Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Farooqi IS, Wangensteen T, Collins S, et al. Clinical and molecular genetic spectrum of congenital deficiency of the leptin receptor. N Engl J Med 2007;356:237-47.

Clinical and Molecular Genetic Spectrum of
Congenital Deficiency of the Leptin Receptor
Farooqi IS, Wangensteen T, Collins S, et al.

Online Supplementary Material

Methods

Studies of Mutant Receptor Function

The wild-type (normal) *LEPR* construct (gift provided by C. Bjorbaeck, Boston) was subcloned into the mammalian expression vector pcDNA3 (Invitrogen). The wild-type construct was N-terminally tagged with MYC using site directed mutagenesis and this construct was then used to generate MYC-tagged mutant constructs using the QuikChangeTM site directed mutagenesis kit (Stratagene) according to manufacturer's protocols. All constructs were verified by direct nucleotide sequencing. HEK293 cells cultured in 6 well plates were transfected with N-terminal MYC-tagged constructs encoding wild type and mutant leptin receptors using the polyethylenimine (PEI) transfection reagent. For each well, 2.5 ug of DNA was transfected using 7.5 ul of PEI reagent (1mg/ml). 40 hours post transfection, cells were serum starved for 8 hours then either left unstimulated or stimulated with 100 ng/ml leptin for 10 mins. Cells were lysed on ice with 200ul of ice cold lysis buffer (50mM Tris-HCl pH7.4, 1mM EGTA, 1mM EDTA, 1mM sodium orthovanadate, 50mM sodium fluoride, 5mM sodium pyrophosphate, 1% (w/v) Triton-X 100, 0.27M sucrose, 0.1% (v/v) 2-mercaptoethanol and ‘Complete’ protease inhibitor cocktail (Roche). Lysates were cleared by centrifugation at 16,000g, 4ºC for 15 mins. The resulting supernatants were analysed for protein content against a BSA standard. Equal amounts of protein were analysed by SDS-PAGE using an 8% gel, transferred to PVDF membrane and blocked in 3% BSA for phosphoSTAT3 (Cell Signaling) immunoblots and 5% milk for anti-myc (Upstate) or total STAT3 (Cell Signaling) immunoblots. BSA or milk was dissolved in 50 mM Tris-HCl pH7.5, 150 mM NaCl, 0.1% (v/v) Tween (TBST). Antibody incubations were carried out overnight at 4ºC and detected after several washes in TBST using horse-radish peroxidase conjugated secondary antibodies (Pierce) and the enhanced chemiluminesence reagent (GE Healthcare). Immunoblots shown are representative of 4 separate experiments.

Sequencing Of The Leptin Receptor Gene In Obese Subjects

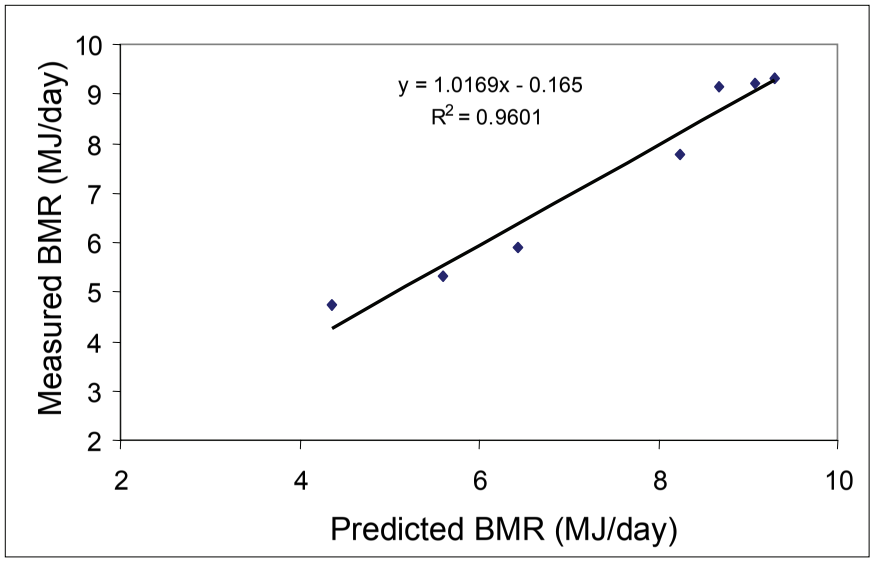
The entire coding region of the LEPR gene was amplified from genomic DNA using the primers listed. PCR was performed using BioTaq (Bioline, London, U.K.) and carried out under standard conditions. Thirty-five cycles of 30 s at 95°C, 30 s at 55°C, and 30 s at 72°C were performed using a PTC-225 Peltier Thermal Cycler (MJ Research, Watertown, MA). The PCR product was sequenced on both strands to determine the nucleotide sequence. The same primers were used for sequencing. Sequencing reactions were carried out using BigDye Terminator chemistry (Perkin-Elmer, Foster City, CA) and analyzed on an ABI 377 automated DNA sequencer (Perkin-Elmer).

PRIMERS

EXON1F: 5'-TAAATTTAGAGACTTATCTATAATCCC-3'
EXON 1R: 5'-TAACTAGAAATAGGAAATTCTGTTAGC-3'
EXON 2F: 5'- TCAGATACTTTCTATTCATGTCTTAGT-3'
EXON 2R: 5'-GTACAAAGAATTAAAAAACATTGTTC-3'
EXON 3F: 5'- TTTTTTTTAAATTCAGATGCAAACTGGA-3'
EXON 3R: 5'-TAAAAAAACTGTGTATTAGAAATGC-3'
EXON 4F: 5'-TCCTCTTTAAAAGCCTATCCAGTATTT-3'
EXON 4R: 5'-AGCTAGCAAATATTTTTGTAAGCAAT-3'
EXON 5F: 5'-GACTTTATTTTATTCAGCTATAATTGT-3'
EXON 5R: 5'-GCAGAGGGTAATTGCTATGGGAC-3'
EXON 6F: 5'-AGTAACGGTTCCACATCAACTTG-3'
EXON 6R: 5'-GGCCTCAAAATGTAAGATGCTTATAC-3'
EXON 7F: 5'-CAGAATGTTTGTCTTCATCTGATATCC-3'
EXON 7R: 5'-ATTCAAGTTGTGGAACAAAATGAACA-3'
EXON 8F: 5'-TATATTAGAGTCCTGTTAAAGATG-3'
EXON 8R: 5'-ATTTTTATCTTCACTGTGCCCAC-3'
EXON 9F: 5'-GAATTCTCAGATATCTTCTTGTTGC-3'
EXON 9R: 5'-CATTAAATCTGCATCAATCTGCATAC-3'
EXON 10F: 5'-CACAATGTTTTTAGGCATTATTAC-3'
EXON 10R: 5'-TCTAATGCAATTAAACTCTTACATATT-3'
EXON 11F: 5'-GTACTTCAGGGCCCCTTTAGATACATA-3'
EXON 11R: 5'-TTTGAAGAAATACTTTTCAGCCATA-3'
EXON 12F: 5'-GCATAAGTGTGTGCTTCAAATATGG-3'
EXON 12R: 5'-CGAAGATTAATAACAGGATTATGGACC-3'
EXON 13F: 5'- TCAGTTAGTATAAAAAGCACTGCAGC-3'
EXON 13R: 5'- TGCAAAAGTTAAATATTAAAAGAGGC-3'
EXON 14F: 5'-TAAGTTCCTCAAGAGTATTAGTAG-3'
EXON 14R: 5'-TTTTGAAGTTTTCATTAACTGGC-3'
EXON 15F: 5'-ATGTATGTTCCACTCATTACTATTA-3'
EXON 15R: 5'-CAATATTACTGCAAACAAATTAGGCAC-3'
EXON 16F: 5'-GTGATGAATTCAGAAAATGTCTACT-3'
EXON 16R: 5'-AATCAGGGTTTGAATACGCGTA
EXON 17F: 5'-ACTAACTGTTCACATTTTCCATATGG-3'
EXON 17R: 5'-ATAACAGATATATTAAGATG-3'
EXON 18AF: 5'-GCAAAATTTTTTAACATAATTGAGCC-3'
EXON 18AR: 5'-GCCTCATAGGTTACCTCAGTACC-3'
EXON 18BF: 5'-CAGTTCAACAGTGTTAACTTCTCTG-3'
EXON 18BR: 5'-TGCTGATCTGATAATATAAAAAATG-3'
EXON 18CF: 5'- AATAGCTCATGGGAGATAGAG-3'
EXON 18CR: 5'-GTCTTCTTACTAGAAGTTCCTAAGTTG
EXON 18DF: 5'-GGACAGTTGCTCACACTTTGTAG-3'
EXON 18DR: 5'-CACCCACAACTATAATCTATTACAC-3'

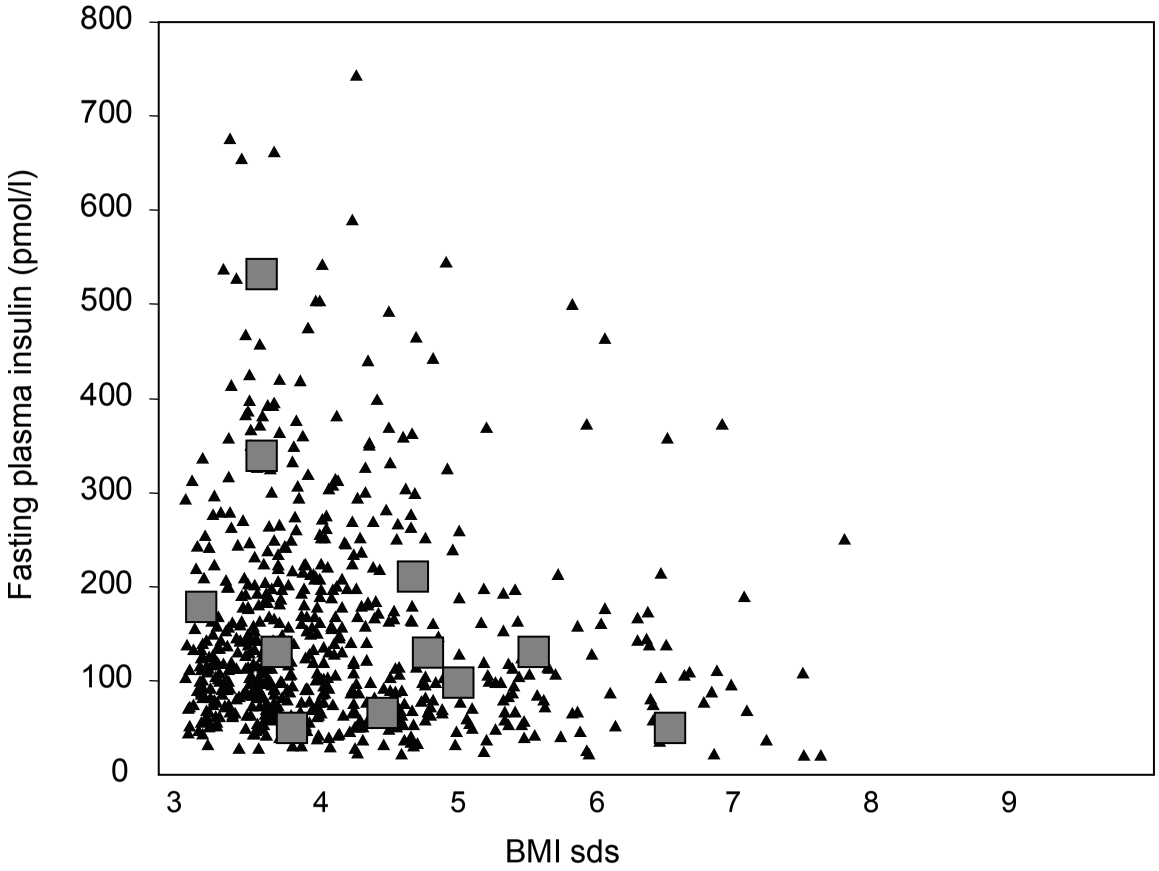
Supplementary Figure 1

A – Measured versus predicted BMR in leptin receptor deficient subjects



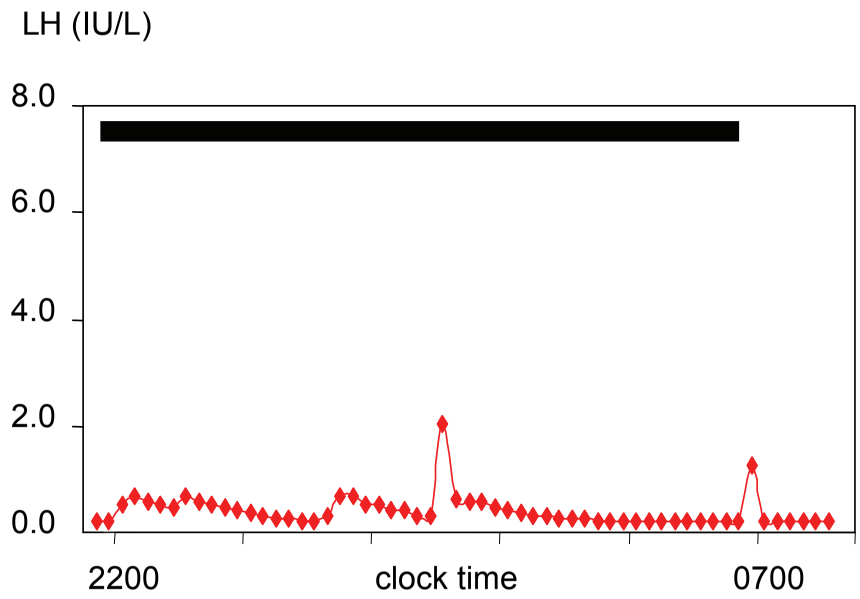
Supplementary Figure 1

B – Fasting plasma insulin in leptin receptor deficient subjects (grey symbols) and severely obese subjects from the GOOS cohort (black symbols)



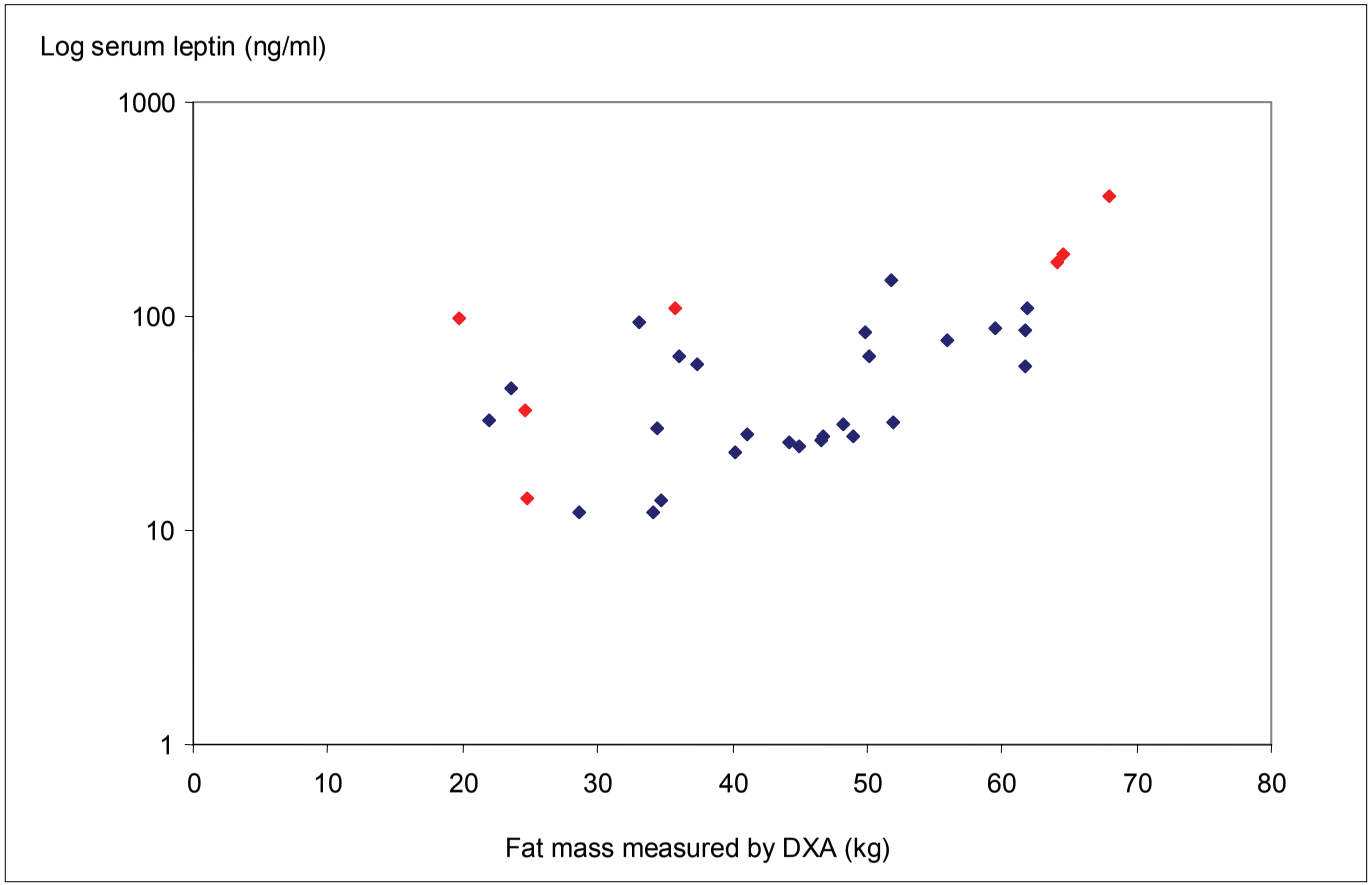
Supplementary Figure 1

C – Absence of LH pulsatility in samples obtained at 10 minute intervals over 8hrs overnight. Hours of sleep indicated by solid bar.

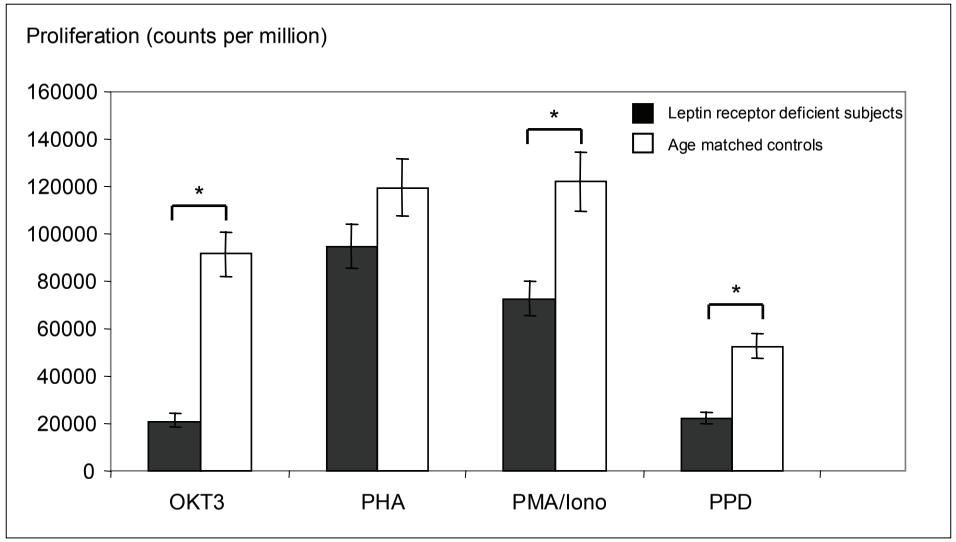


Supplementary Figure 1

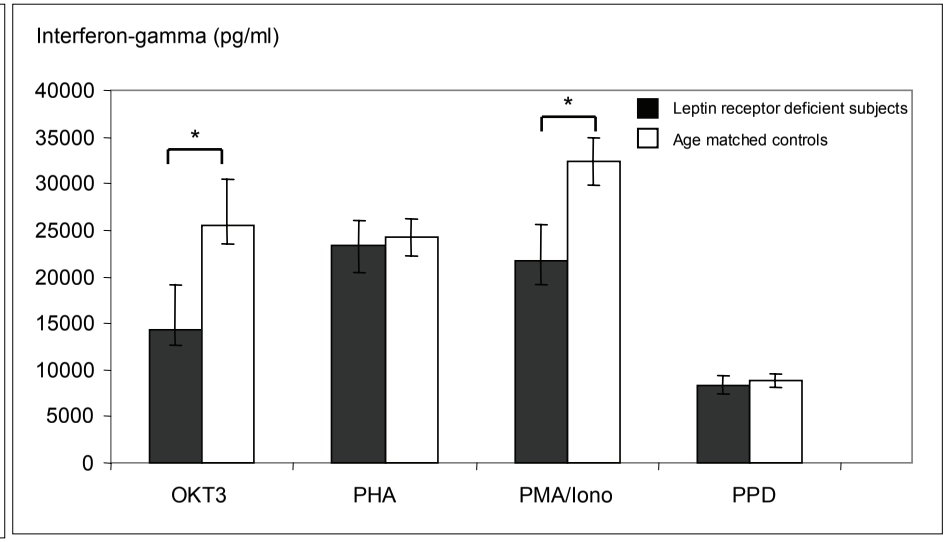
D – Log serum leptin versus fat mass measured by DXA in leptin receptor deficient subjects (red symbols) and severely obese subjects from the GOOS cohort (blue symbols)



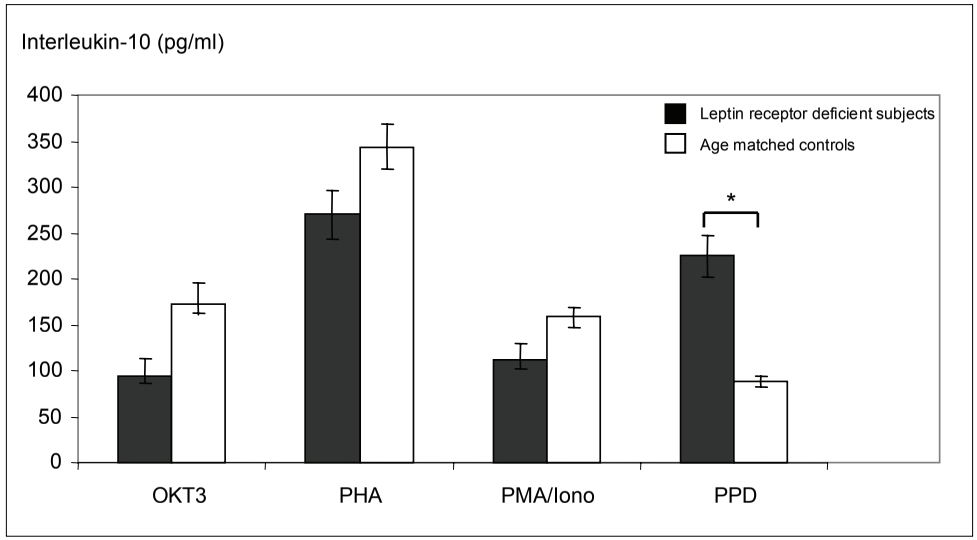
Supplementary Figure 1 **T cell proliferation (Panel E), Interferon gamma secretion (Panel F) and Interleukin-10 secretion (Panel G) in response to different antigens in leptin receptor deficient subjects and age matched controls.**



E



F



G

Supplementary Figure 1

H - Interleukin-4 secretion in response to different antigens in leptin receptor deficient subjects and age matched controls.