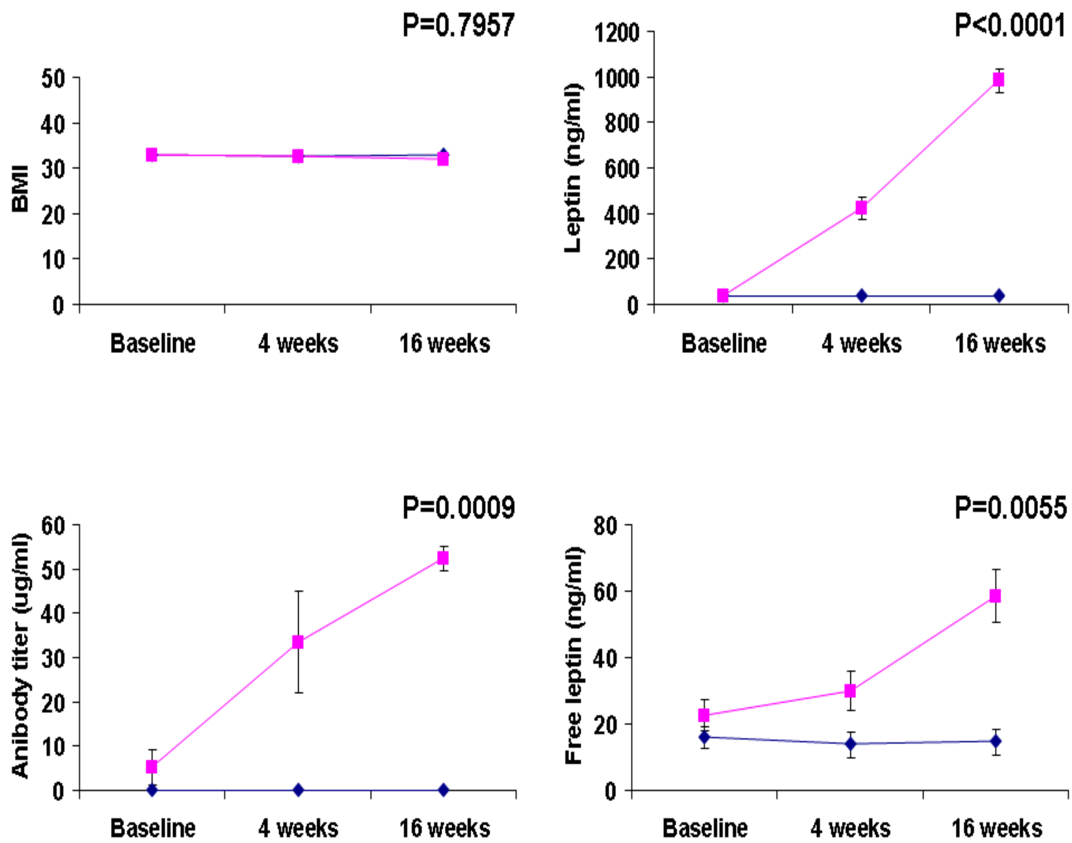
SUPPLEMENTARY DATA

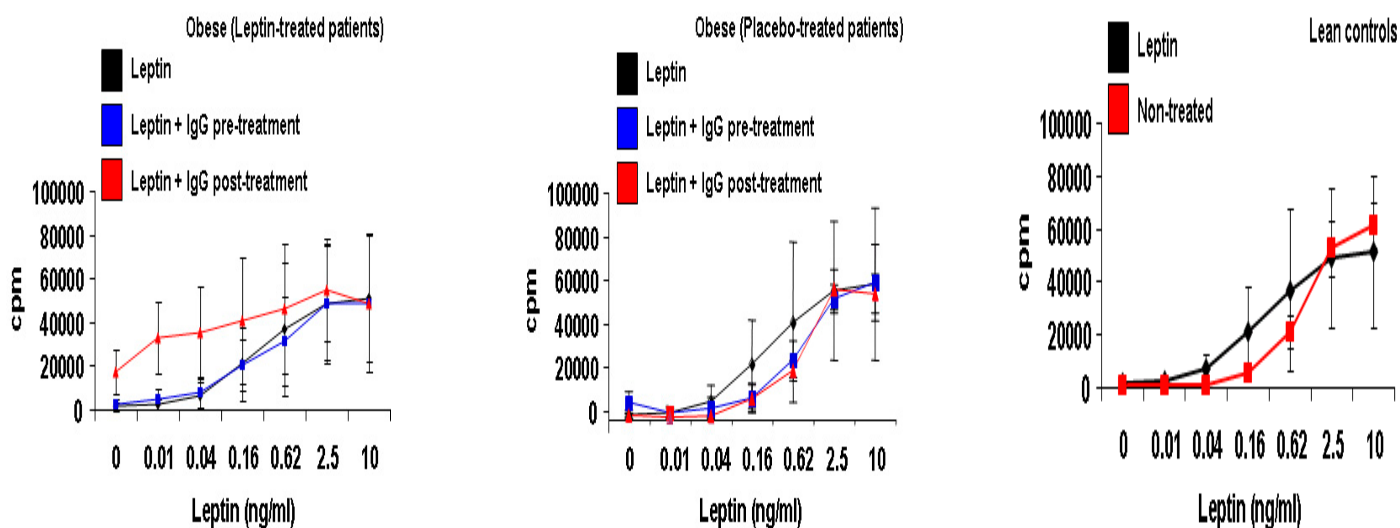
Supplementary Figure 1. Clinical Study I: Body weight, metabolic and immune responses to metreleptin vs. placebo treatment in obese hyperleptinemic subjects with diabetes mellitus - Clinical study I was performed as described in the Methods. BMI, leptin, free leptin and anti-leptin antibody titer were measured as described in detail in the Supplementary Methods. Mean measures of study variables for women on placebo at baseline, 4, and 16 wk follow-up. Values are means (placebo-treated: n=21, leptin-treated: n=50) ± SD. P-values calculated from repeated measures analysis of variance comparing placebo- and metreleptin-treated groups and evaluating the change in study variables over time, adjusted for age and sex. (■: Metreleptin-treated, ♦: Placebo-treated)



©2011 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db10-1791/-/DC1

Supplementary Figure 2. Laboratory Study I: Standard curves of anti-leptin antibodies for detection of anti-leptin antibodies and *in vitro* metreleptin signaling in BAF3 cells - (A) Standard curves of anti-leptin antibodies were performed as described in detail in the Methods. Standard curves of anti-leptin antibodies developed in each assay using an anti-leptin monoclonal antibody generated in our laboratory (971212 mAb). Quantification of each value was performed after extrapolation from standard curves of known concentration of anti-leptin antibodies. **(B)** Biochemical level the capacity of anti-leptin IgGs isolated from leptin or placebo-treated subjects and healthy controls, to affect LepR signalling in BAF3 cells, was performed as described in detail in the Methods. The cells were administered metreleptin (2 ng/ml) in the presence or absence of IgGs (50 µg/ml). All lysates were examined by Western blot with primary STAT3 and p-STAT3 antibodies. Secondary antibodies used were horseradish peroxidase-conjugated anti-goat antibodies. All values shown are representative of an experiment performed at least 6 times. Values are means (n=6) ± SD.

A

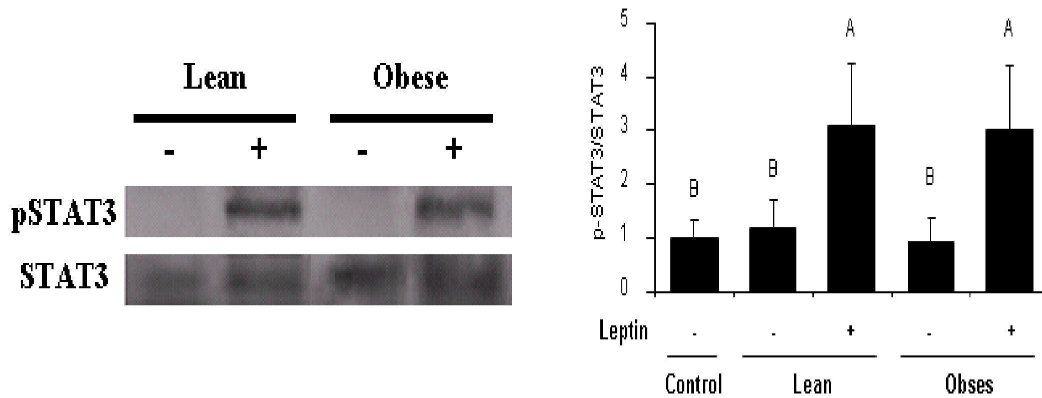


B

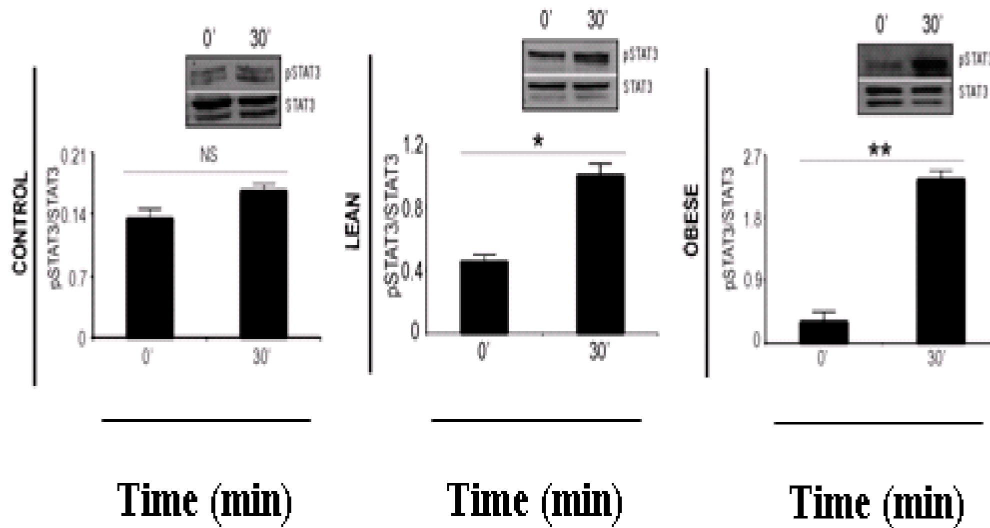
©2011 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db10-1791/-/DC1

Supplementary Figure 3. Clinical Study II: *In Vivo* metreleptin signaling in human adipose tissue (hAT) and human peripheral blood mononuclear cells (hPBMC) from lean and obese subjects - *In vivo* metreleptin signaling study was performed as described in detail in the Methods. The hAT **(A)** and hPBMC **(B)** from 6 control, 6 lean, and 6 obese subjects after *in vivo* metreleptin administration for 30 min. All immunoblots shown are representative of an experiment performed at least 6 times. All density values for each protein band of interest are expressed as a fold increase. Data were analyzed using one-way ANOVA followed by post-hoc test for multiple comparisons. Values are means (n=6) ± SD. Means with different letters are significantly different, $p<0.05$.

A

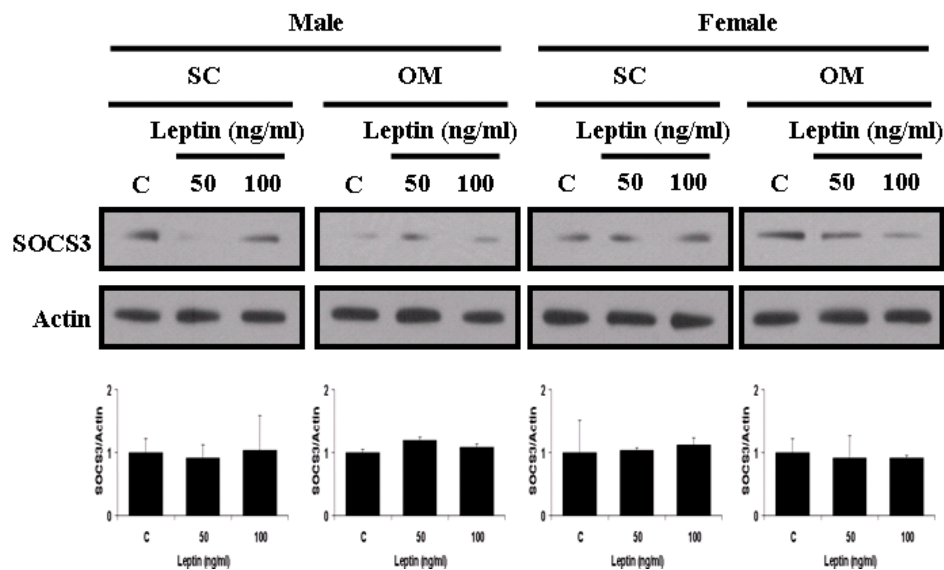


B



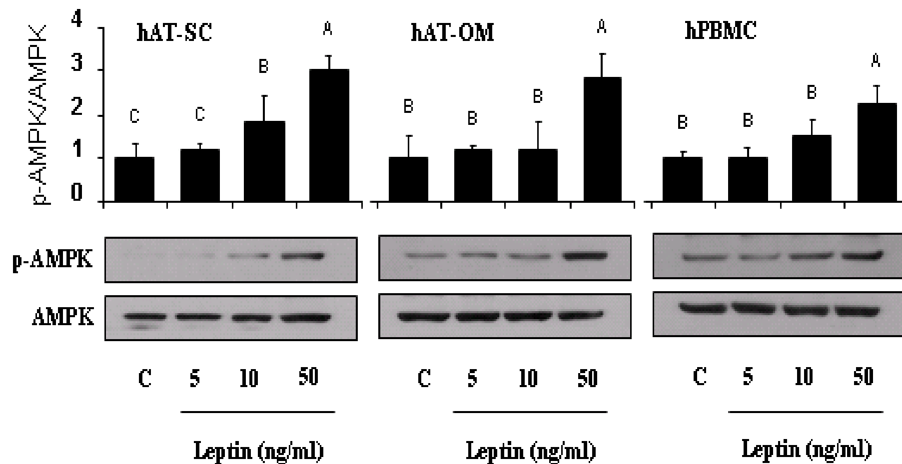
©2011 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db10-1791/-/DC1

Supplementary Figure 4. Laboratory Study II: No differential activation of inhibitors of *ex vivo* metreleptin administration in human adipose tissue (hAT) from obese male and female subjects - *Ex vivo* metreleptin administration in hAT was performed as described in detail in the Methods. The hAT was pre-incubated and stimulated with or without metreleptin (0 to 100 ng/ml) for 30 min. All tissue lysates were examined by Western blot with primary SOCS3 and β-actin antibodies. Secondary antibody used was horseradish peroxidase-conjugated anti-mouse antibody. All immunoblots shown are representative of an experiment performed at least 3 times. All density values for each protein band of interest are expressed as a fold increase. All data were analyzed using one-way ANOVA followed by post-hoc test for multiple comparisons. Values are means (n=3) ± SD. Means with different letters are significantly different, $p<0.05$. SC: subcutaneous, OM: omental.



©2011 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db10-1791/-/DC1

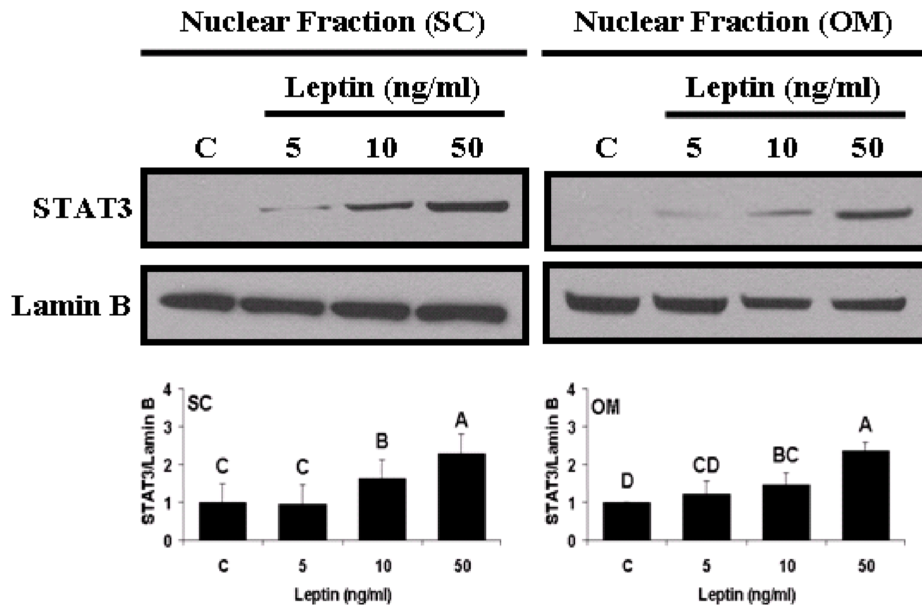
Supplementary Figure 5. **Laboratory Study II: No differential *a*ctivation of AMPK signaling by *ex vivo* metreleptin administration in human adipose tissue (hAT) and human peripheral blood mononuclear cells (hPBMC) from obese female subjects** - *Ex vivo* metreleptin administration in hAT and hPBMC was performed as described in detail in the Methods. The hAT and hPBMC was pre-incubated and stimulated with or without metreleptin (0 to 50 ng/ml) for 30 min. All tissue lysates were examined by Western blot with primary p-AMPK and AMPK antibodies. Secondary antibody used was horseradish peroxidase-conjugated anti-mouse antibody. All immunoblots shown are representative of an experiment performed at least 3 times. All density values for each protein band of interest are expressed as a fold increase. All data were analyzed using one-way ANOVA followed by post-hoc test for multiple comparisons. Values are means (n=3) ± SD. Means with different letters are significantly different, p<0.05. SC: subcutaneous, OM: omental.



©2011 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db10-1791/-/DC1

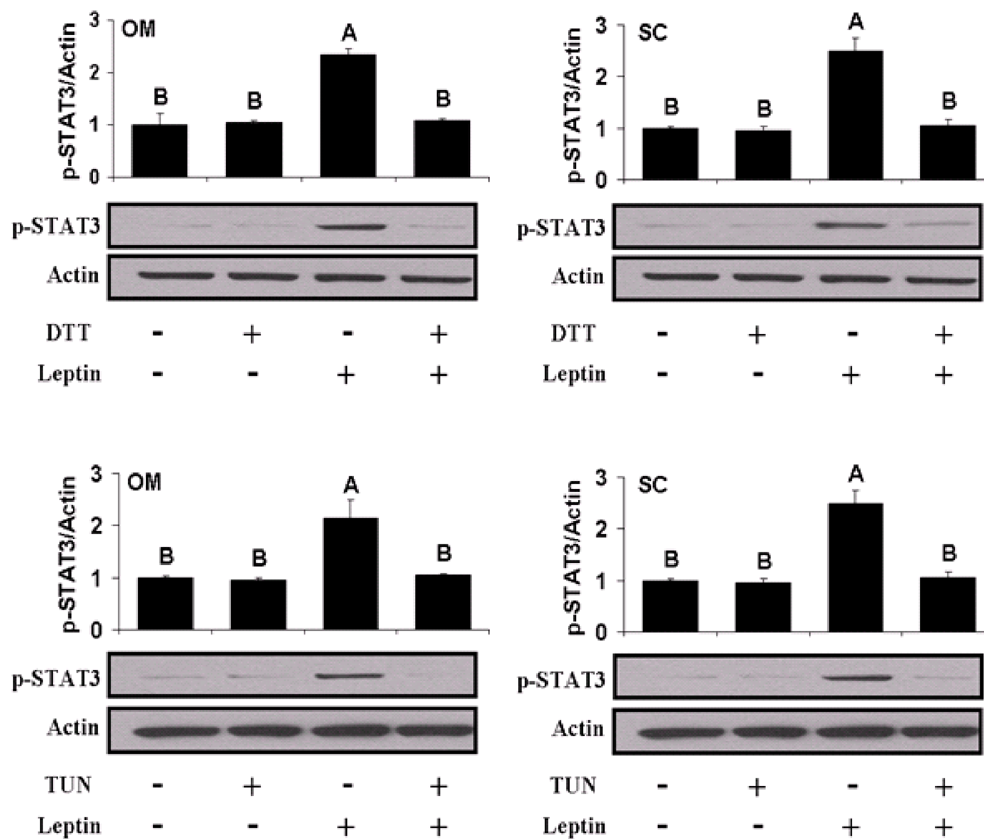
SUPPLEMENTARY DATA

Supplementary Figure 6. Laboratory Study III: No differential *a*ctivation of STAT3 signaling by *in vitro* metreleptin administration in human primary adipocytes (hPA) from obese female subjects - *In vitro* metreleptin administration in hPA was performed as described in detail in the Methods. After 28 days of culture, the cells were treated with metreleptin at indicated concentrations for 30 min and nuclear was then extracted as described in the Supplemental Methods. All lysates were examined by Western blot with primary p-STAT3 and Lamin B antibodies. Secondary antibodies used were horseradish peroxidase-conjugated anti-mouse and -goat antibodies. All immunoblots shown are representative of an experiment performed at least 3 times. All density values for each protein band of interest are expressed as a fold increase. Data were analyzed using one-way ANOVA followed by post-hoc test for multiple comparisons. Values are means (n=3) ± SD. Means with different letters are significantly different, $p<0.05$. SC: subcutaneous, OM: omental.



©2011 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db10-1791/-/DC1

Supplementary Figure 7. Laboratory Study III: Down-regulation of *in vitro* metreleptin-stimulated STAT3 signaling by endoplasmic reticulum (ER) stress in human primary adipocytes (hPA) from obese female subjects - *In vitro* metreleptin administration in hPA was performed as described in detail in the Methods. After 28 days of culture, the cells were either pre-incubated with dithiothreitol (DTT, 1 mM) or tunicamycin (TUN, 3 mg/ml) for 5 hr and subsequently taken to metreleptin (50 ng/ml) stimulation for 30 min. All lysates were examined by Western blot with primary p-STAT3 and STAT3 antibodies. Secondary antibodies used were horseradish peroxidase-conjugated anti-mouse and -goat antibodies. All immunoblots shown are representative of an experiment performed at least 3 times. All density values for each protein band of interest are expressed as a fold increase. Data were analyzed using one-way ANOVA followed by post-hoc test for multiple comparisons. Values are means (n=3) ± SD. Means with different letters are significantly different, $p<0.05$. SC: subcutaneous, OM: omental.



©2011 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db10-1791/-/DC1