 2k,l), indicating no changes in substrate oxidation. Despite the reduction in dietary protein, the fasting plasma total amino acid levels remained unchanged during both the LPHC and LPHF interventions (Extended Data Fig. 2g,h). During both the LPHC and LPHF interventions, fasting plasma urea levels

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Table 2 | Macronutrients in the LPHC diet and the habitual HPD in study 2 from healthy, lean men

	LPHC		HPD	
	Week 0	Week 5	Week 0	Week 5
Energy consumption (MJ)	13.5±0.6	16.1±0.9[a,d]	16.1±0.8[a,d]	14.3±0.7[b,c]
Protein (E%)	9.3±0.0	9.3±0.0	18.3±0.0	18.3±0.0
Protein (g kg^{-1} body weight)	0.89±0.00	1.06±0.02[a,c,d]	2.06±0.04[a,b,d]	1.84±0.07[a,b,c]
Histidine (mg)	2170±96	2584±126[a,c,d]	4401±214[a,b,d]	3895±191[a,b,c]
Isoleucine (mg)	3646±162	4340±211[a,c,d]	7097±345[a,b,d]	6281±308[a,b,c]
Leucine (mg)	2320±103	2762±134[c,d]	11874±577[a,b,d]	10509±516[a,b,c]
Lysine (mg)	846±38	1007±49[c,d]	10256±498[a,b,d]	9078±446[a,b,c]
Methionine (mg)	2388±106	2842±138[a,c,d]	3451±168[a,b,d]	3054±150[a,b,c]
Threonine (mg)	1755±78	2089±102[c,d]	5700±277[a,b,d]	5045±248[a,b,c]
Tryptophan (mg)	760±34	904±44[a,c,d]	1820±88[a,b,d]	1611±79[a,b,c]
Tyrosine (mg)	1343±60	1599±78[c,d]	5301±258[a,b,d]	4692±230[a,b,c]
Valine (mg)	2783±123	3313±161[c,d]	8652±420[a,b,d]	7658±376[a,b,c]
Carbohydrate (E%)	70.0±0.0	70.0±0.0	48.7±0.0	48.7±0.0
Glucose (g)	44±2	52±3[a,c,d]	21±1[a,b]	18±1[a,b]
Fructose (g)	54.2±2.4	64.5±3.1[a,c,d]	23.1±1.1[a,b]	20.5±1.0[a,b]
Dietary fibre (g)	53±2	63±3[a,c,d]	50±2[b,d]	44±2[a,b,c]
Fat (E%)	21.0±0.0	21.0±0.0	33.0±0.0	33.0±0.0
Saturated fatty acids (E%)	5.2±0.0	5.2±0.0	12.4±0.0	12.4±0.0
Monounsaturated fatty acids (E%)	7.0±0.0	7.0±0.0	10.1±0.0	10.1±0.0
Polyunsaturated fatty acids (E%)	3.3±0.0	3.3±0.0	4.3±0.0	4.3±0.0

Data are mean±s.e.m. Repeated measures one-way ANOVA with a Bonferroni post hoc test was applied to test for differences in macronutrients between the first (week 0) and the last (week 5) day on the LPHC diet and the habitual HPD. [a]Different from LPHC at week 0. [b]Different from LPHC at week 5. [c]different from HPD at week 0. [d]Different from HPD at week 5; n=8.

were lower than during the HPD intervention (Extended Data Fig. 2i,j). Notably, lean body mass was not compromised during the 5 weeks of protein restriction, and fat mass also remained unchanged with the protein-restricted diet (Table 1). This could suggest that the protein intake during the LPD interventions is sufficient and the excess protein from the HPD might have been excreted as urea. It is noteworthy that the preservation of lean body mass occurred when energy intake was adequate to maintain body weight.

Protein-restricted diet and whole-body insulin sensitivity

Pre-intervention fasting plasma glucose level averaged 5.3 ± 0.1 mmol l^{-1} and 5.2 ± 0.1 mmol l^{-1} in the LPHC and LPHF groups (studies 2 and 3), respectively and remained unchanged during both interventions (Fig. 4a,b). Plasma insulin levels remained unchanged at an average of 6.4 ± 0.6 µIU ml^{-1} and 3.8 ± 0.3 µIU ml^{-1} in the LPHC and LPHF treatments, respectively (Fig. 4c,d), resulting in an unchanged HOMA-IR index during the interventions (Extended Data Fig. 4a,b). When whole-body insulin sensitivity was assessed at end-intervention by the hyperinsulinemic-euglycemic clamp, a 16% increase in glucose infusion rate for the last 60 min of the clamp was observed after the LPHC intervention compared with after the HPD (Fig. 4e). Despite the high fat intake during LPHF, the glucose infusion rate remained unchanged after the LPHF intervention (−1.5%) compared to after the HPD intervention (Fig. 4f). During the clamp, plasma FGF21 levels started higher and remained elevated by 389–210% during insulin stimulation after the LPHC and LPHF diet, respectively compared to the HPD (Extended Data Fig. 4c,d).

The hepatic glucose production in the fasting state was similar after both protein-restricted interventions, in line with unchanged plasma glucose concentrations (Fig. 4g,h). Moreover, insulin-mediated suppression of hepatic glucose production remained unchanged (Fig. 4g,h). Metabolic flexibility, assessed as a change in RER from basal to insulin stimulation (Fig. 4i,j), remained unchanged after both interventions. During the clamp, plasma glucose and insulin concentrations were similar after the low protein and control interventions (Extended Data Fig. 4e–h).

Prolonged protein restriction and adipose tissue proteome

Human adipose tissue proteomic analysis was performed by liquid chromatography–mass spectrometry to investigate whether the increase in energy requirement to maintain body weight, owing to reduced protein intake, was reflected in alterations in the subcutaneous adipose tissue proteome (studies 2 and 3). This analysis revealed that out of 4,438 (LPHC) and 4,436 (LPHF) detected proteins, 318 (LPHC) and 149 (LPHF) proteins were upregulated, and among these, 21 similar proteins were found in both interventions (Fig. 5a). Of the 318 (LPHC) and 120 (LPHF) downregulated proteins, 17 decreased in both interventions (Fig. 5a). The volcano plots illustrate the overall upregulated and downregulated proteins after the LPHC and LPHF diets (Fig. 5b,c). Of note, we did not detect uncoupling protein 1 (UCP1) mRNA by qPCR or UCP1 protein by proteomic analysis in human white subcutaneous adipose tissue.

Among the upregulated proteins were the large protein complexes in the electron transport chain: complex I (NDUFA4 (fold change (FC), 1.5), NDUFS6 (FC, 1.4), NDUFS7 (FC, 2.5), NDUFV3 (FC, 1.6) and NDUFB9 (FC, 1.4)), the electron carrier COQ3 (FC, 1.5) and CYB5A (FC, 1.7); complex III (UQCRH (FC, 1.5)) and complex IV (COX7B (FC, 6.3), COX17 (FC, 2.1) and COX6B1 (FC, 1.2)) (Fig. 5b,c), all involved in pumping protons from the mitochondrial matrix space into the intermembrane space. Such upregulation of proteins in complex I–IV indicates an increase in the capacity for establishing a greater proton motive force (Δp), which would be expected to increase the need for proton re-entry through the ATP-synthase (complex V) coupling the release of Δp to ATP synthesis. However, among the downregulated proteins were proteins involved in ATP-synthase (MT-ATP6 (FC, 6.7), ATP5I (FC, 6.2), ATP5L (FC, 1.4)

and ATP5O (FC, 1.2)) and other proteins involved in the final step of oxidative phosphorylation (SLC25A4-ANT1 (FC, 2.4–2.8)) (Fig. 5b,c). These opposite changes could suggest inefficient coupling of respiration and Δp generation to ATP synthesis, and hence energy dissipating mitochondrial uncoupling. In addition, significant upregulation was obtained for ATP2A1 (FC, 2.5) (Fig. 5b), a SERCA isoform catalysing the hydrolysis of ATP coupled with the translocation of calcium from the cytosol to the sarcoplasmic reticulum[22], suggesting increased calcium cycling. These findings were most pronounced after consumption of the LPHC diet.

As a consequence of these findings, the question was raised whether FGF21 is involved in the changes obtained in the adipose tissue mitochondrial machinery. To evaluate such potential causality, we took advantage of FGF21-KO mice and compared them to WT mice on a protein-restricted diet. When WT mice were fed a protein-restricted diet (5 E%) for 10 weeks, either enriched in carbohydrate (85 E%) or fat (60 E%), their body weight remained unchanged (Extended Data Fig. 5a) despite higher energy intake during both interventions (Extended Data Fig. 5b) compared with the consumption of a standard HPD group (20 E% protein), as also observed in the humans. Proteomic analysis of inguinal white adipose tissue (iWAT) from WT mice obtained after 10 weeks of feeding revealed that out of 5,170 (LPHC) and 4,436 (LPHF) detected proteins, 108 (LPHC) and 117 (LPHF) proteins were upregulated, and of these, 12 proteins were increased in both interventions (Fig. 5e); 174 (LPHC) and 224 (LPHF) were downregulated. Among these, ten proteins were downregulated in both diets (Fig. 5e). Similar to the human data, proteins in the electron transport chain were upregulated: complex I (Ndufa4, Ndufb9, Nduf6f, Ndufs7, Ndufv3), Complex III (Uqcrh) and Complex IV (Cox7b) (Fig. 5f,g). We also observed downregulation of ATP-synthase complex V proteins (Atp6v0a1, Atp6v1a, Atp6v1e1 and ATP6v1g1), primarily after the LPHF intervention (Fig. 5g). When FGF21-KO mice were fed the similar protein-restricted diets as the WT mice, 5,170 and 5,172 proteins were detected after the LPHC and LPHF interventions, respectively, whereby 147 (LPHC) and 133 (LPHF) proteins were upregulated and 176 (LPHC) and 301 (LPHF) were downregulated (Fig. 5i). Among these proteins, 35 proteins were similarly upregulated after the LPHC and LPHF interventions and 46 proteins were similarly downregulated (Fig. 5i). Interestingly, the changes of proteins in the electron transport chain obtained in WT mice (and in humans) were blunted in FGF21-KO mice fed a protein-restricted diet (Fig. 5j,k). These data suggest an impact of FGF21 on the LPD-induced alterations in adipose tissue electron-transport-linked phosphorylation system (Fig. 5i).

Discussion

Overall, our findings in lean men indicate that a longer-term protein-restricted diet requires a higher energy intake to maintain body weight regardless of whether the protein is replaced by carbohydrates or fat, indicating an increase in energy turnover on a protein-restricted diet, still within minimum protein requirements. Proteomic analysis on human and mouse adipose tissue revealed that this effect appears to be at least partially mediated by adaptations in the adipose tissue electron transport system, potentially driven by FGF21, supported by FGF21-KO mouse studies. The protein-restricted diet also improved whole-body insulin sensitivity when proteins were replaced by carbohydrates and remained unchanged after the high-fat diet.

In the present study, we first show in healthy, lean male volunteers that a protein-restricted, carbohydrate-rich diet (that is, LPHC), meeting the minimum protein requirements, consumed for 5 weeks, required a successive increase in energy provision to maintain body weight. The increase in energy provision reached 20–21% (2.5 MJ day^{-1}) to maintain body weight. Notably, energy provision was not increased until 7–10 days after initiating the protein-reduced intervention, which may indicate that the increased energy provision was not caused by an initial underestimation of energy requirement. Emphasizing the important role of dietary protein intake on energy provision was further supported by our findings that when the participants returned to their habitual higher protein intake for the following 5 weeks (that is, HPD), energy provision was again successively reduced after 6–8 days to baseline levels to prevent weight gain.

Whether the simultaneous increase in dietary carbohydrates is required for the protein-restricted effects has been debated[23]. To test whether the effect of reducing protein intake is independent of the simultaneous increase in carbohydrate intake, we also included a diet in which reduced protein was replaced by dietary fat instead of carbohydrates (that is, LPHF). Interestingly, like our findings in the LPHC experiments, energy consumption had to be successively increased by 21% at the end of the LPHF intervention to maintain stable body weight. Again, we needed to reduce the energy provision to baseline levels to prevent weight gain when individuals returned to their standard higher protein intake. Thus, we show in lean men that regardless of whether the protein reduction in the protein-reduced diets was replaced by carbohydrates or fat, energy provision had to be increased similarly to maintain body weight. This is in line with some[21], but not all[24], previous findings on LPHC dietary interventions, probably related to much greater protein restriction markedly below the minimum requirements of protein (0.83 g protein per kg body weight per day) in those studies[24]. Notably, we also revealed an enhanced energy expenditure after a meal with restricted dietary protein compared with a meal of high dietary protein. Although previous human studies studying hypercaloric diets with low protein content (3–5% of energy) found less weight gain compared to diets with higher protein content partly owing to less muscle mass gain and hence shift in body composition[17,18], the present findings of unchanged fat and lean body mass and body water content with eucaloric protein restriction do not suggest re-partitioning of muscle to fat mass as being a contributing factor to the observed increased energy demand to maintain body weight. Collectively, these findings indicate that dietary protein restriction during eucaloric conditions, rather than the change in dietary content of fat or carbohydrates, impacted energy homeostasis in men, presumably by increased energy expenditure. Our data suggest that increased energy expenditure during the LPDs occurs mainly during meals, and possible additional increases in RMR in the fasted state may have been too low to be detected by indirect calorimetry.

Diets low in proteins have been shown to elevate hepatic and circulating levels of FGF21 in rodents[11,13,14,20] and humans[10,14,19,21,25]. Furthermore, FGF21 has been shown to be an important regulator of energy

Fig. 3 | Effect of dietary protein-restricted diet either rich in carbohydrates or fat on energy balance. a, Illustration of studies 2 and 3 in healthy, lean men ingesting a eucaloric LPHC or LPHF diet for 5 weeks followed by another 5 weeks on a eucaloric habitual HPD. **b**,**c**, Daily energy provision during the LPHC, LPHF and HPD interventions. **d**,**e**, Daily measurement of body weight and **f**,**g**, number of steps taken during the LPHC, LPHF and HPD interventions (in **g**, activity recording is missing in one participant because of an allergy to the band). **h**,**i**, Change in fasting plasma FGF21 levels during the LPHC, LPHF and HPD interventions. **j**, rmcorr plot illustrating the association between changes in energy consumption (%) and changes in circulating FGF21 (%). Each subject is presented as a colour and two points; the circles show the difference in energy intake (%) and FGF21 (%) between week 0 (baseline) and week 5 for LPHC and LPHF; the triangles show the difference in energy intake (%) and FGF21 (%) between week 5 and week 10 for HPDs. *P* values in **b**–**i** determined by repeated measures one-way ANOVA/mixed-effects model with a Bonferroni post hoc test; in **b**, **c** and **f**–**i**, statistics were only applied to the bar graphs to test for effects of diets. In **j**, a repeated measures correlation was applied; correlation coefficient (*r*) and *P* value are shown in the figure. All data are presented as mean ± s.e.m. LPHC, $n = 8$; LPHF, $n = 6$. In **g**, $n = 5$ because of technical issues with the accelerometer. In **j**, $n = 14$, all participants of studies 2 and 3. Graphical illustration created in BioRender.com.

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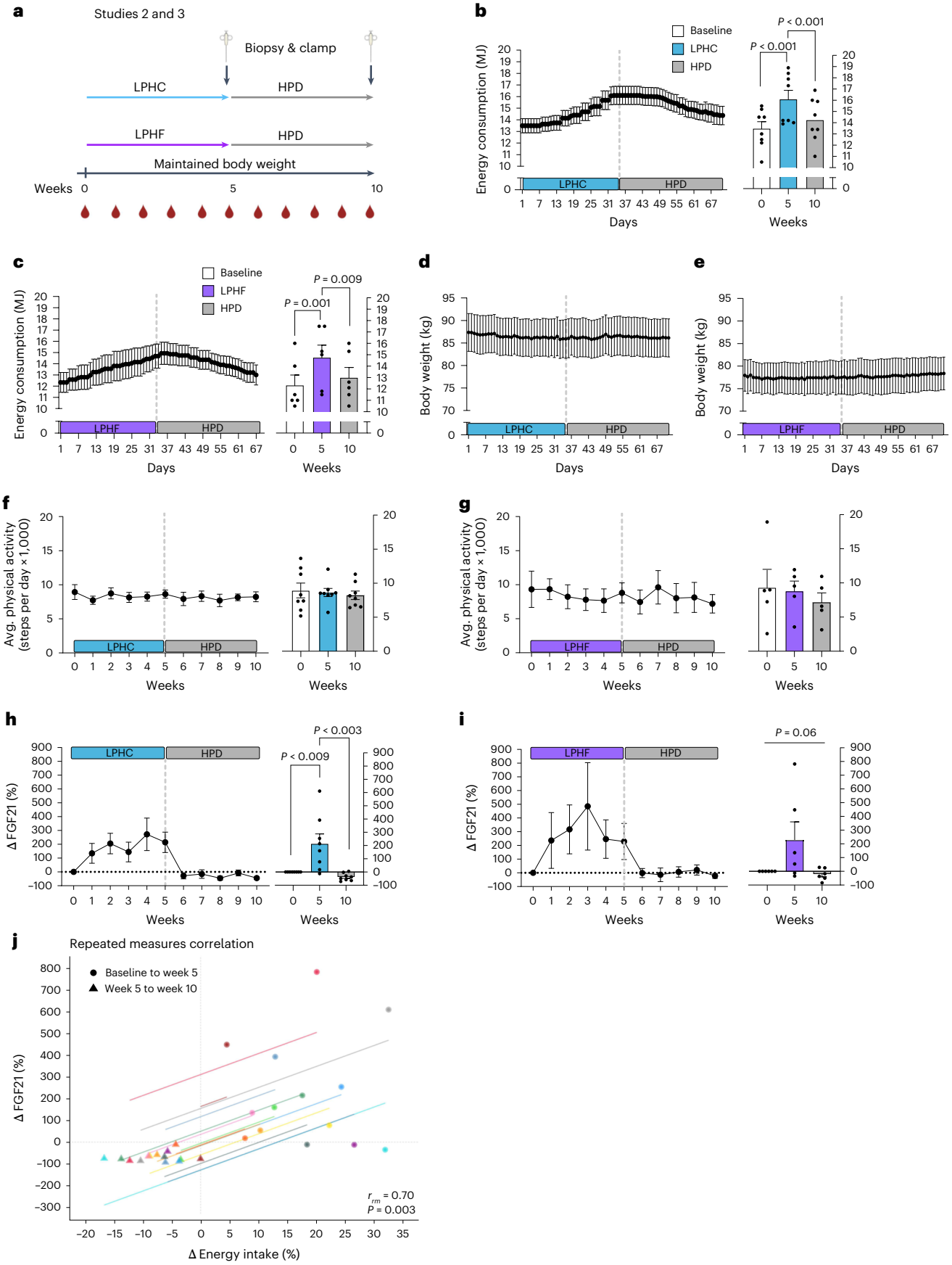

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Table 3 | Macronutrients in the LPHF diet and the habitual HPD in study 3 from healthy, lean men

	LPHF		HPD	
	Week 0	Week 5	Week 0	Week 5
Energy consumption (MJ)	12.3±0.9	14.8±1.1[a,d]	14.8±1.1[a,d]	13.0±0.9[b,c]
Protein (E%)	9.2±0.0	9.2±0.0	18.3±0.0	18.3±0.0
Protein (g kg^{-1} body weight)	0.85±0.03	1.02±0.04[a,c,d]	2.04±0.08[a,b,d]	1.78±0.05[a,b,c]
Histidine (mg)	1340±97	1605±117[c,d]	4037±294[a,b,d]	3554±243[a,b,c]
Isoleucine (mg)	2441±176	2922±213[c,d]	6509±474[a,b,d]	5730±392[a,b,c]
Leucine (mg)	4021±291	4815±351[c,d]	10890±794[a,b]	9588±656[a,b]
Lysine (mg)	2784±201	3333±243[c,d]	9407±686[a,b,d]	8281±567[a,b,c]
Methionine (mg)	961±69	1150±84[c,d]	3165±231[a,b,d]	2786±191[a,b,c]
Threonine (mg)	1824±132	2184±159[c,d]	5228±381[a,b,d]	4602±315[a,b,c]
Tryptophan (mg)	691±50	828±60[c,d]	1669±122[a,b,d]	1470±101[a,b,c]
Tyrosine (mg)	1632±118	1954±142[c,d]	4862±354[a,b,d]	4281±293[a,b,c]
Valine (mg)	2976±215	3563±260[c,d]	7936±578[a,b,d]	6986±478[a,b,c]
Carbohydrate (E%)	41.4±0.0	41.4±0.0	48.7±0.0	48.7±0.0
Glucose (g)	16±1	19±1[a,d]	19±1[a,d]	17±1[b,c]
Fructose (g)	19.7±1.4	23.6±1.7[a,c,d]	21.2±1.5[b,d]	18.7±1.3[b,c]
Dietary fibre (g)	36±3	43±3[a,c,d]	46±3[a,d]	40±3[b,c]
Fat (E%)	50.0±0.0	50.0±0.0	33.0±0.0	33.0±0.0
Saturated fatty acids (E%)	19.7±0.0	19.7±0.0	12.4±0.0	12.4±0.0
Monounsaturated fatty acids (E%)	17.4±0.0	17.4±0.0	10.1±0.0	10.1±0.0
Polyunsaturated fatty acids (E%)	6.5±0.0	6.5±0.0	4.3±0.0	4.3±0.0

Data are mean±s.e.m. Repeated measures one-way ANOVA with a Bonferroni post hoc test was applied to test for differences in macronutrients between the first (week 0) and the last (week 5) day on the LPHF diet and the habitual HPD. [a]Different from LPHC at week 0. [b]Different from LPHC at week 5. [c]Different from HPD at week 0. [d]Different from HPD at week 5. n=6.

expenditure in mice because, unlike WT mice, whole-body FGF21-KO mice[11–13] or liver-specific FGF21-KO mice[14] failed to increase energy expenditure on a protein-restricted, carbohydrate-rich diet. In the present study, we showed that fasting plasma FGF21 levels increased by an average of 280% after the LPHC and the LPHF interventions, demonstrating the importance of low dietary intake of proteins and amino acids for the endogenous increase in plasma FGF21 levels in humans. Notably, this was independent of which macronutrient was substituted for protein. Our findings that plasma FGF21 levels returned to baseline levels immediately after the participants returned to their habitual higher protein ingestion underpin the importance of protein restriction in inducing FGF21. Furthermore, we demonstrate a significant correlation between the increase in plasma FGF21 and the increase in energy intake needed to maintain body weight, which is in line with the current concept derived from rodent studies that increased circulating FGF21 is obligatory for the effect of protein restriction to increase energy expenditure[11–14,19]. Furthermore, a study involving obese rhesus macaque monkeys treated with human FGF21 analogue for 12 weeks showed 18% weight reduction without a reduction in food intake[26], suggesting that the effect of FGF21 on energy expenditure extends to other mammals in addition to rodents.

In mice, the effect of a protein-restricted diet on body weight regulation appears related to enhanced energy expenditure, which has been suggested to be mediated almost exclusively by elevated adipose thermogenesis[20], although the exact mechanisms remain to be established. These findings are supported by findings in adipose-specific FGF receptor 1 (FGFR1)-KO mice, who failed to increase energy expenditure when treated with FGF21 (ref. 27). Additionally, the FGF21 co-receptor βKlotho specifically in the brain appears particularly important for increasing energy expenditure in response to protein restriction[28], while FGF21 has also been suggested to mediate effects on energy expenditure through adipose tissue independent effects as well[29]. Increased expression of UCP1 has been discussed as an important mediator of elevated thermogenesis[12]. UCP1 has a central role in non-shivering thermogenesis and is specific for brown and beige adipose tissue. The role of UCP1 in white adipose tissue is unclear. Some studies in mice have suggested that at least a part of the body weight-lowering effect with protein restriction is mediated via UCP1 (ref. 12), whereas other studies in mice suggest that increased FGF21 levels either pharmacologically or in response to a protein-restricted diet can regulate energy expenditure independently of UCP1 (refs. 14,30,31). In the present study, we did not detect UCP1 mRNA by qPCR or UCP1 protein in human white subcutaneous adipose tissue, consistent with a previous finding failing to detect UCP1 mRNA in this tissue (average cycle threshold values from qPCR analysis of 38 indicative of no or very little expression of UCP1)[32]. These findings suggest that UCP1-independent mechanisms are responsible for the increased energy turnover observed after protein restriction in humans. Although it cannot be excluded that other tissues, such as skeletal muscle, could also contribute to this phenomenon, it seems less likely, as skeletal muscle does not—or only to a neglectable extent—express the receptor and co-receptor, FGFR1 or βKlotho, important for FGF21 tissue effects[33–36]. Indeed, the proteomic analysis of the human white adipose tissue provided interesting clues. Thus, among the upregulated proteins (NDUFS7, COX7B, COX17, CYB5A) were proteins belonging to complexes I–IV in the electron transport chain. An upregulation of these proteins might increase the transport of protons across the inner mitochondrial membrane, resulting in an amplified proton gradient across this membrane. Our data also reveal that among the downregulated proteins were proteins in ATP-synthase complex V (ATP5I, ATP5L, ATP5O, MT-ATP6, SLC25A4/ANT1), which oversee ADP import into the mitochondria matrix for ATP synthesis as well as ATP

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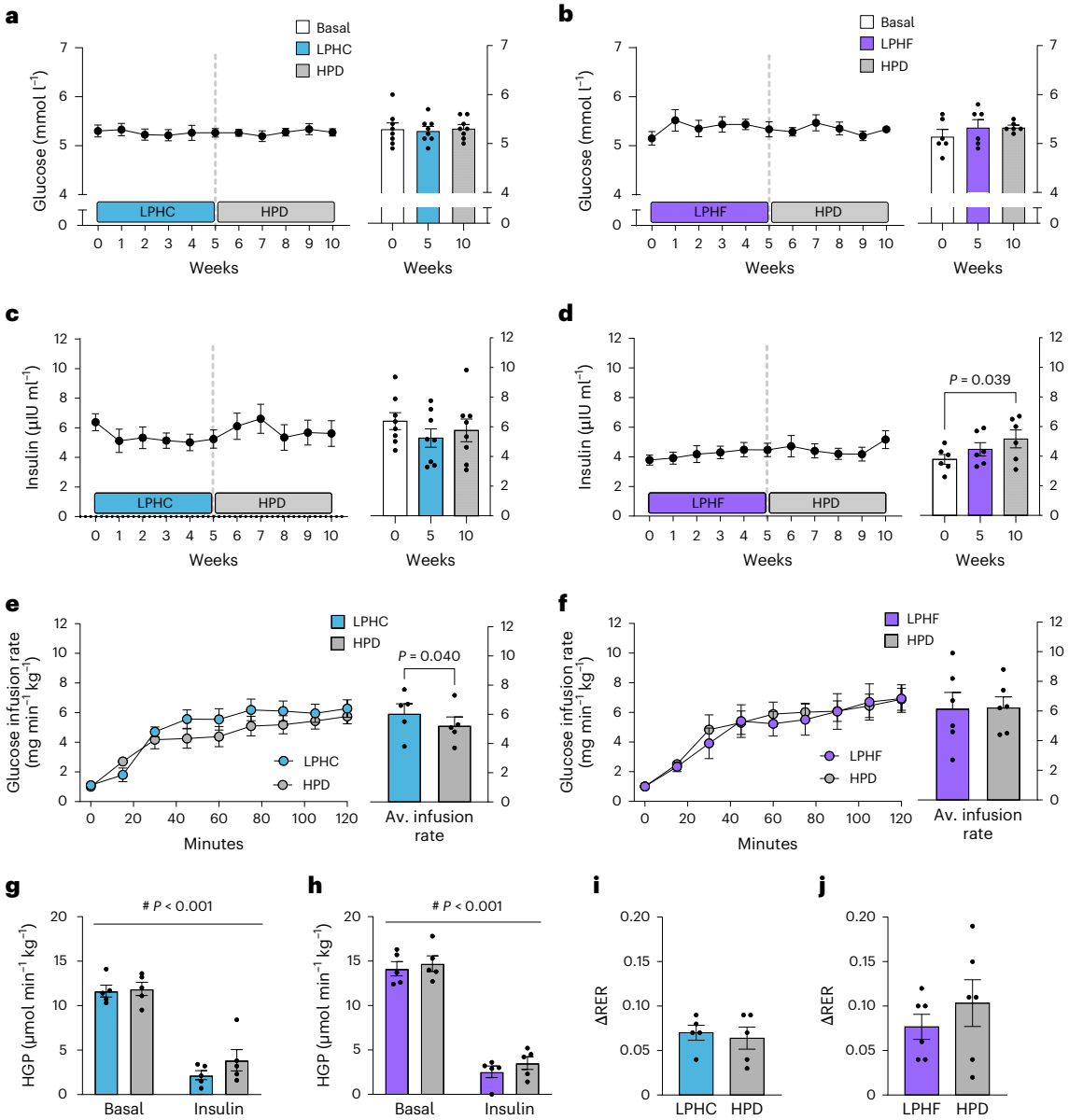

Fig. 4 | Effect of dietary protein restriction on whole-body insulin sensitivity in healthy, lean men. a,**b**, Fasting plasma glucose and **c**,**d**, insulin levels during the LPHC, LPHF and habitual HPD interventions. **e**,**f** Glucose infusion rate following the LPHC, LPHF and HPD interventions (Av. infusion rate, average infusion rate during the last 60 min). **g**,**h**, Hepatic glucose production (HGP) in the basal state and during the hyperinsulinemic-euglycemic clamp following the LPHC, LPHF and HPD interventions. **i**,**j**, Delta values of RERs after the LPHC, LPHF and HPD interventions. *P* values in **a**–**d** determined by repeated measures one-way ANOVA or **g**,**h** repeated measures two-way ANOVA with a Bonferroni post hoc test; in **e**, **f**, **i** and **j**, two-tailed paired *t*-test; in **a**–**f**, statistics were only applied to the bar graphs to test for effects of diets. The # represents the effect of insulin. All data are presented as mean ± s.e.m. LPHC, $n = 8$; clamp data from three subjects (LPHC intervention, study 2) were excluded: one subject fainted after the basal biopsy and two were excluded owing to technical problems during the clamp (LPHC, $n = 5$). LPHF, $n = 6$; data on hepatic glucose production is missing from one subject (LPHF, study 3) owing to lack of glucose tracer ($n = 5$).

export for cellular fuel. Notably, the ADP/ATP carrier SLC25A4/ANT1 exhibits reduced expression in this study, indicating a potential slowdown in ADP import, subsequently leading to impaired ATP synthesis and reduced ATP production. Therefore, it would also be expected that the inner mitochondrial membrane would display increased leakiness for protons bypassing the complex V ATPase. Altogether, such mitochondrial uncoupling effect might result in increased energy dissipation in the white adipose tissue and may be responsible for the higher energy needed to keep body weight stable when consuming a protein-diluted diet.

The observation in mitochondrial proteins was mainly seen after the LPHC intervention, probably because of the higher number of participants in the proteomic analysis.

In large human studies, fat mass has been established to be a significant contributor to basal metabolic rate, with at least 6% of the variation in basal metabolic rate being attributable to fat mass[37] and white adipose tissue estimated to contribute with more than 280 kJ day^{-1} (ref. 38). Although this is fairly minor amount considering the observed increased energy demand to maintain body weight with LPDs in the present study, mitochondrial leak in respiratory electron transport chain of human white adipose tissue in individuals suffering from burn—comparable to the adipose tissue proteome in the present study—has been estimated to increase the contribution of white adipose tissue to whole-body energy expenditure threefold, up to 861 kJ day^{-1} (refs. 39,40). This could potentially explain much of the observed increased energy demand on LPDs of 1.0–1.3 MJ day^{-1} (incremental

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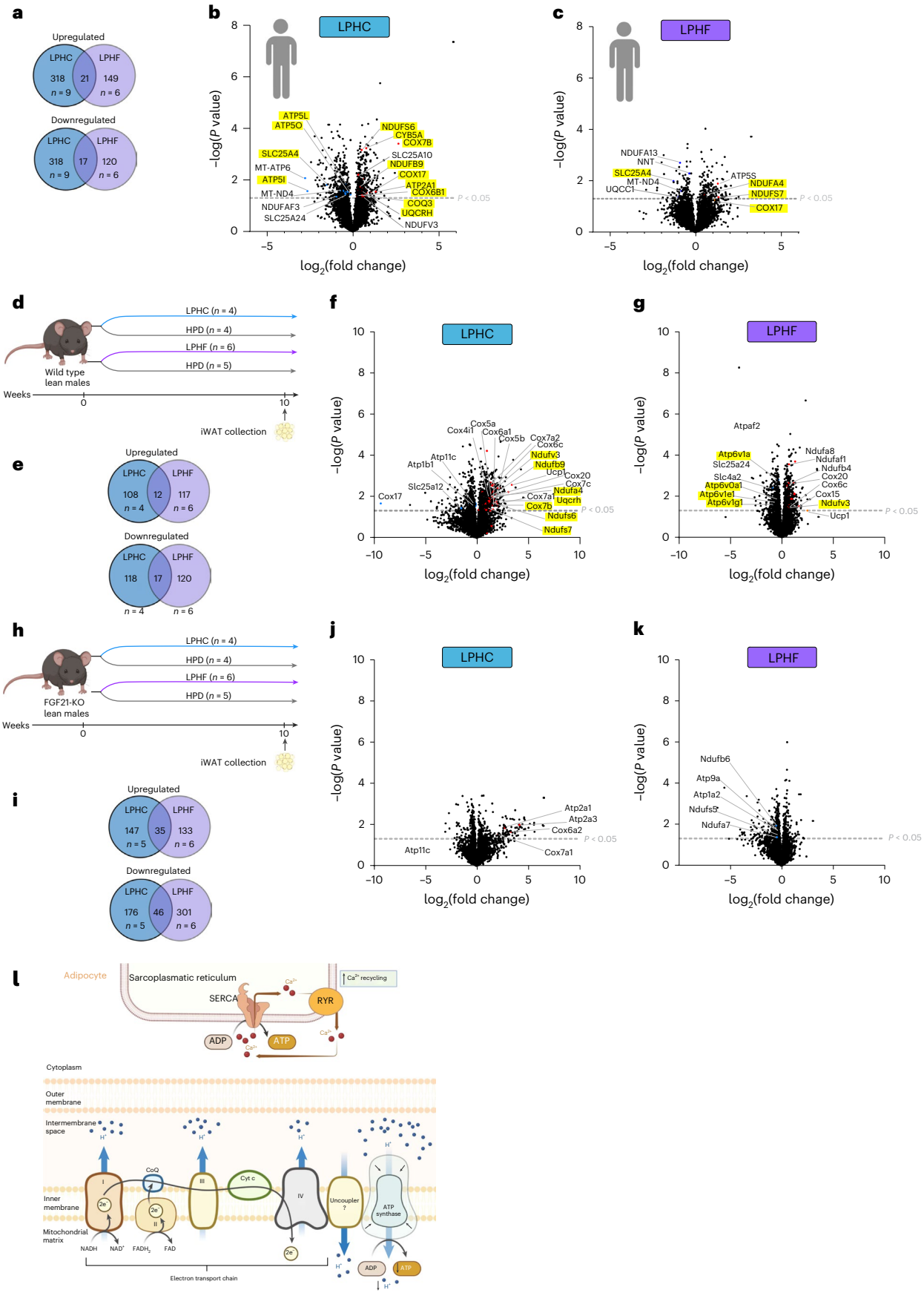

Fig. 5 | Adipose tissue proteome after dietary protein restriction in healthy lean men and male mice. a, Venn diagram showing the upregulated and downregulated proteins in white adipose tissue from healthy lean men after the LPHC and LPHF interventions. **b**,**c**, Volcano plot comparing the *P* value ($P < 0.05$) and fold change after the LPHC and LPHF interventions relative to the respective habitual HPD intervention. **d**, Illustration of the study in which WT male mice were fed either a LPHC, LPHF or standard HPD for 10 weeks. **e**, Venn diagram showing the upregulated and downregulated proteins in iWAT in WT mice after the LPHC and LPHF interventions **f**,**g**, Volcano plot comparing the *P* value ($P < 0.05$) and fold change after the LPHC and LPHF intervention relative to the HPD intervention in iWAT. Proteins highlighted in **b**, **c**, **f** and **g** represent proteins of the electron transport chain, which are discussed in the text. **h**, Illustration of study in which FGF21-KO male mice were fed either a LPHC, LPHF or HPD for 10 weeks. **i**, Venn diagram showing the upregulated and downregulated proteins in iWAT in FGF21-KO male mice after the LPHC and LPHF interventions. **j**,**k** Volcano plot comparing the *P* value ($P < 0.05$) and fold change after the LPHC and LPHF interventions relative to the HPD intervention in iWAT. Unadjusted two-sided Student's *t*-tests were used to identify differentially regulated proteins between two conditions in all proteome analyses. **i**, Working hypothesis of how a prolonged protein-restricted diet increases thermogenesis in adipose tissue obtained from lean men. Graphical illustrations created in BioRender.com.

area under the curve of 35.4–43.8 MJ over 35 days in increased energy intake of the LPDs). However, future studies should investigate whether similar mitochondrial adaptations occur in other tissues as well and contribute to the observed increased energy demand to maintain body weight on LPDs.

To obtain further insight into mechanisms leading to these changes in mitochondrial oxidative capacity and investigate the role of FGF21 in these mitochondrial adaptations, we fed WT and FGF21-KO mice with diets similar to the human diets: LPHC, LPHF and HP diets for 10 weeks. Proteomic analysis on iWAT in WT mice revealed that proteins in complex I–IV (COX and NDUFs) were increased, whereas proteins in complex V (for example, the SLC25 isoform) were decreased, similar to what was observed in humans. Excitingly, mitochondrial oxidative proteins in iWAT were not regulated when FGF21 was lacking, both when FGF21-KO mice were fed LPHC and LPHF diets. These findings reveal thermogenic mechanisms in adipose tissue mediated by protein-restricted FGF21 induction and suggest that the increased energy consumption in response to a protein-restricted diet in humans could be caused by uncoupling or a leak within the electron transport chain in white adipose tissue, potentially induced by FGF21.

In a previous study[41], in which mice were fed LPDs (5 E% casein protein), the particular amino acids required for induction of the systemic FGF21 response to a protein-restricted diet were examined. Data showed that restriction of only one or few specific essential amino acids, especially threonine and tryptophan, were sufficient to induce the systemic metabolic effects such as observed with overall protein restriction. We calculated the essential amino acid content in our human diets from dietary databases and found that threonine and tryptophan were decreased by 80% and 90%, respectively (Extended Data Table 4). Despite a 50% reduction in total protein intake, all nine essential amino acids in the restricted diets remained within the daily recommendations by the WHO (Extended Data Table 4). Additionally, we did not observe any changes in fasting plasma levels of total amino acids. This is in line with our recent study in healthy male volunteers on a protein-restricted diet (0.9 g kg^{-1} day^{-1}) like the diet in the present study[42]. The results from a different study[29] revealed that very few plasma amino acids were affected by the protein-restricted diet in the fasted state. However, several plasma amino acids were significantly lower in the hours after a protein-restricted meal, especially leucine, methionine and threonine, compared with a normal protein meal[42]. Together, these observations indicate that it is primarily the low plasma amino acids after a meal that induce metabolic changes. In agreement, in the present study, we observed a higher metabolic rate in the hours after ingestion of a protein-restricted meal rather than in the fasting state, together with a 57% increase in plasma FGF21 level compared with a meal with the habitual protein content.

Another key measure of metabolic health is glucose homeostasis. In mice on a short-term protein-restricted diet, plasma glucose and plasma insulin levels were markedly reduced during a glucose tolerance test, indicating improved glucose homeostasis[14]. In the present study, fasting plasma glucose and insulin levels remained unchanged during the whole intervention. However, whole-body insulin sensitivity, measured by the hyperinsulinemic-euglycemic clamp technique, was increased (16%) after the protein-restricted diet, when the reduced protein was replaced by carbohydrates. Even when proteins were replaced with fat, whole-body insulin action remained unchanged. That high intake of dietary fat in a eucaloric diet does not compromise insulin sensitivity is in line with our recent findings in humans who were overweight[43]. Hepatic glucose production in the present study was not affected by dietary protein restriction in the basal or insulin-stimulated state. This indicates that the enhanced insulin sensitivity after the protein-restricted diet with upregulation of carbohydrates was primarily a result of enhanced glucose disposal in peripheral tissues rather than suppression of endogenous glucose production, probably related to both enhanced glycogen deposition and glucose oxidation in the skeletal muscle as previously suggested[44,45]. Even though 60–80% of the insulin-stimulated glucose uptake takes place in skeletal muscle, it was shown that improved glucose homeostasis in mice on a protein-restricted diet was ascribed to an enhanced glucose disposal in brown adipose tissue and iWAT[14]. Given that finding, it could be speculated that part of the increased peripheral glucose disposal when ingesting the LPHC diet in the present human study may be directed towards white adipose tissue, and this might be governed by FGF21. Support for this notion is the findings in mouse and human adipocytes that FGF21 is a potent activator of glucose uptake[46].

Limitations

To allow comparison with existing literature, only men were studied in the present mechanistic study. It would be of great interest in future studies to investigate whether women are affected similarly by dietary protein restriction.

In the present study, we used indirect calorimetry to estimate acute fasting and postprandial energy expenditure. To determine total energy expenditure more comprehensively over 24 h or longer, it would have been of interest to apply 24 h direct calorimetry or double-labelled water techniques; however, this was not possible owing to technical limitations.

In conclusion, a diet with a protein content that is lower than a typical Western diet but still within levels recommended by the WHO increases energy demand to maintain body weight and FGF21 plasma levels and mediates adaptations in the respiratory chain in adipose tissue in healthy young men. Results from our mouse studies suggest that the increase in FGF21 may be responsible for this effect of a protein-restricted diet by, at least in part, inducing leak or uncoupling processes within the mitochondria of adipose tissue, thereby increasing the body's energy requirements to maintain body weight. Collectively, these findings reveal interesting physiological effects and molecular signatures associated with reducing dietary protein intake in humans, raising questions about the metabolic and energy homeostasis benefits of the typically HP Western diet.

Methods

Human studies

All subjects gave their written consent to take part in the experiment, executed at the Copenhagen University Department for Nutrition, Exercise and Sports, approved by the Copenhagen Ethics Committee

(H-18005023) and registered in a public database, Clinical Trials.gov (NCT06267235).

Three studies were performed in three groups of individuals (Fig. 1).

Study 1, acute and prolonged reduced protein intake replaced by carbohydrates. To evaluate the acute postprandial metabolic effects, ingestion of a LPHC meal was compared with a meal with a HP content (Fig. 1), which was equivalent to the participants' habitual protein intake. Eight healthy, young (age 27 ± 2 years), lean (body mass index (BMI), 25 ± 2 kg m^{-2}), moderately physically active (peak oxygen uptake (VO_{2peak}), 44 ml min^{-1} kg^{-1} body mass) men (Table 1) with a daily protein intake of ≥1.5 g kg^{-1} body weight were recruited. The LPHC and HP meal tests were performed in a randomized order, separated by 72 h. The LPHC meal consisted of 8 E% protein, 71 E% carbohydrate and 21 E% fat; the HP meal consisted of 16 E% protein, 57 E% carbohydrate and 27 E% fat (Extended Data Table 1) and reflected the habitual dietary macronutrient composition of the participants. After arrival at the institute in the morning in an overnight-fasted state (10 h), a catheter was inserted into an antecubital vein for blood sampling. Then the participants ingested the meal within 10 min, together with 200 ml of water. The energy intake amounted to 55 KJ kg^{-1} body weight. Blood samples were drawn before (0 min) the meal and at 15, 30, 45, 60, 90, 120, 180 and 240 min after ingestion. Indirect calorimetry was applied at 0, 30, 60, 90, 120, 180 and 240 min. After the second meal test, the participants continued a eucaloric diet reduced in protein (0.8 g kg^{-1} body weight per day) replaced by carbohydrates (LPHC) for 5 weeks (9 E% protein, 70 E% carbohydrate and 21 E% fat) (Fig. 1 and Extended Data Table 1).

Study 2, protein-reduced diet substituted with carbohydrates for 5 weeks followed by 5 weeks on habitual HPD. Eight healthy, young (age, 26 ± 2 years), lean (BMI, 25 ± 3 kg m^{-2}), moderately physically active (VO_{2peak}, 44 ± 5 ml min^{-1} kg^{-1}) men (Table 1), with a daily protein intake of ≥1.5 g kg^{-1} body weight were recruited to this study. The participants consumed the eucaloric LPHC as described in study 1 (Extended Data Table 2) for 5 weeks, followed by another 5 weeks on a eucaloric diet consisting of the participants' habitual HPD (18 E% protein, 49 E% carbohydrate, 33 E% fat) (Fig. 1 and Table 2).

Study 3, protein-reduced diet substituted with fat for 5 weeks followed by 5 weeks on the habitual HPD. Seven healthy, young (age, 25 ± 1 years), lean (BMI, 23 ± 2 kg m^{-2}), moderately physically active (VO_{2peak}, 41 ± 8.0 ml min^{-1} kg^{-1}) men (Table 1), with a daily protein intake of ≥1.5 g kg^{-1} body weight matching the participants in study 1 and 2 were enrolled. One participant dropped out because of illness. The participants consumed a eucaloric LPHF diet, consisting of 9 E% protein, 41 E% carbohydrate and 50 E% fat for 5 weeks, followed by another 5 weeks on their habitual HPD (18 E% protein, 49 E% carbohydrate, 33 E% fat) (Fig. 1 and Table 3).

Experimental diets and eucaloric weight maintenance

In a run-in period of at least 1 week before each of the dietary interventions (Fig. 1), energy content and macronutrient composition of the participants' habitual diet were evaluated from a weighed dietary registration for four non-consecutive days (Extended Data Table 3). All food and fluids were weighed to an accuracy of 1 g and analysed with computer software (Vitakost, Denmark). The daily energy provision on the experimental diets was based on the individual diet registrations adjusted based on previously published basal metabolic rate equations[47] and multiplied by the physical activity level value, estimated from fitness level (VO_{2peak}), measured on a bicycle ergometer and pediometrics and accelerometrics. To maintain the baseline body weight of the participants throughout the interventions, participants registered their morning weight daily. Energy provision was adjusted during the diet intervention if body weight, based on a 3-day average, changed by ±0.5 kg. All foods in the experimental diets were weighted to 1 g of accuracy and pre-packed in meal portions in a 7-day menu rotation, and food was picked up by participants every third day at the institute. The LPHC diet contained a high proportion of carbohydrates derived from white bread, rice, pasta and fruits. The LPHF diet contained a high proportion of fat derived from vegetable oil, nuts, butter, cheese, milk and yoghurt with high fat content. The HPD reflected the habitual mixed diet of the participants.

Experimental study

Before and after the LPHC diet (study 1) and before and weekly during the LPHC and LPHF diets as well as the HPD (studies 2 and 3, Fig. 1), participants arrived in the morning at the institute by passive transport in an overnight-fasted state (10 h). After resting in the supine position for at least 30 min under thermoneutral conditions, RMR was measured by indirect calorimetry (Masterscreen CPX SBX, CareFusion) at week 0 and week 5 (studies 2 and 3). Then a catheter was inserted into the antecubital vein from which blood samples were obtained. In study 1, body composition was assessed using DXA-scanning (Lunar Corporation) before and at week 5. In studies 2 and 3, body composition was evaluated before, at week 5 and at week 10. Additionally, at weeks 5 and 10, body water content was measured by bio-impedance (InBody270).

Whole-body insulin sensitivity. Participants in studies 2 and 3 underwent a hyperinsulinemic-euglycemic clamp at the end of week 5 of the LPHC, LPHF and HPD diet (Fig. 1). Participants arrived at the institute in the morning after an overnight fast (10 h) by passive transport. A catheter was inserted in the antecubital vein and fasting blood samples were drawn for basal measurements and 2D glucose background enrichment. Then, a bolus injection of [6,6-D2] glucose tracer (2.6 mg kg^{-1}) was given, followed by constant infusion (0.044 mg kg^{-1} min^{-1}) to determine basal hepatic glucose production. Another catheter was inserted into a dorsal hand vein in the other arm, and a heating pad was wrapped around the hand to arterialize the venous blood from the hand. After 120 min tracer infusion, a 120 min hyperinsulinemic-euglycemic clamp was initiated by a bolus of insulin (9.0 mU kg^{-1}) (Actrapid, Novo Nordisk) followed by a constant infusion rate (1.0 mU insulin per kg per min). During the clamp, 20% glucose solution enriched with 1.9% [6.6-D2]-glucose tracer was infused at a rate ensuring euglycemia, matching the fasting arterialized blood glucose level determined from three blood samples obtained before initiating the insulin infusion. The same glucose target was used after the HPD. Blood samples were drawn before (0 min) and at 30, 90 and 120 min during the clamp, and indirect calorimetry was applied at 0, 45, 60 and 120 min. Biopsies were obtained from the periumbilical subcutaneous adipose tissue by a modified Bergström needle with suction under local anaesthesia (~2 ml of xylocaine 1%; AstraZeneca) before the clamp. Biopsies were rinsed in ice-cold saline, snap-frozen in nitrogen and stored at −80 °C until analysis. Clamp data from two subjects (LPHC intervention, study 2) were excluded: one subject fainted after the basal biopsy and one was excluded because of technical problems during the clamp. Data on hepatic glucose production are missing from one subject (LPHF, study 3) because of a lack of glucose tracer.

Physical activity. Participants were instructed to maintain their physical activity level during each intervention. Before and after the LPHC, LPHF and HPD interventions, maximal oxygen uptake (VO_{2peak}) was measured (Masterscreen CPX SBX, CareFusion) using an incremental test to exhaustion on a bicycle ergometer. To monitor daily physical activity levels during the interventions, participants wore either a pedometric watch (Polar Loop-watch or a Garmin Vivofit3-watch; study 1) or a triaxial accelerometer (SENS motion system, Denmark; study 2 and 3) attached to the skin on the lateral thigh approximately 10 cm from the knee to measure daily number of steps and activities.

Plasma analysis
See resource table (Extended Data Table 5).

Calculations
The HOMA-IR index was calculated as ($insulin_{fasting}$) × ($glucose_{fasting}$) / 22.5.

Hepatic glucose production in the fasting state and during insulin stimulation was calculated from three blood samples obtained during the last 20 min of the basal period and again during the last 20 min of the clamp period using Steele's equation, taking into account both the 'cold' glucose concentration and the enrichment of glucose tracer in the blood during these periods as previously described[48]. RMR was calculated based on a previous publication[49] as EE (kJ min^{-1}) = VO_2 × (4.686 + (RQ − 0.707) × 0.361 / 0.293) × 4.14 kJ $kcal^{-1}$, where EE is energy expenditure and RQ is the respiratory quotient.

Animal study
Animal handling and experimentation was done at the German Cancer Research Centre (Heidelberg) in accordance with European Union directives and the German Animal Welfare Act and approved by local authorities (Regierungspräsdidium Karlsruhe) and conformed to ARRIVE guidelines. WT C57BL/6J male mice were obtained from Charles River Laboratories. All mice were maintained on a 12 h light–dark cycle (06:00–18:00 h) at 22 °C with unrestricted access to food and water. Germline FGF21-KO male mice were generated on a C57BL/6 background as previously described[14]. $FGF21^{+/-}$ mice were crossed by het × het littermate pairing to generate FGF21-KO ($^{-/-}$) and WT ($^{+/+}$) littermates, bred at the German Cancer Research Center. All experimental procedures have been described in detail elsewhere[14]. In short, 8-week-old mice were housed with two to three mice per cage and fed ad libitum with either a LPHC diet (5 E% protein and 85 E% carbohydrate; Research Diets, D10062201) or a LPHF diet (5 E% protein and 60 E% fat; Research Diets, D12020703), with representative mice fed on a standard HPD (protein 20 E%; Research Diets, D12450B and D12492). Mice were weighed before allocation to diet groups by counterbalancing. Food intake and body weight were measured before and after the diet interventions. After 10 weeks, mice were killed and iWAT was dissected and snap-frozen in nitrogen. No animals were excluded from the analysis.

Adipose tissue proteomics and bioinformatics. A total of 50 mg adipose tissue in 5× volume lysis buffer (2% sodium deoxycholate, 100 mM Tris pH 8.5) was boiled for 10 min at 99 °C. After homogenization with pestle, samples were sonicated for 15 min (Branson probe sonifier, output 3–4, 50% duty cycle, 15 min with 30 s × 30 s cycles), and protein content was determined with the bicinchoninic acid method (BCA no. 23225, Pierce) and adjusted to a protein level at 0.25 µg $µl^{-1}$. Then, proteins were alkylated (40 mM 2-chloroacetamide and 10 mM trifluoroacetic acid (TFA)) in the dark for 10 min at 45 °C at 1,000 rpm in a thermoshaker and digested with LysC and trypsin (1:50 protein to enzyme) overnight at 37 °C, 1,000 r.p.m. in a thermoshaker. Digested peptides were acidified by adding 1:1 isopropanol with 1% TFA and loaded in triple layer Styrene Divinylbenzene–Reversed Phase Sulfonate STAGE tips (SDB–RPS; 3M Empore). The STAGE tips were first cleaned (100 µl 80% acetonitrile (ACN)) then activated (100 µl 30% methanol + 1% TFA) and acidified (150 µl 0.2% TFA). The peptides were eluted with 60 µl SDB–RPS elution buffer (80% ACN, 5% NH_4OH) on a SpeedVac for 45 min at 45 °C and dissolved in 6 µl MS loading buffer (2% ACN, 0.01% TFA).

Proteomic samples were measured at a Thermo Exploris 480 combined with a Thermo EasyNLC 1200 and Thermo FAIMS Pro using a 60 min method with single collision voltage of −50 V in data-independent acquisition (DIA) mode. The input material for each sample was 500 µg of peptides, based on Nanodrop measurement.

Liquid chromatography gradients were provided by incrementally mixing buffer A (0.1% formic acid) and buffer B (80% acetonitrile, 0.1% formic acid). After starting with 5% buffer B, the amount was linearly increased to 20% after 30 min, 29% after 39 min, 45% after 45 min and 95% after 50 min, followed by holding 95% for another 5 min (until min 55) and finally reduced to 5% after 60 min at a flow of 300 nl min^{-1}.

MS1 scans were acquired with an orbitrap resolution of 120,000 in positive ion mode. HCD collision energy was 30%. For MS2 scans, an orbitrap resolution of 15,000 was used with 66 DIA windows of variable size.

Statistical analyses
Data collection and analysis were not performed blind to the conditions of the experiments. Statistical analyses were performed in GraphPad Prism (v.8 and v.9). Data distribution was assumed to follow the Gaussian normal distribution as well as variance homogeneity, but this was not formally tested. To assess differences between interventions and/or the effect of time, repeated measures one-way or two-way ANOVA was performed, or a mixed-effects model in the case of missing values. When ANOVA revealed significant interactions, Bonferroni post hoc testing with correction for multiple testing was applied. When comparing pre-intervention versus post-intervention or comparing between groups, a two-tailed paired *t*-test or unpaired *t*-test was used. To assess the relationship between changes in energy intake and circulating FGF21 levels, a repeated measures correlation was conducted using percentage delta values from baseline to week 5 and from week 5 to week 10 among participants enrolled in studies 2 and 3. This method addresses the issue of non-independence among observations, providing a common within-individual association. The analysis was conducted using the rmcorr package in R (v.4.3.3) with RStudio. For proteome analyses, the raw data were analysed using Spectronaut (Biognosis) in directDIA mode, using a library-free approach. All searches were performed against the human UniProt FASTA database, with MaxLFQ settings enabled for protein-level label-free quantification. The MaxQuant contaminant FASTA file was included in the search, and default parameters were retained unless explicitly stated otherwise. Bioinformatics analysis was conducted using Perseus, applying default settings unless specified. Before analysis, common contaminants were filtered out. For total proteome analysis, LFQ values were transformed to a logarithmic scale (log_2), and proteins were selected if they had at least two valid measurements across biological replicates for any given condition. Missing values were imputed based on a Gaussian normal distribution model with a width of 0.3 and a downshift of 1.8. Differentially regulated proteins between two conditions were identified using an unadjusted two-sided Student's *t*-test. No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications[14]. All data are presented as mean ± s.e.m. except for subject characteristics in Table 1 (mean ± s.d.). For all statistical tests, $P < 0.05$ was considered statistically significant.

Reporting summary
Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability
The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE[50] partner repository with the dataset identifier PXD047177. Any additional information required to reanalyse the data reported in this paper is available from the corresponding author upon request. Source data are provided with this paper.

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Author contributions

T.S.N. and B.K. planned and designed the experiments. T.S.N., B.K., E.A.R., K.A.S., A.E.L., C.T.V., J.K.J., E.M.M., C.K.N., M.B.-I. and N.J.W.A. carried out the human experiments and/or analysis. A.J.R. performed the mouse studies. D.T.H. and N.K. analysed adipose tissue proteome. T.S.N., A.M.L., A.M.F., C.C., E.A.R. and B.K. interpreted data and T.S.N. and B.K. wrote the draft of the manuscript; all authors contributed to the final version.

Competing interests

The authors declare no competing interests.

Additional information

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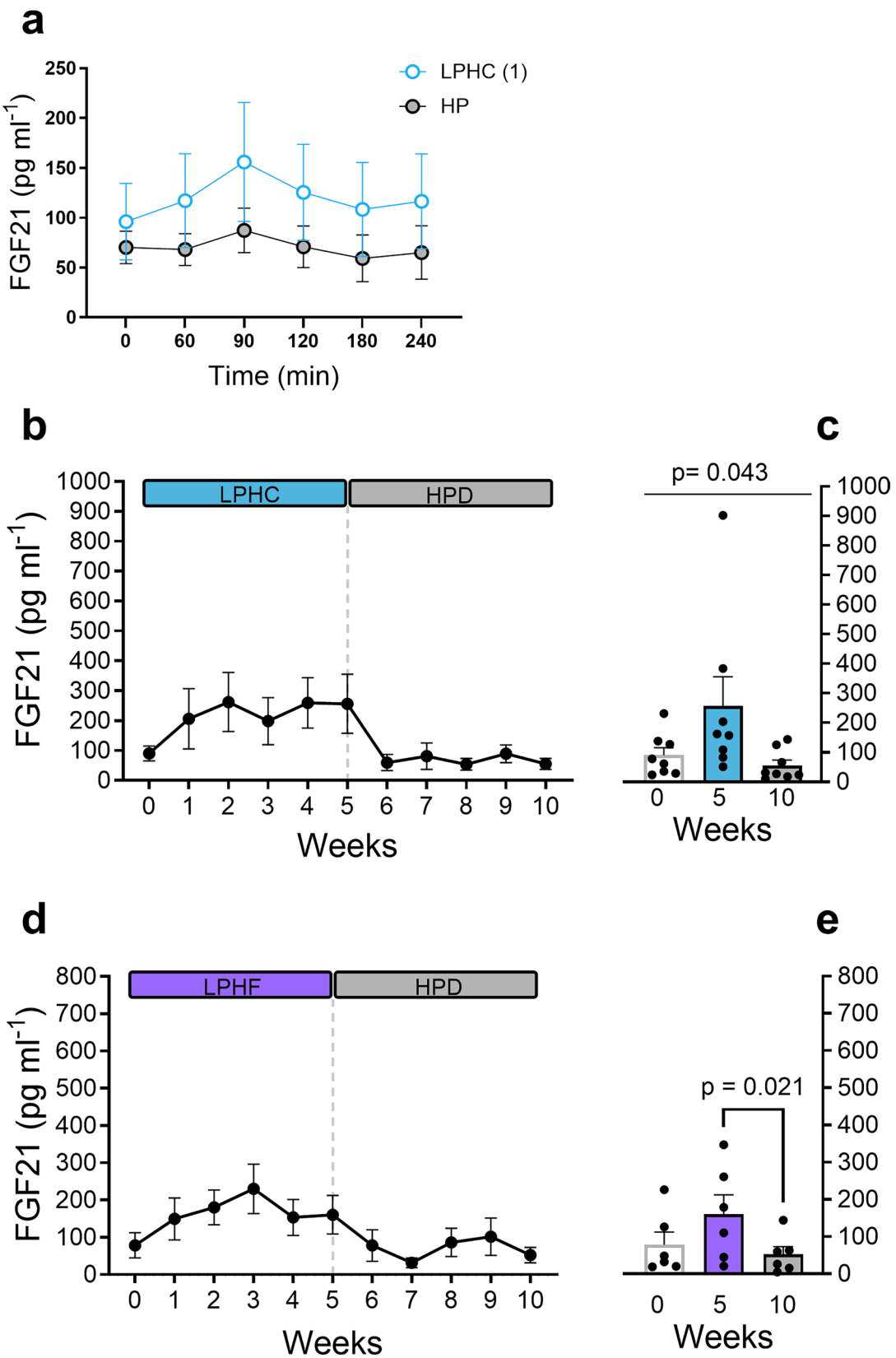

Extended Data Fig. 1 | Effects of dietary protein-restriction on plasma FGF21 concentrations. (**a**) plasma FGF21 levels during meal test in Low protein, high carbohydrates Study 1 (LPHC (1)) (**b**) fasting plasma FGF21 levels during low protein, high carbohydrates study 2 (LPHC (2)) and high protein diet (HPD), (**c**) before, after LPHC (2) and after HPD, (**d**) fasting plasma FGF21 levels during low protein, high fat (LPHF) and HPD, (**e**) before, after LPHF and after HPD. Statistics were only applied to bar graphs to test for effects of diet: **a** Repeated measures Two-way ANOVA. **c**, **e** Repeated measures one-way ANOVA with a Bonferroni post hoc test. Data are presented as means ± SEM. in **a**: n = 9, in **b**, **c**: n = 8 and in **d**, **e**: n = 6.

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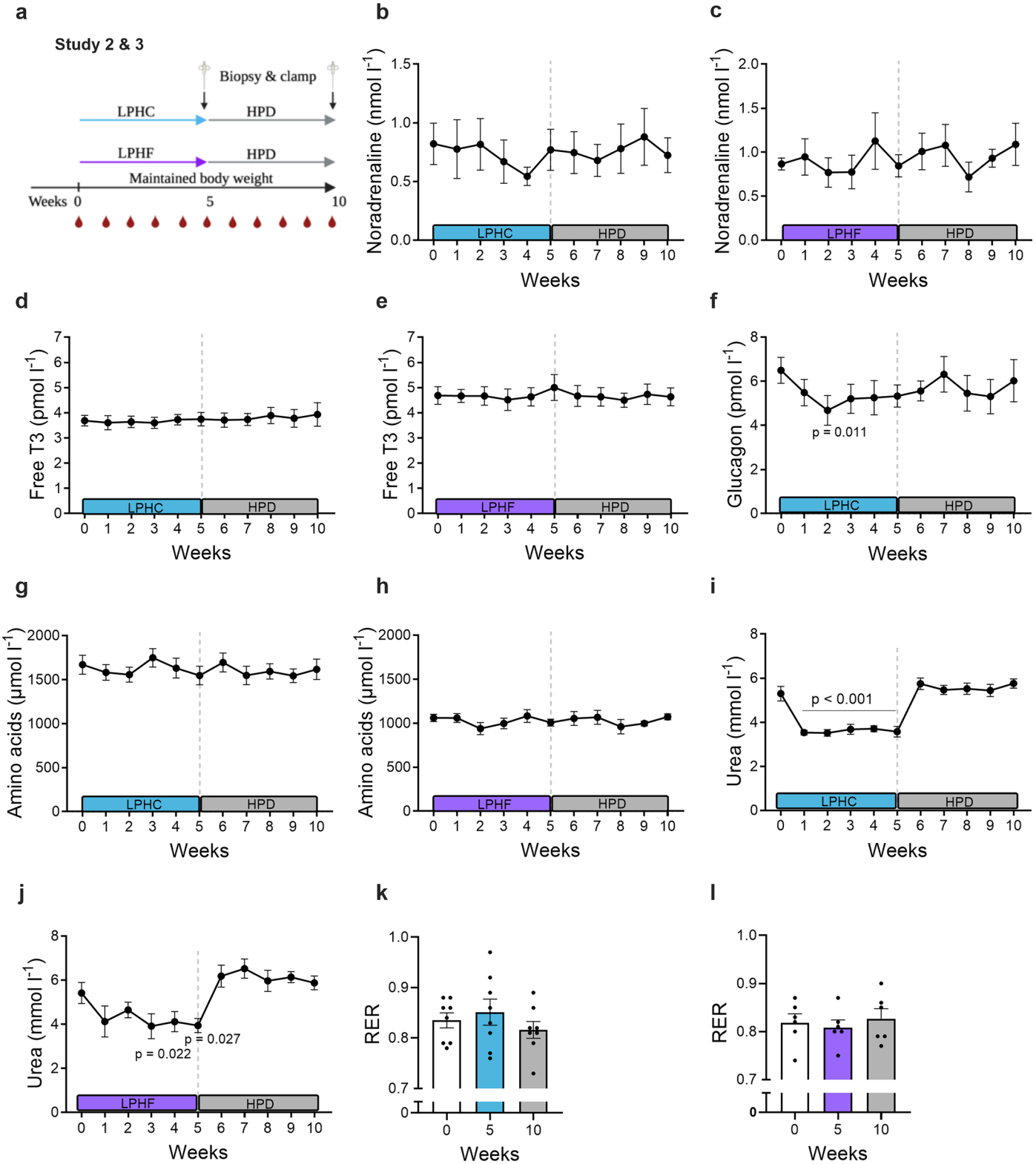

Extended Data Fig. 2 | Effect of prolonged protein-restricted diet on hormones regulating energy expenditure and amino acid balance. (**a**) Illustration of study 2 and 3 in human participants ingesting a eucaloric low-protein, high-carbohydrate (LPHC) or low-protein, high-fat (LPHF) diet for 5 weeks followed by another 5 weeks on a eucaloric habitual higher protein diet (HPD). (**b**, **c**) Fasting plasma noradrenaline, and (**d**, **e**) T3 levels during the LPHC, LPHF and HPD interventions. (**f**) Fasting plasma glucagon levels during the LPHC and HPD interventions. (**g**, **h**) Fasting plasma total amino acids levels during the LPHC and HPD interventions. (**i**, **j**) fasting plasma urea levels during the LPHC, LPHF, and HPD interventions. (**k**) RER before LPHC, after LPHC and after HPD, (**l**) RER before LPHF, after LPHF and after HPD. Repeated measures one-way ANOVA/ mixed-effects model with Bonferroni post hoc test to test for differences from week 0. Data are presented as means ± SEM. In **b**, **d**, **f**, **g**, **i**, **k**: n = 8 and in **c**, **e**, **h**, **j**, **l**: n = 6.

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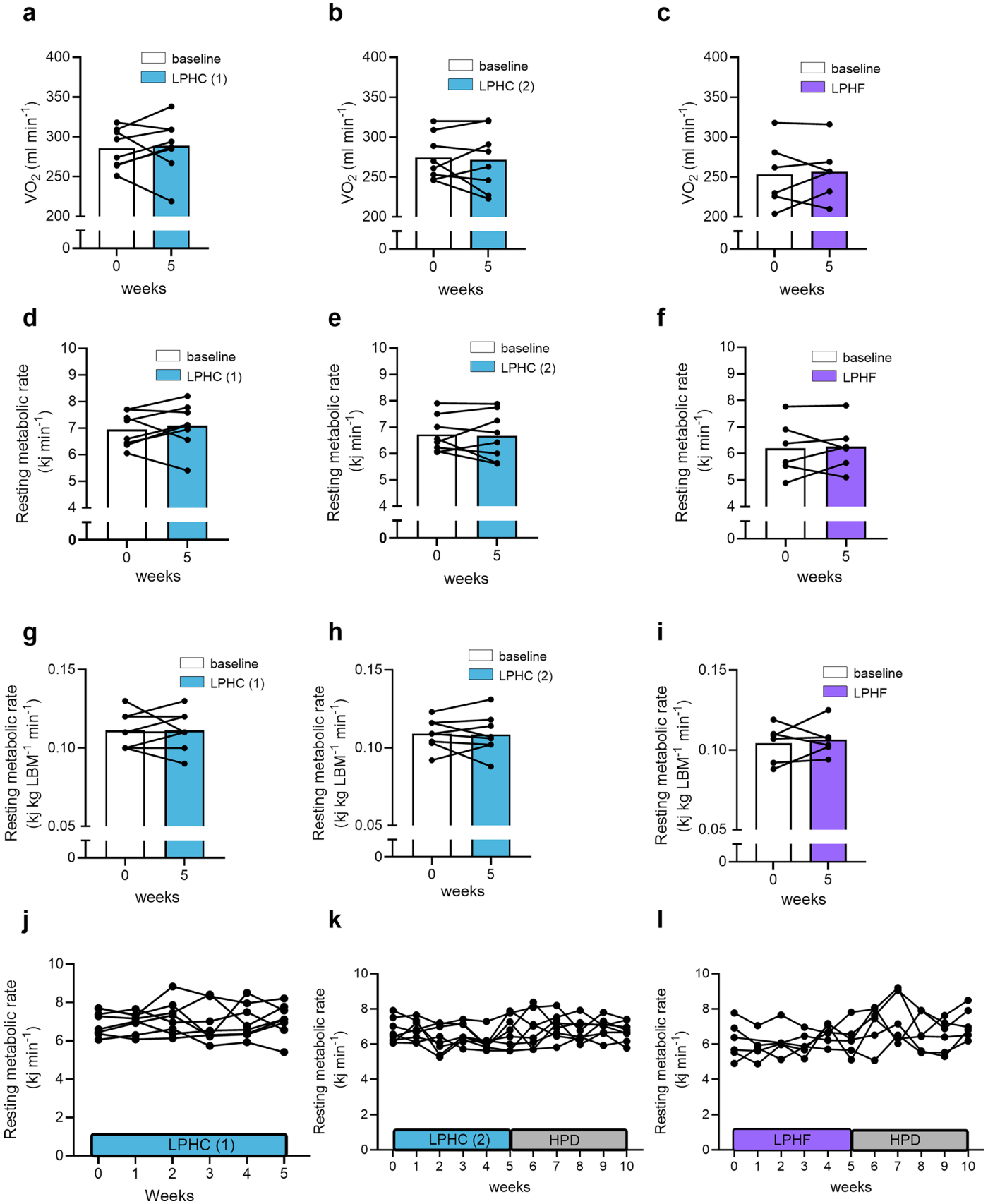

Extended Data Fig. 3 | The effect of dietary protein-restriction on resting metabolic rate. (**a**–**c**) VO_2 baseline and after low protein, high carbohydrates study 1 (LPHC (1)), low protein, high carbohydrates study 2 (LPHC (2)) and low protein, high fat, (LPHF) study, (**d**–**f**) Resting metabolic rate baseline and after LPHC (1), LPHC (2) and LPHF, (**g**–**i**) Resting metabolic rate related to LBM baseline and after LPHC (1), LPHC (2) and LPHF interventions, (**k**, **j**, **m**) weekly resting metabolic rate during LPHC (1), and LPHC (2) and LPHF followed by high protein diet (HPD). Statistics were applied to bar graphs to test for effect of diets. For **a**–**i** a two-tailed paired t-test was conducted. **a**–**i** are presented as means and individual values. **j**–**l** are presented as individual values. In **a**, **d**, **g**, **j:** n = 8, in **b**, **e**, **h**, **k**: n = 8 and in **c**, **f**, **i**, **l**: n = 6.

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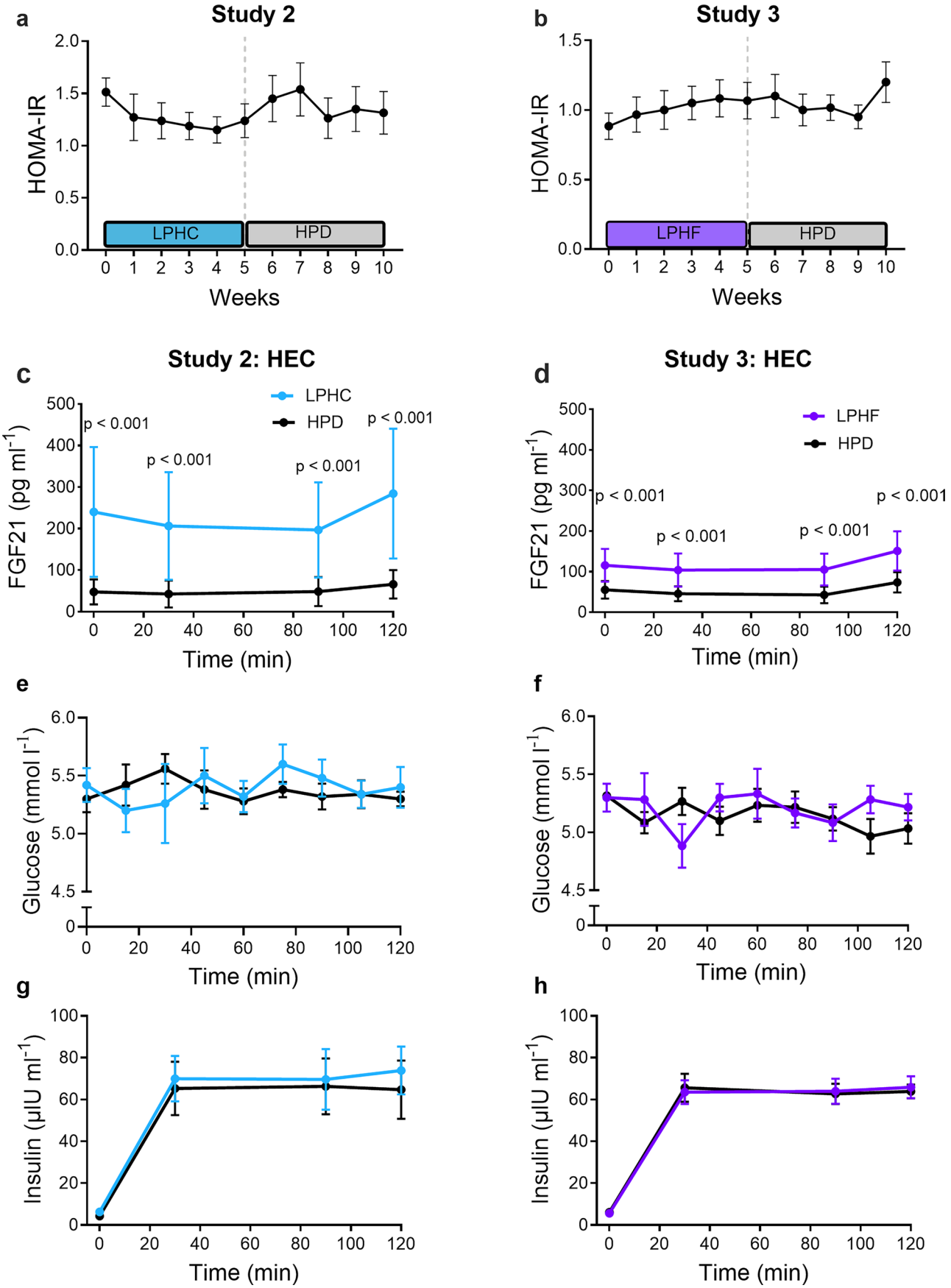

Extended Data Fig. 4 | See next page for caption.

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Extended Data Fig. 4 | Effect of a prolonged protein-restricted diet on HOMA-IR and plasma FGF21 levels, plasma glucose and plasma insulin during a hyperinsulinemic-euglycemic clamp. (**a**, **b**) HOMA-IR during the low-protein, high-carbohydrate (LPHC), low-protein, high-fat (LPHF), and habitual higher protein diet (HPD) interventions. (**c**, **d**) Plasma FGF21 levels during a hyperinsulinemic-euglycemic clamp (HEC). (**e, f**) Plasma glucose concentrations during HEC. (**g**, **h**) Plasma insulin concentrations during HEC. **a**, **b** Repeated measures one-way ANOVA or in **c**–**h** repeated measures two-way ANOVA/mixed-effects model with a Bonferroni post hoc test to test for differences between diet interventions. Data are mean ± SEM. In **a**: n = 8, in **b**: n = 6. In **c**, **e**, **g**: n = 5, in **d**, **f**, **h**: n = 6.

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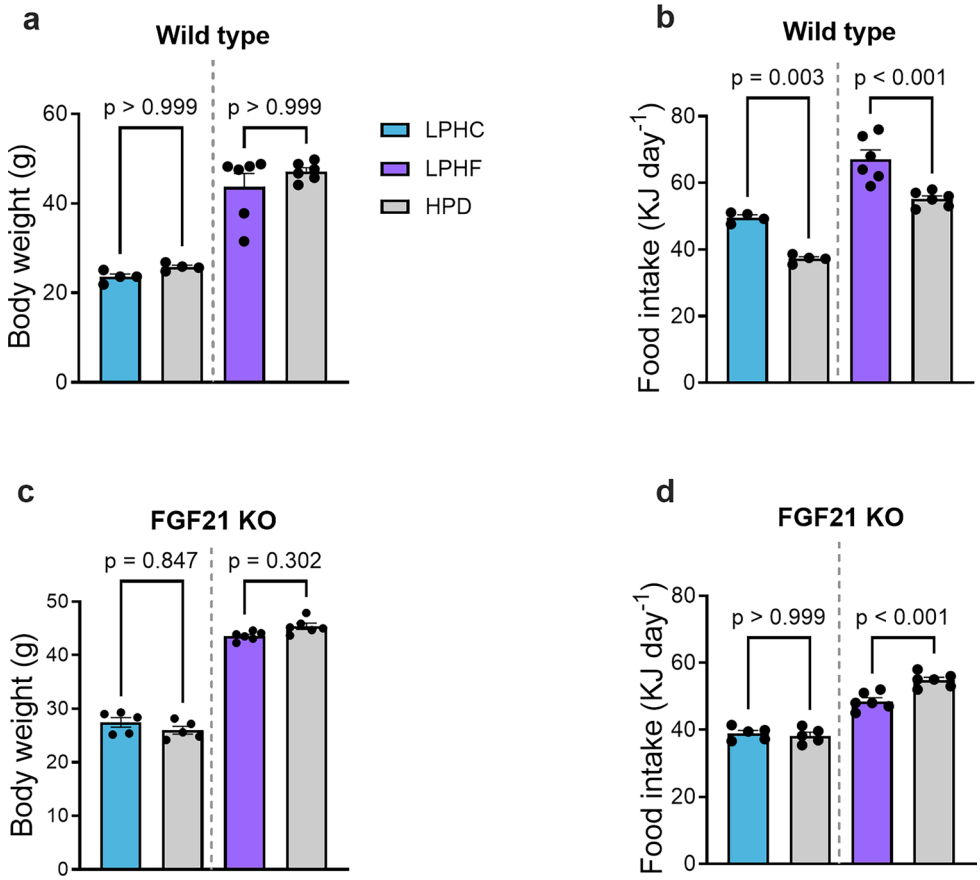

Extended Data Fig. 5 | Effects of a protein-restricted diet on body weight and food intake is FGF21-dependent in mice. (**a**) Body weight and (**b**) food intake in wild type mice fed a low-protein, high-carbohydrate (LPHC) (n = 4), low-protein, high-fat (LPHF) (n = 6) or a standard higher protein diet (HPD) (controls for LPHC; n = 4, controls for LPHF; n = 6) for 12 weeks. (**c**) Body weight and (**d**) food intake in FGF21 KO mice fed a LPHC (n = 5), LPHF (n = 6) or HPD (controls for LPHC; n = 5, controls for LPHF; n = 6) diet for 12 weeks. One-way ANOVA with a Bonferroni post hoc test. Data are mean ± SEM.

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Extended Data Table 1 | Macronutrients in the low-protein, high-carbohydrate (LPHC) meal and higher, habitual protein (HP) meal (study 1)

	LPHC meal	HP meal
Energy (KJ/kg BW)	55	55
Energy (MJ)	4.4	4.4
Protein (E%)	8	16
Protein (g)	21	42
Carbohydrate (E%)	71	57
Fat (E%)	21	27

Values are calculated for an 80 kg individual.

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Extended Data Table 2 | Macronutrients in the low-protein, high-carbohydrate (LPHC) diet in study 1

	LPHC	
	Week 0	Week 5
Energy consumption (MJ)	12.6 ± 0.8	15.0 ± 1.1*
Protein (E%)	9.3 ± 0.0	9.3 ± 0.0
Protein (g/kg body weight)	0.80 ± 0.05	0.96 ± 0.05*
Histidine (mg)	2025 ± 132	2413 ± 182*
Isoleucine (mg)	3402 ± 222	4053 ± 306*
Leucine (mg)	2165 ± 141	2580 ± 195*
Lysine (mg)	790 ± 52	941 ± 71*
Methionine (mg)	2228 ± 145	2655 ± 200*
Threonine (mg)	1638 ± 107	1951 ± 147*
Tryptophan (mg)	709 ± 46	845 ± 64*
Tyrosine (mg)	1254 ± 82	1493 ± 113
Valine (mg)	2597 ± 170	3094 ± 233*
Carbohydrate (E%)	70.0 ± 0.0	70.0 ± 0.0
Glucose (g)	41 ± 3	49 ± 4*
Fructose (g)	50.5 ± 3.3	60.2 ± 4.5*
Dietary fiber (g)	49 ± 3	59 ± 4*
Fat (E%)	21.0 ± 0.0	21.0 ± 0.0
Saturated fatty acids (E%)	5.2 ± 0.0	5.2 ± 0.0
Monounsaturated fatty acids (E%)	7.0 ± 0.0	7.0 ± 0.0
Polyunsaturated fatty acids (E%)	3.3 ± 0.0	3.3 ± 0.0

Data are mean±s.e.m. Two-tailed paired *t*-test was applied to test for differences in macronutrients between the first (week 0) and the last day (week 5) on the LPHC diet. *$P<0.05$; $n=8$.

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Extended Data Table 3 | Registered energy intake

	Registered diet (Study 2: LPHC)	Registered diet (Study 3: LPHF)
Energy consumption (MJ)	13.5 ± 0.6	12.3 ± 0.9
Protein (E%)	20 ± 1	24 ± 2
Protein (g/kg body weight)	1.8 ± 0.1	2.2 ± 0.2
Fat (E%)	36 ± 1	31 ± 2
Carbohydrate (E%)	44 ± 1	43 ± 2

Registered energy intake from dietary registration. Data are mean±s.e.m. LPHC (n=8) and LPHF (n=6).

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Extended Data Table 4 | Calculated essential amino acids in the low-protein, high-carbohydrate (LPHC) diet, low-protein, high-fat (LPHF) diet, and higher habitual protein diet (HPD) in study 2 and 3

	LPHC	LPHF	HPD	WHO, 2007[1]
Histidine	26 ± 1	17 ± 1	52 ± 2	10
Isoleucine	43 ± 2	31 ± 1	84 ± 3	20
Leucine	27 ± 1	51 ± 2	140 ± 6	39
Lysine	10 ± 0	35 ± 1	121 ± 5	30
Methionine	28 ± 1	12 ± 0	41 ± 2	10
Threonine	21 ± 1	23 ± 1	112 ± 4	15
Tryptophan	9 ± 0	9 ± 0	86 ± 3	4
Tyrosine	16 ± 1	21 ± 1	67 ± 3	25
Valine	33 ± 1	38 ± 1	21 ± 1	26

Data are mean±s.e.m. LPHC (n=8), LPHF (n=6) and HPD (n=14). The essential amino acids were calculated in all diet interventions using the database (Vitakost).

https://doi.org/10.1038/s42255-025-01236-7

Extended Data Table 5 | Specification of kits, tracer, insulin, anaesthesia and software used in the studies

PLASMA ANALYSIS	SOURCE	IDENTIFIER
Glucose (whole blood)	ABL800 (Radiometer Medical A/S)	N/A
Insulin ELISA Kit	Mercodia	Cat#10-1113-01
Noradrenaline ELISA Kit	Labor Diagnostika Nord GmbH	Cat# BA E-5400R
Free triiodothyronine (T3) ELISA Kit	Abnova Corporation	Cat# BOXM01090J00056
FGF21 ELISA Kit	R&D Systems	Cat# FGF-21
Amino acids ELISA Kit	Abcam	Cat# ab65347
Glucagon ELISA Kit	Mercodia A/S	Cat# 10-1271-01
Urea Pentra	Trio Lab	Cat#A11A01640
HYPERINSULINEMIC-EUGLYCEMIC CLAMP		
[6,6-D2] glucose tracer. Bolus injection: 2.6 mg/kg. Constant infusion rate: 0.044 mg/kg/min.	Chromatography-mass spectrometry (Automass II)	N/A
Humant insulin Bolus injection: 9.0 mU/kg. Constant infusion rate: 1.0 mU insulin kg/min.	Actrapid, Novo Nordisk A/S	N/A
Xylocaine 2%	AstraZeneca	Cat# 012641
SOFTWARE		
Vitakost	Computer software (2018-2020)	https://app.vitakost.dk/da
SENS	SENS motion system (v5.2)	https://www.sens.dk/da/
Polar Loop-watch or Garmin Vivofit3-watch	Fitnesstracker	N/A
Biorender	Cartoon illustrations were created using Biorender	https://www.biorender.com
Graphpad Prism v8-9	Statistical software	https://www.graphpad.com
Perseus V1.6.14.0	Statistical software	https://maxquant.net/perseus/

nature portfolio

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Reporting Summary

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

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

Data

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

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

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

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

Research involving human participants, their data, or biological material

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

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

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

Field-specific reporting

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

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

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Life sciences study design

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

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

Reporting for specific materials, systems and methods

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

Materials & experimental systems

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

Methods

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

Animals and other research organisms

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

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

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

Plants

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