Supporting Information

Matarese et al. 10.1073/pnas.1214554110

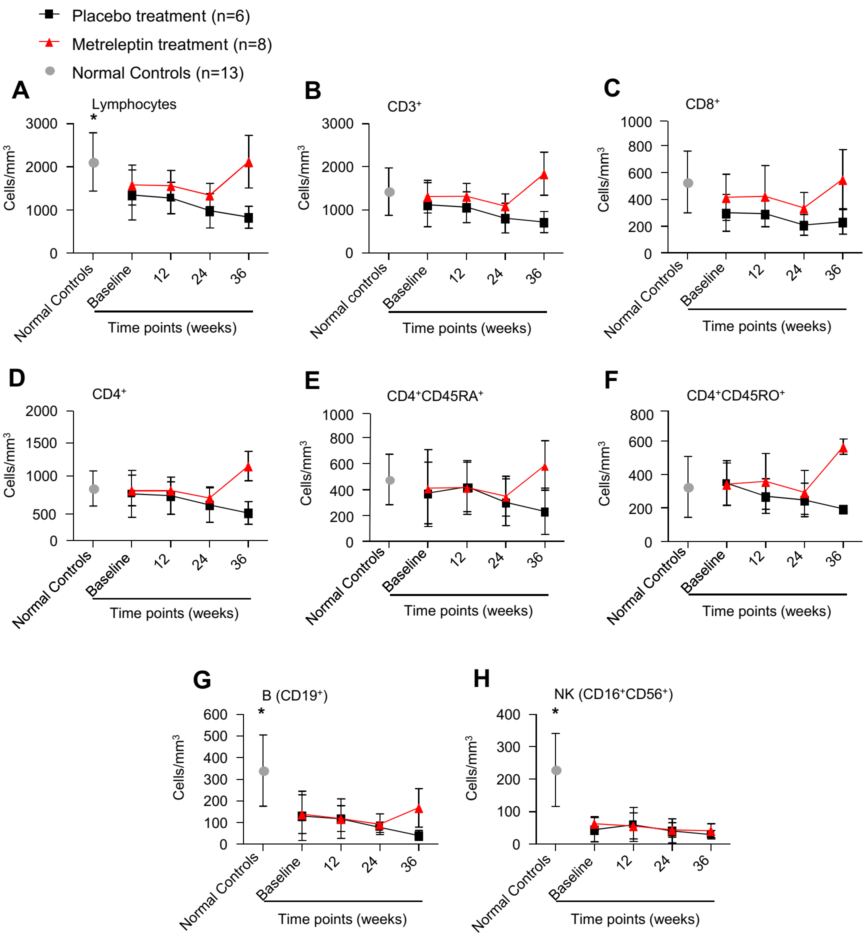

Fig. S1. Absolute number of lymphocytes, T cells, B cells, and natural killer (NK) cells in hypothalamic amenorrhea (HA) subjects over time compared with healthy controls. (*A*, *G*, and *H*) At baseline, the total populations of lymphocytes, B cells, and NK cells were significantly lower in HA subjects than in controls (*$P < 0.05$). (*B–F*) CD3$^+$, CD4$^+$, CD8$^+$ T, CD4$^+$CD45RA$^+$, and CD4$^+$CD45RO$^+$ cell populations were not different in HA subjects and normal controls at baseline.

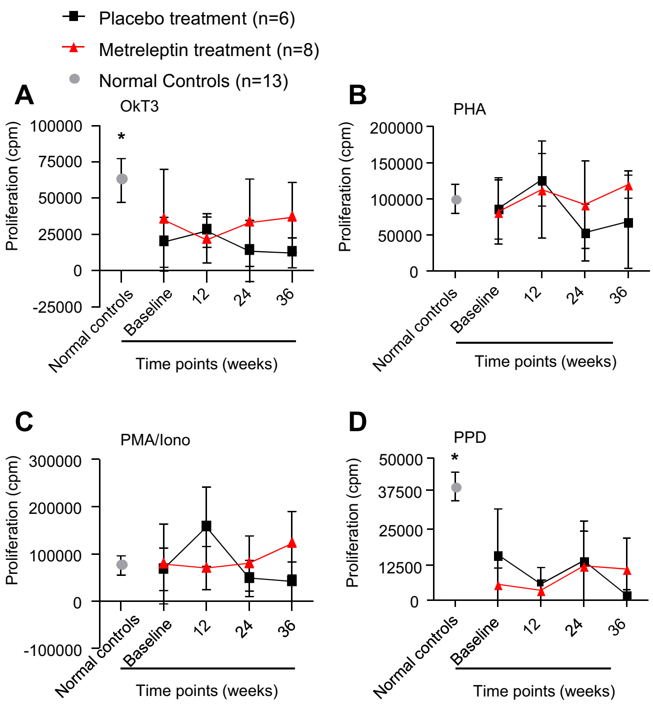

Fig. S2. Proliferative response of peripheral blood mononuclear cells (PBMCs) to OKT3 (*A*), phytohemagglutinin (PHA) (*B*), phorbol 12-myristate 13-acetate plus ionomycin (PMA/Iono) (*C*), and purified protein derivative (PPD) (*D*) in HA subjects over time compared with healthy controls. At baseline, the proliferative response to OKT3 (*A*) and PPD (*D*) stimulation was significantly lower (*$P < 0.05$) in HA subjects than in healthy controls.

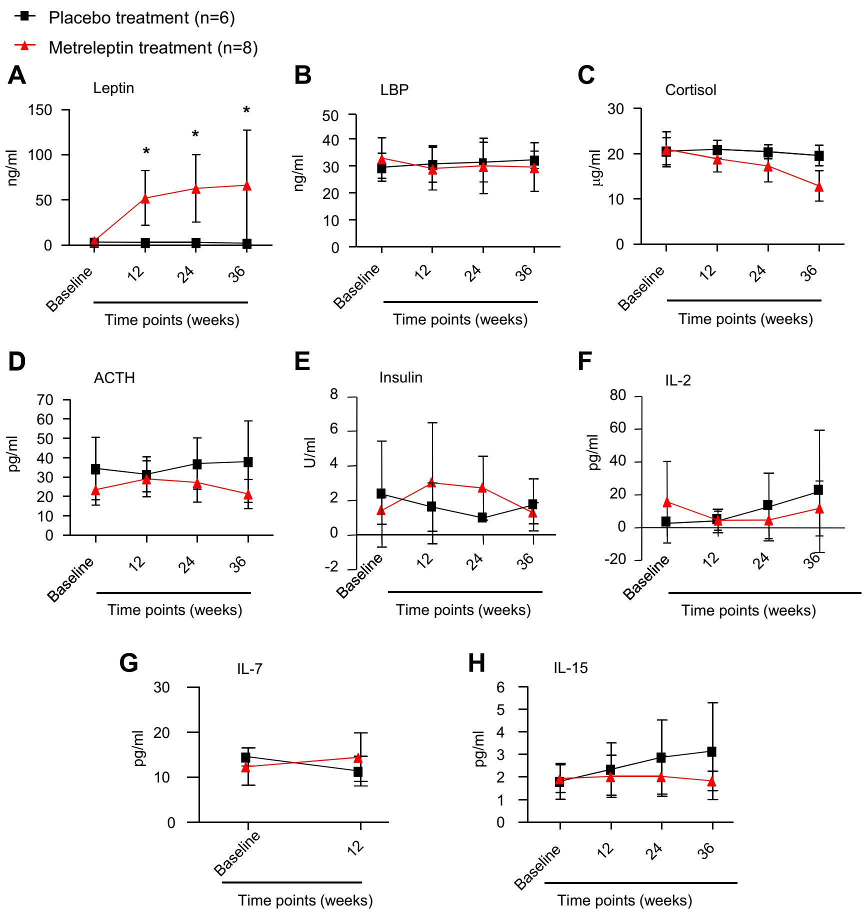

Fig. S3. Levels of hormones and cytokines in HA subjects over time. Only leptin levels (*A*) were significantly higher in the time course. There were no significant differences in the parameters analyzed in *B–H*.

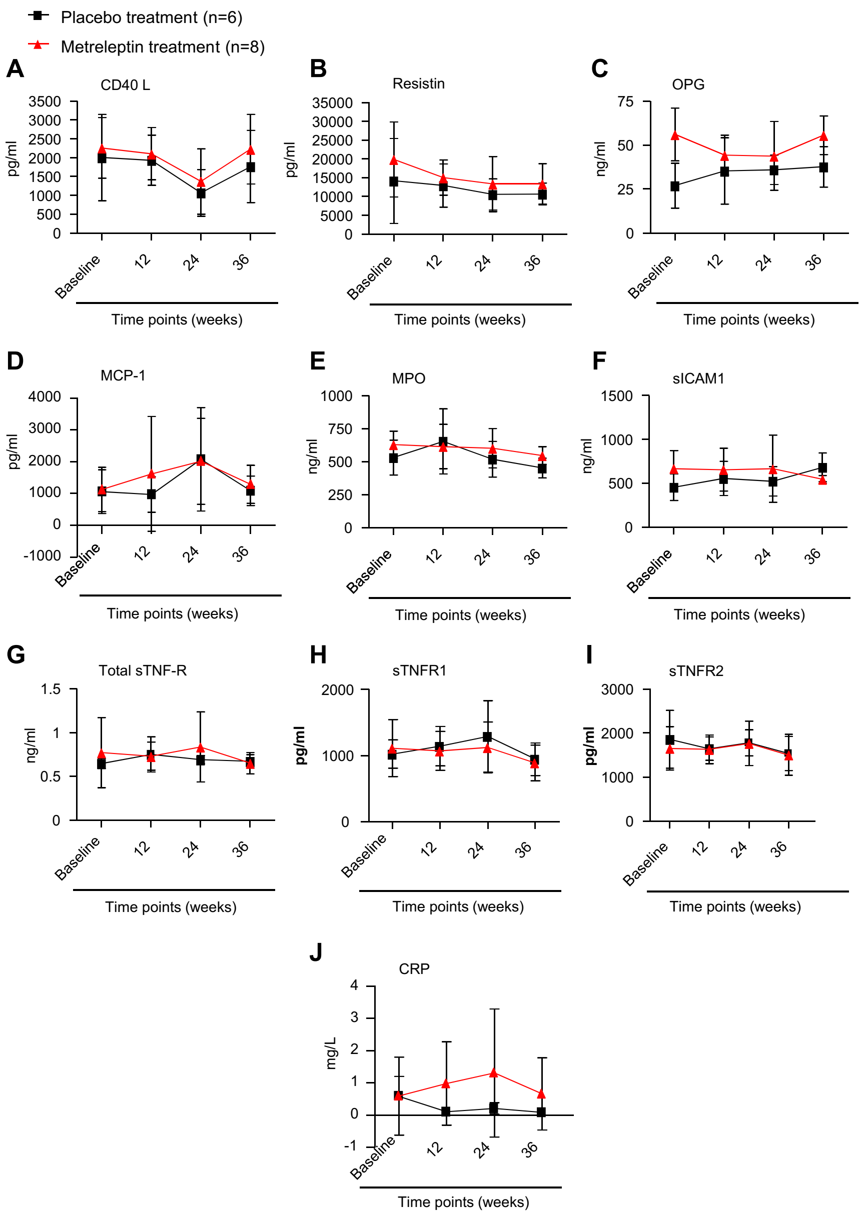

Fig. S4. Levels of inflammatory and metabolic parameters in HA subjects over time. There were no statistically significant differences in the metabolic/inflammatory parameters analyzed in *A*–*J*.

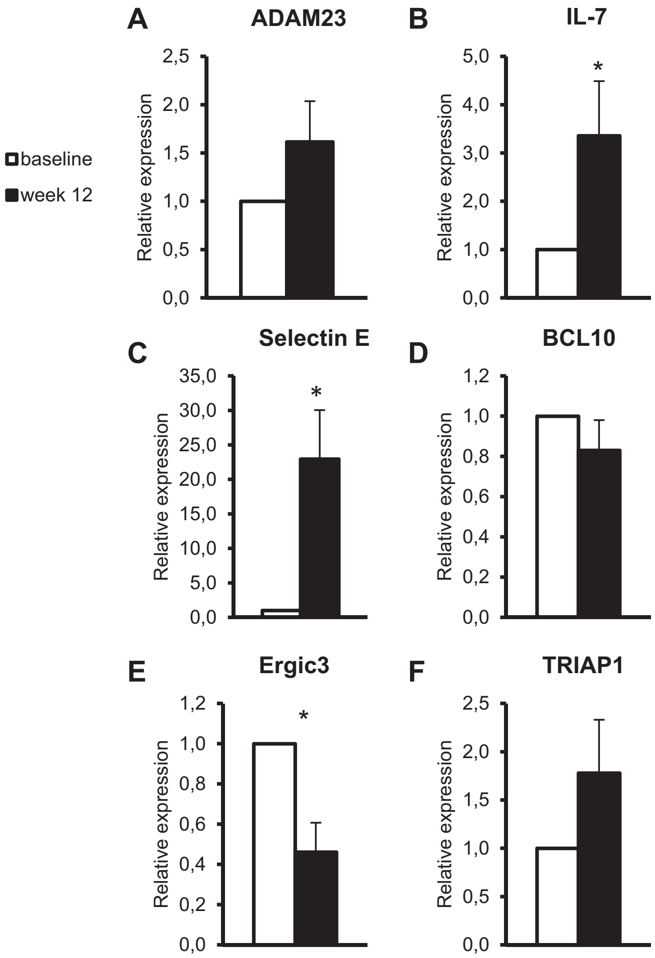

Fig. S5. Gene-expression levels were verified by real-time PCR. Metreleptin-treated groups at week 12 were compared with the baseline levels. Gene-expression levels of IL-7 (*B*), selectin E (*C*), and Ergic3 (*E*) were changed significantly (*P ≤ 0.05) compared with their baseline levels. Gene-expression levels of ADAM-23 (*A*), BCL-10 (*D*), and TRIAP-1 (*F*) did not change significantly from baseline.

Table S1. Differences in gene-expression profile in response to metreleptin treatment at week 24 vs. baseline

Gene symbol	Gene assignment	Gene ID	*P* value	Fold change
Cacng8	Calcium channel, voltage-dependent, gamma subunit 8	59283	0.00197222	0.74792967
ZNF490	Zinc finger protein 490	57474	0.00107306	0.59417048
CENPI	Centromere protein I	2491	0.00724578	0.45477823
LELP1	Late cornified envelope-like proline-rich 1	149018	0.00013992	0.42264562
DPPA5	Developmental pluripotency associated 5	340168	0.00743253	0.33456177
ARNT	Aryl hydrocarbon receptor nuclear translocator	405	0.00860958	0.31569146
WDR53	WD repeat domain 53	348793	0.00494466	0.29463523
SPHKAP	SPHK1 interactor, AKAP domain containing	80309	0.00351039	0.2461562
DBT	Dihydrolipoamide branched chain transacylase E2	1629	0.0099502	0.23485338
BCL2L11	BCL2-like 11 (apoptosis facilitator)	10018	0.00543426	−0.3127599
PRDM2	PR domain containing 2, with ZNF domain	7799	0.00790638	−0.322973
Cnr2	Cannabinoid receptor 2 (macrophage)	1269	0.00416617	−0.3281563
TGFBRAP1	Transforming growth factor beta receptor-associated protein 1	9392	0.00359646	−0.3439002
ZNF592	Zinc finger protein 592	9640	0.00782736	−0.3622582
MED10	Mediator complex subunit 10	84246	0.00406945	−0.547997
STX10	Syntaxin 10	8677	0.00218949	−0.751147

Table S2. Differences in gene-expression profile in response to metreleptin treatment at week 36 vs. baseline

Gene symbol	Gene assignment	Gene ID	*P* value	Fold change
TNFRSF10C	Tumor necrosis factor receptor superfamily, member 10c, decoy without an intracellular domain	8794	0.006717	0.90305714
ZNF200	Zinc finger protein 200	7752	0.00610212	0.54233656
f2rl1	Coagulation factor II (thrombin) receptor-like 1	2150	0.00829106	0.51888316
GAST	Gastrin	2520	0.00128913	0.46499107
GLI1	GLI family zinc finger 1	2735	0.00939256	0.45136181
CYTH4	Cytohesin 4	27128	0.00437337	0.36625752
bbs7	Bardet–Biedl syndrome 7	55212	0.00857128	0.27351589
ilf2	Interleukin enhancer binding factor 2, 45kDa	3608	0.00451506	−0.692222
TXNIP	Thioredoxin interacting protein	10628	0.00791743	−0.4120624
Cdk5r1	Cyclin-dependent kinase 5, regulatory subunit 1 (p35)	8851	0.00957235	−0.4554088
GFI1	Growth factor independent 1 transcription repressor	2672	0.0062539	−0.5224277
ZBTB16	Zinc finger and BTB domain containing 16	7704	0.00228704	−0.7820146

Table S3. Sequence of primers for RT-PCR microarray validation

Gene name	Forward	Reverse
18s	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG
ADAM23	GCTACAATGGCGAGTGCAAG	CTCCATCCTTCCCGCAGTTT
SELE	GTGAAGCTCCCACTGAGTCC	AGCCAGAGGAGAAATGGTGC
IL7	TTTATTCCGTGCTGCTCGC	CCAGGGCAGCTGGTTTTCTT
Ergic3	GAAGTCAATAAGGTGGCCGGA	AGCTCTGCAAGTCATGGATCT
TRIAP1	CTGTCGCCATGAACAGTGTG	AAATTTCTCGGCGAACCAGC
BCL10	TTCCAGATGGAGCCACGAAC	CGTCGTGCTGGATTCTCCTT

Other Supporting Information Files

Dataset S1 (XLSX)
Dataset S2 (XLSX)
Dataset S3 (XLSX)