Supplementary Material

Bone mineral analysis. Femora of all different groups were prepared for bone mineralogy analysis. For more details, please see the Materials and Methods section in the main text. After digestion, bone samples were analyzed by inductively coupled plasma-optical emission spectometry (ICP-OES) for sodium, potassium, magnesium, zinc, calcium, and phosphorus quantities. All determinations were realized in duplicates. The parameters used for the ICP-OES operation (Optima 2000DV; PerkinElmer, Waltham, Massachusetts, USA) are described in Supplementary Table i.

Hormonal effects on body, morphometric, and serum parameters. The ovariectomy led to a substantial reduction in the relative uterus weight of ovariectomized rats (OVX) and ovariectomized rats subjected to a low-protein diet (OVX-LP), but the oestrogen replacement by hormone replacement therapy (HRT) partially recovered the uterus mass in ovariectomized rats subjected to HRT and fed a normoprotein diet (OVX-HRT) and ovariectomized rats subjected to HRT and fed a low-protein diet (OVX-HRT-LP). The hormonal effect accounted for 76% of total variance ($p < 0.001$, two-way ANOVA test) (Supplementary Table ii). Moreover, ovariectomized groups (OVX and OVX-LP) had increased serum luteinizing hormone (LH) and serum follicle-stimulating hormone (FSH) levels ($p < 0.001$, two-way ANOVA test), and protein restriction also reduced LH and FSH levels ($p = 0.041$ for LH and $p = 0.019$ for FSH, two-way ANOVA test) (Supplementary Table ii). In our experimental conditions, the HRT did not reverse the higher LH and FSH in both experimental groups (Supplementary Table ii). In relation to muscle mass, hormone replacement increased the relative femoral weight significantly (accounting for

Table i. Experimental conditions used on inductively coupled plasma–optical emission spectrometry (ICP-OES) (Optima 2000DV; PerkinElmer, Waltham, Massachusetts, USA)

Parameter	Value
Radio frequency power, W	1300
Sample flow, ml min⁻¹	1.5
Integration and reading time, s	10
Main argon flow rate, l min⁻¹	15
Auxiliary argon flow rate, l min⁻¹	0.20
Nebulizing flow rate, l min⁻¹	0.50
Wave length, nm	
Sodium	589.592
Potassium	766.490
Magnesium	285.213
Phosphorus	213.617
Calcium	317.933
Zinc	206.200

18% of total variance; OVX-HRT > C; $p = 0.042$, two-way ANOVA test) (Supplementary Table ii).

Ovariectomized groups (OVX and OVX-LP) had a non-significant increase in total body fat (Supplementary Fig. aa). On the other hand, the metabolic rate showed a non-significant decrease in the OVX group, and was influenced by HRT (hormonal effect accounted for 31% of total variance; $p = 0.0018$, using two-way ANOVA) (Supplementary Fig. ab). The OVX-HRT group had a significant increase in the metabolic rate ($p = 0.02$ vs C; $p = 0.001$ vs OVX, using two-way ANOVA test followed by Fisher's LSD), and the OVX-HRT-LP group slightly enhanced the metabolic rate compared with the LP group (OVX-HRT-LP had $p = 0.0688$ vs LP, using two-way ANOVA test followed by Fisher's LSD) (Supplementary Fig. ab).

Table ii. Additional morphometric and serum parameters in different experimental groups

Parameter	Group						p-value*		
	C	OVX	OVX-HRT	LP	OVX-LP	OVX-HRT-LP	Hormonal effect	Diet effect	Interaction
Mean relative uterus weight, % (SD)	0.19 (0.07)	0.03 (0.01)†	0.14 (0.03)†‡	0.20 (0.05)	0.02 (0.05)†	0.12 (0.01)†‡	< 0.001§	0.673	0.658
Mean relative femur weight, % (SD)	0.27 (0.04)	0.27 (0.01)	0.29 (0.02)‡	0.27 (0.04)	0.25 (0.03)	0.28 (0.02)	0.042§	0.195	0.436
Mean LH, pg/ml (SD)	559 (509)	6869 (4471)†	8249 (6248)†	3146 (222)	4737 (3178)†	3586 (3029)¶	< 0.001§	0.041§	0.271
Mean FSH, pg/ml (SD)	752 (434)	15 439 (11 234)†	19 439 (14 550)†	264 (146)	7589 (3167)	9359 (6401)†¶	< 0.001§	0.019§	0.274

*Significant difference accounted for hormonal, diet or interaction effects, analyzed by two-way analysis of variance (ANOVA), followed by Fisher's least significant difference (LSD) method
†Hormonal effect: $p \leqslant 0.05$ versus control-matched group (C or LP)
‡$p \leqslant 0.05$ vs OVX-matched group (OVX or OVX-LP)
§Statistically significant
¶Diet effect: $p \leqslant 0.05$ vs control hormonal-matched group (C, OVX, or OVX-HRT)
C, control; OVX, ovariectomized rats; OVX-HRT, ovariectomized rats subjected to HRT and fed a normoprotein diet; LP, low-protein; OVX-LP, ovariectomized rats subjected to a low-protein diet; OVX-HRT-LP, ovariectomized rats subjected to HRT and fed a low-protein diet; LH, serum luteinizing hormone; FSH, serum follicle-stimulating hormone
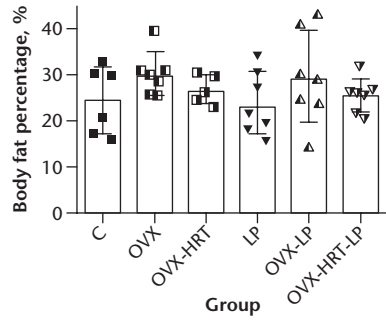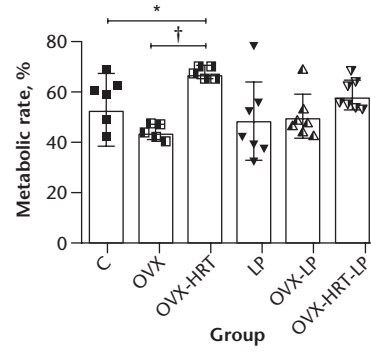
Fig. aa

Fig. ab

a) Percentage of body fat assessed by dual-energy x-ray absorptiometry (DXA), and b) metabolic rate from different experimental groups. Results are expressed as the mean (SD). Significant difference accounted for hormonal, diet, or interaction effects, analyzed by two-way analysis of variance (ANOVA), followed by Fisher's least significant difference (LSD) method. *Hormonal effect: $p \leq 0.05$ versus control-matched group (C or LP); †$p \leq 0.05$ versus OVX-matched group (OVX or OVX-LP). C, control; OVX, ovariectomized rats; OVX-HRT, ovariectomized rats subjected to HRT and fed a normoprotein diet; LP, low-protein; OVX-LP, ovariectomized rats subjected to a low-protein diet; OVX-HRT-LP, ovariectomized rats subjected to HRT and fed a low-protein diet.