ELECTRONIC SUPPLEMENTARY INFORMATION

METHODS

Leukocyte isolation

Peripheral blood leukocytes were immediately obtained after blood draw from EDTA anticoagulated blood samples after incubation for 60 minutes with cold dextran 3% (1:1) at 4ºC. Cells in the supernatant were diluted in NaCl 0.9% up to 50 ml and centrifuged at 525 x g for 10 min at 4ºC. Cells were then lysed by osmotic shock during 50 seconds and subsequently washed twice at 325xg and 250xg in NaCl 0.9% at 4ºC. Cell pellets were resuspended in cold PBS, counted in a Neubauer chamber and finally centrifuged at 250xg for 10 min to be stored in RNAlater (Sigma-Aldrich Química S.A., Alcobendas, Spain) at -80ºC.

Quality control and relative quantification (qPCR)

RNA yield, quality and purity were assessed with a NanoDrop2000 spectrophotometer and the Qubit RNA HS Assay kit in a Qubit3.0 fluorimeter (ThermoFisher Scientific – Spain). We randomly selected more than 30 samples to evaluate the RNA integrity number (RIN) with the High Sensitivity RNA ScreenTape in a TapeStation2200 (Agilent Technologies). All the samples evaluated presented RINs > 8.5.

To test our samples for factors that could lead to unreliable results in SYBR Green qPCR we evaluated 24 unselected samples with the Human RT2 RNA QC PCR array (Qiagen, Hilden, Germany) prior to proceeding with complete gene expression experiments. We also evaluated seven genes (*B2M*, *GUSB*, *ACTB*, *HPRT1*, *TBP*, *RPS18* and *GADPH*) to determine their usability as reference genes. *RPS18* and *HPRT1* showed a more consistent expression in our samples and therefore were selected as reference genes for data normalization (Primer sets, buffer and enzyme are available from AnyGenes, Paris, France). For the relative quantification of gene expression levels we used the comparative threshold cycle method.[1] A quantification cycle (Cq) was obtained for each amplification curve and a ΔCq value was first calculated by subtracting the average Cq value of *RPS18* and *HPRT1* from the Cq value of the gene of interest. Data were expressed as arbitrary units using the following transformation [expression = $\log2^{-\Delta Cq}$].

1.   K. J. Livak and T. D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, *Methods*, 2001, **25**, 402-408.

Table S1. Correlations between the responses during the oral loads (AUCs) and their corresponding fasting levels.

Fasting	AUC	All subjects (n = 53)	Subjects without obesity (n = 28)	Subjects with obesity (n = 25)
Serum Ghrelin	Ghrelin during glucose load	ρ = -0.843, *P* < 0.001	ρ = -0.854, *P* < 0.001	ρ = -0.740, *P* < 0.001
	Ghrelin during lipid load	ρ = -0.716, *P* < 0.001	ρ = -0.852, *P* < 0.001	ρ = -0.425, *P* = 0.043
	Ghrelin during protein load	ρ = -0.661, *P* < 0.001	ρ = -0.739, *P* < 0.001	ρ = -0.310, *P* = 0.161
GHRL expression	*GHRL* expression during glucose load	r = -0.327, *P* = 0.017	r = -0.403, *P* = 0.030	r = -0.214, *P* = 0.305
	GHRL expression during lipid load	r = -0.495, *P* < 0.001	r = -0.501, *P* = 0.007	r = -0.464, *P* = 0.020
	GHRL expression during protein load	r = -0.135, *P* = 0.336	r = -0.195, *P* = 0.320	r = -0.085, *P* = 0.686
Serum Leptin	Leptin during glucose load	ρ = -0.306, *P* = 0.026	ρ = -0.563, *P* = 0.002	ρ = -0.144, *P* = 0.493
	Leptin during lipid load	ρ = -0.319, *P* = 0.020	ρ = -0.577, *P* = 0.001	ρ = -0.234, *P* = 0.261
	Leptin during protein load	ρ = -0.737, *P* < 0.001	ρ = -0.857, *P* < 0.001	ρ = -0.628, *P* = 0.001
LEPR expression	*LEPR* expression during glucose load	r = -0.575, *P* < 0.001	r = -0.691, *P* < 0.001	r = -0.352, *P* = 0.085
	LEPR expression during lipid load	r = -0.499, *P* < 0.001	r = -0.526, *P* = 0.004	r = -0.421, *P* = 0.036
	LEPR expression during protein load	r = -0.409, *P* = 0.002	r = -0.461, *P* = 0.014	r = -0.487, *P* = 0.014
Serum sLepR	sLepR during glucose load	ρ = -0.287, *P* = 0.037	ρ = -0.065, *P* = 0.741	ρ = -0.575, *P* = 0.003
	sLepR during lipid load	ρ = -0.209, *P* = 0.133	ρ = -0.305, *P* = 0.114	ρ = -0.041, *P* = 0.844
	sLepR during protein load	ρ = -0.377, *P* = 0.005	ρ = -0.485, *P* = 0.009	ρ = -0.199, *P* = 0.341
Serum Adiponectin	Adiponectin during glucose load	ρ = -0.055, *P* = 0.696	ρ = -0.187, *P* = 0.342	ρ = 0.298, *P* = 0.148
	Adiponectin during lipid load	ρ = -0.440, *P* = 0.001	ρ = -0.252, *P* = 0.206	ρ = -0.685, *P* < 0.001
	Adiponectin during protein load	ρ = -0.542, *P* < 0.001	ρ = -0.691, *P* < 0.001	ρ = -0.280, *P* = 0.175
ADIPOR1 expression	*ADIPOR1* expression during glucose load	r = -0.272, *P* = 0.049	r = -0.184, *P* = 0.349	r = -0.403, *P* = 0.046
	ADIPOR1 expression during lipid load	r = -0.567, *P* < 0.001	r = -0.525, *P* = 0.004	r = -0.609, *P* = 0.001
	ADIPOR1 expression during protein load	r = -0.101, *P* = 0.473	r = -0.190, *P* = 0.333	r = -0.056, *P* = 0.791

Data were submitted to Pearson's (r) or Spearman's (ρ) correlation analysis as needed.

Table S2. Fasting levels and during the distinct macronutrient challenges of circulating ghrelin and *GHRL* expression.

		Control women		Women with PCOS		Control men		P value	P value	P value
		No obesity (n = 9)	Obesity (n = 8)	No obesity (n = 9)	Obesity (n = 8)	No obesity (n = 10)	Obesity (n = 9)	Oral load	Oral load x Group	Oral load x Obesity
Serum levels										
Ghrelin (pg/ml)	Glucose Load (0 h)	27.5 ± 21.7	11.5 ± 3.1	24.4 ± 9.8	17.6 ± 8.7	21.1 ± 15.0	15.2 ± 9.1			
	Glucose Load (1 h)	19.5 ± 13.7	10.2 ± 2.3	14.3 ± 8.4	10.9 ± 5.0	15.0 ± 10.8	10.7 ± 2.9	<0.001	0.125	0.034
	Glucose Load (2 h)	15.8 ± 8.0	13.5 ± 7.1	14.2 ± 7.0	16.8 ± 9.8	21.4 ± 16.5	14.2 ± 5.5			
	Lipid Load (0 h)	33.7 ± 19.0	19.5 ± 17.8	26.1 ± 15.0	17.7 ± 7.3	24.9 ± 29.9	14.3 ± 5.5			
	Lipid Load (2 h)	22.7 ± 11.3	15.5 ± 10.2	17.7 ± 8.4	15.3 ± 9.9	21.8 ± 17.7	13.9 ± 4.8	0.001	0.022	0.364
	Lipid Load (4 h)	16.2 ± 7.4	13.1 ± 4.2	23.1 ± 18.5	12.9 ± 2.5	22.1 ± 19.2	15.6 ± 5.2			
	Protein Load (0 h)	36.4 ± 35.5	16.9 ± 7.1	42.5 ± 23.5	22.8 ± 14.6	38.1 ± 20.1	20.6 ± 9.8			
	Protein Load (1 h)	39.5 ± 31.0	17.6 ± 11.8	25.7 ± 10.6	30.3 ± 17.4	23.0 ± 8.1	20.7 ± 10.4	0.495	0.098	0.091
	Protein Load (2 h)	40.7 ± 37.2	17.7 ± 7.9	28.4 ± 26.9	19.3 ± 7.3	46.6 ± 40.6	19.8 ± 7.4			
Leukocyte gene expression										
GHRL (a.u.)	Glucose Load (0 h)	-1.59 ± 0.14	-1.62 ± 0.11	-1.58 ± 0.16	-1.61 ± 0.13	-1.66 ± 0.16	-1.53 ± 0.17			
	Glucose Load (1 h)	-1.50 ± 0.15	-1.55 ± 0.12	-1.62 ± 0.14	-1.57 ± 0.17	-1.60 ± 0.22	-1.42 ± 0.19	0.006	0.130	0.265
	Glucose Load (2 h)	-1.46 ± 0.15	-1.60 ± 0.10	-1.62 ± 0.10	-1.61 ± 0.16	-1.57 ± 0.20	-1.45 ± 0.14			
	Lipid Load (0 h)	-1.61 ± 0.20	-1.63 ± 0.18	-1.65 ± 0.17	-1.63 ± 0.10	-1.67 ± 0.18	-1.54 ± 0.13			
	Lipid Load (2 h)	-1.61 ± 0.24	-1.66 ± 0.17	-1.59 ± 0.14	-1.65 ± 0.14	-1.64 ± 0.14	-1.53 ± 0.11	0.259	0.750	0.486
	Lipid Load (4 h)	-1.60 ± 0.20	-1.68 ± 0.12	-1.71 ± 0.13	-1.64 ± 0.10	-1.66 ± 0.18	-1.56 ± 0.15			
	Protein Load (0 h)	-1.59 ± 0.15	-1.58 ± 0.15	-1.63 ± 0.10	-1.57 ± 0.07	-1.65 ± 0.15	-1.50 ± 0.08			
	Protein Load (1 h)	-1.64 ± 0.17	-1.61 ± 0.18	-1.62 ± 0.14	-1.57 ± 0.19	-1.65 ± 0.17	-1.47 ± 0.17	0.859	0.565	0.647
	Protein Load (2 h)	-1.64 ± 0.21	-1.62 ± 0.09	-1.60 ± 0.11	-1.56 ± 0.15	-1.63 ± 0.18	-1.52 ± 0.15			

Data are means ± SD. Data were analyzed by univariate general linear models for repeated measures introducing fasting and postprandial levels as within-subjects factor (to evaluate differences from fasting levels: *Oral load P* value), and Obesity and Group as between-subjects factor (to evaluate their influence in the postprandial response: *Oral load x* Group and *Oral load x Obesity P* values). PCOS, polycystic ovary syndrome.

Table S3. Fasting levels and during the distinct macronutrient challenges of circulating leptin, sLepR and *LEPR* expression.

		Control women		Women with PCOS		Control men		*P* value Oral load	*P* value Oral load x Group	*P* value Oral load x Obesity
		No obesity (n = 9)	Obesity (n = 8)	No obesity (n = 9)	Obesity (n = 8)	No obesity (n = 10)	Obesity (n = 9)			
Serum levels										
Leptin (ng/ml)	Glucose Load (0 h)	43 ± 33	131 ± 118	38 ± 36	125 ± 93	7 ± 4	25 ± 16			
	Glucose Load (1 h)	40 ± 26	140 ± 132	35 ± 31	118 ± 83	6 ± 4	24 ± 15	0.069	0.360	0.508
	Glucose Load (2 h)	42 ± 34	144 ± 152	35 ± 32	119 ± 94	6 ± 4	26 ± 17			
	Lipid Load (0 h)	61 ± 51	158 ± 110	52 ± 33	145 ± 69	9 ± 4	35 ± 22			
	Lipid Load (2 h)	61 ± 52	154 ± 109	46 ± 29	147 ± 68	8 ± 4	31 ± 21	<0.001	0.024	0.181
	Lipid Load (4 h)	55 ± 47	143 ± 95	45 ± 29	147 ± 71	7 ± 4	30 ± 22			
	Protein Load (0 h)	62 ± 32	147 ± 97	54 ± 38	128 ± 56	7 ± 3	42 ± 32			
	Protein Load (1 h)	57 ± 29	122 ± 80	49 ± 35	122 ± 58	7 ± 3	33 ± 20	<0.001	0.750	0.675
	Protein Load (2 h)	52 ± 27	122 ± 85	47 ± 35	116 ± 58	6 ± 3	31 ± 20			
sLepR (ng/ml)	Glucose Load (0 h)	21.0 ± 5.7	16.4 ± 2.1	18.6 ± 4.4	16.3 ± 2.6	19.8 ± 5.2	14.1 ± 2.0			
	Glucose Load (1 h)	20.6 ± 6.9	15.3 ± 2.2	17.6 ± 4.0	14.3 ± 2.1	18.8 ± 5.9	13.8 ± 2.1	0.001	0.661	0.773
	Glucose Load (2 h)	20.4 ± 7.1	15.0 ± 2.1	18.1 ± 3.3	14.7 ± 2.1	18.5 ± 4.9	14.1 ± 2.1			
	Lipid Load (0 h)	19.9 ± 4.1	14.2 ± 6.3	16.6 ± 8.3	13.6 ± 6.2	17.0 ± 6.2	13.4 ± 4.8			
	Lipid Load (2 h)	17.5 ± 4.5	14.4 ± 6.8	17.3 ± 9.0	13.3 ± 6.2	15.3 ± 5.5	13.1 ± 6.2	0.042	0.410	0.178
	Lipid Load (4 h)	17.9 ± 4.7	14.2 ± 6.8	16.8 ± 6.8	12.7 ± 5.0	13.2 ± 6.2	13.4 ± 5.7			
	Protein Load (0 h)	22.9 ± 4.8	15.0 ± 3.3	17.4 ± 5.3	15.7 ± 5.7	18.2 ± 6.2	14.7 ± 5.6			
	Protein Load (1 h)	20.0 ± 4.7	14.3 ± 4.5	14.5 ± 3.7	14.0 ± 5.6	17.2 ± 5.8	14.7 ± 6.3	0.001	0.128	0.139
	Protein Load (2 h)	20.1 ± 4.9	14.6 ± 4.8	16.8 ± 5.1	15.6 ± 5.9	17.6 ± 6.5	14.6 ± 5.7			
Leukocyte gene expression										
LEPR (a.u.)	Glucose Load (0 h)	-1.91 ± 0.18	-1.96 ± 0.15	-1.91 ± 0.19	-1.90 ± 0.14	-1.95 ± 0.15	-1.87 ± 0.11			
	Glucose Load (1 h)	-1.87 ± 0.16	-1.91 ± 0.15	-1.89 ± 0.08	-1.90 ± 0.15	-1.89 ± 0.15	-1.77 ± 0.14	0.004	0.670	0.951
	Glucose Load (2 h)	-1.85 ± 0.14	-1.91 ± 0.14	-1.90 ± 0.16	-1.84 ± 0.07	-1.86 ± 0.16	-1.80 ± 0.08			
	Lipid Load (0 h)	-1.89 ± 0.13	-1.90 ± 0.11	-1.85 ± 0.12	-1.76 ± 0.13	-1.91 ± 0.18	-1.79 ± 0.11			
	Lipid Load (2 h)	-1.90 ± 0.11	-1.87 ± 0.13	-1.82 ± 0.11	-1.82 ± 0.14	-1.88 ± 0.17	-1.84 ± 0.13	0.655	0.265	0.238
	Lipid Load (4 h)	-1.91 ± 0.12	-1.92 ± 0.13	-1.85 ± 0.14	-1.73 ± 0.14	-1.85 ± 0.12	-1.79 ± 0.10			
	Protein Load (0 h)	-1.91 ± 0.14	-1.87 ± 0.09	-1.87 ± 0.13	-1.82 ± 0.12	-1.85 ± 0.15	-1.85 ± 0.06			
	Protein Load (1 h)	-1.92 ± 0.16	-1.91 ± 0.12	-1.91 ± 0.12	-1.83 ± 0.11	-1.87 ± 0.12	-1.82 ± 0.10	0.662	0.817	0.089
	Protein Load (2 h)	-1.92 ± 0.10	-1.90 ± 0.14	-1.93 ± 0.14	-1.76 ± 0.08	-1.88 ± 0.15	-1.80 ± 0.10			

Data are means ± SD. Gene expression values are showed as arbitrary units (a.u.) using the equation $\log 2^{-\Delta Cq}$. Data were analyzed by univariate general linear models for repeated measures introducing fasting and postprandial values as within-subjects factor (to evaluate differences from fasting levels: *Oral load P* value), and Obesity and Group as between-subjects factor (to evaluate their influence in the postprandial response: *Oral load x Group* and *Oral load x Obesity P* values). PCOS, polycystic ovary syndrome.

Table S4. Fasting levels and during the distinct macronutrient challenges of circulating adiponectin and *ADIPOR1* expression.

		Control women				Women with PCOS				Control men				*P* value	*P* value	*P* value
		No obesity (n = 9)		Obesity (n = 8)		No obesity (n = 9)		Obesity (n = 8)		No obesity (n = 10)		Obesity (n = 9)		Oral load	Oral load x Group	Oral load x Obesity
Serum levels																
	Glucose Load (0 h)	59.6 ± 95.9		16.8 ± 12.4		58.8 ± 44.0		34.9 ± 33.5		13.3 ± 9.1		13.8 ± 9.1				
	Glucose Load (1 h)	54.9 ± 70.7		20.6 ± 23.8		54.7 ± 44.1		32.6 ± 27.6		10.4 ± 6.5		13.1 ± 9.1		0.582	0.060	0.286
	Glucose Load (2 h)	51.0 ± 64.3		17.3 ± 11.7		59.3 ± 53.1		47.2 ± 53.7		10.8 ± 7.1		14.8 ± 13.6				
Adiponectin (µg/ml)	Lipid Load (0 h)	28.1 ± 27.4		24.1 ± 30.4		48.1 ± 32.5		22.7 ± 19.0		11.0 ± 6.9		16.2 ± 13.9				
	Lipid Load (2 h)	33.0 ± 33.1		21.4 ± 19.7		35.8 ± 15.8		21.6 ± 19.3		12.6 ± 7.6		15.4 ± 11.7		0.994	0.175	0.983
	Lipid Load (4 h)	29.5 ± 18.5		22.0 ± 21.7		42.7 ± 32.5		18.9 ± 12.4		11.3 ± 6.2		14.1 ± 11.2				
	Protein Load (0 h)	59.9 ± 66.7		19.6 ± 17.5		66.9 ± 84.2		26.2 ± 24.0		12.8 ± 6.6		15.3 ± 10.7				
	Protein Load (1 h)	39.0 ± 31.4		18.4 ± 13.8		50.4 ± 47.4		26.1 ± 30.9		10.3 ± 5.6		15.0 ± 10.9		0.050	0.930	0.128
	Protein Load (2 h)	53.5 ± 64.4		18.0 ± 14.4		40.1 ± 20.4		27.2 ± 26.5		11.3 ± 6.4		17.3 ± 13.3				
Leukocyte gene expression																
	Glucose Load (0 h)	-0.18 ± 0.10		-0.21 ± 0.11		-0.22 ± 0.10		-0.18 ± 0.11		-0.25 ± 0.13		-0.12 ± 0.20				
	Glucose Load (1 h)	-0.08 ± 0.17		-0.10 ± 0.13		-0.16 ± 0.14		-0.12 ± 0.12		-0.18 ± 0.15		0.00 ± 0.19		<0.001	0.623	0.764
	Glucose Load (2 h)	-0.08 ± 0.18		-0.14 ± 0.07		-0.19 ± 0.13		-0.14 ± 0.14		-0.17 ± 0.15		-0.04 ± 0.17				
ADIPOR1 (a.u.)	Lipid Load (0 h)	-0.17 ± 0.13		-0.31 ± 0.10		-0.27 ± 0.13		-0.18 ± 0.10		-0.29 ± 0.11		-0.16 ± 0.14				
	Lipid Load (2 h)	-0.13 ± 0.14		-0.25 ± 0.04		-0.20 ± 0.15		-0.19 ± 0.13		-0.19 ± 0.10		-0.11 ± 0.10		0.004	0.765	0.507
	Lipid Load (4 h)	-0.12 ± 0.10		-0.27 ± 0.08		-0.23 ± 0.13		-0.14 ± 0.10		-0.21 ± 0.14		-0.12 ± 0.14				
	Protein Load (0 h)	-0.19 ± 0.11		-0.19 ± 0.07		-0.21 ± 0.09		-0.12 ± 0.08		-0.24 ± 0.11		-0.15 ± 0.10				
	Protein Load (1 h)	-0.19 ± 0.10		-0.19 ± 0.12		-0.20 ± 0.10		-0.17 ± 0.09		-0.25 ± 0.15		-0.07 ± 0.16		0.035	0.399	0.839
	Protein Load (2 h)	-0.16 ± 0.12		-0.16 ± 0.11		-0.18 ± 0.11		-0.09 ± 0.08		-0.23 ± 0.15		-0.10 ± 0.14				

Data are means ± SD. Gene expression values are showed as arbitrary units (a.u.) using the equation $\log2^{-\Delta Cq}$. Data were analyzed by univariate general linear models for repeated measures introducing fasting and postprandial levels as within-subjects factor (to evaluate differences from fasting levels: *Oral load P* value), and Obesity and Group as between-subjects factor (to evaluate their influence in the postprandial response: *Oral load x* Group and *Oral load x Obesity P* values). PCOS, polycystic ovary syndrome.

Table S5. Correlations between ISI and HOMA-IR and the areas under the curve (AUC) of serum ghrelin, leptin, sLepR and adiponectin during the different macronutrient oral challenges.

Oral Challenge	Ghrelin AUC		Leptin AUC		sLepR AUC		Adiponectin AUC	
	ρ	p	ρ	p	ρ	p	ρ	p
Glucose load								
ISI	-0.215	0.126	0.011	0.937	0.001	0.995	0.103	0.463
HOMA-IR	**0.263**	**0.060**	-0.090	0.521	0.109	0.439	-0.010	0.941
Lipid load								
ISI	-0.161	0.260	-0.060	0.672	-0.094	0.504	-0.229	0.102
HOMA-IR	**0.234**	**0.098**	0.055	0.698	0.083	0.556	0.027	0.847
Protein load								
ISI	**-0.251**	**0.082**	**0.315**	**0.023**	**-0.270**	**0.051**	**-0.298**	**0.030**
HOMA-IR	**0.385**	**0.006**	-0.214	0.128	**0.345**	**0.011**	**0.347**	**0.011**

Data were submitted to Spearman´s correlation analysis. Correlations with $p < 0.10$ are highlighted in boldface. HOMA-IR, homeostasis model assessment of insulin resistance; ISI, insulin sensitivity index.