Supplemental Methods

Correlation analysis. For gene selection, a filter of 1.5-fold change and the standard Affymetrix values for change and detection *P* values were used. A gene had to have a significant fold change in at least five samples to pass the filter. Correlation for genes in the intersecting set of pairs was calculated using an absolute log base 2 fold change cutoff greater than 0.6, change *P* value less than 0.0025, and detection *P* value less than 0.04 (these *P* values are the default α_1 and γ_1 values for MAS 5.0, Affymetrix Inc., Santa Clara, California, USA). The criteria used are quite stringent for gene selection, and for most randomly matched pairs, a very small number of genes meet these criteria. This is critical because the distribution of random correlations is highly dependent on the number of points, with a small number of points having a much greater chance of being highly correlated than a large number of points. To minimize this problem, we changed the selection criteria for computing background statistics. This should not affect our results provided the same statistic is used for all computations. We calculated the background correlation using the top 250 genes ranked by absolute log fold change in the union set. Note that 250 is an arbitrary cutoff and we tested cases from 50–500 genes. However, we chose to report the 250 case since it was the most conservative one (i.e., highest *P* value). We calculated the Pearson correlation coefficient:

$$\rho = \frac{\sum_i (X_i - \bar{X})(Y_i - \bar{Y})}{\sqrt{\sum_i (X_i - \bar{X})^2 \sum_i (Y_i - \bar{Y})^2}}$$

where *X* and *Y* are the logs of the fold changes, and a bar over *X* or *Y* indicates the mean of *X* or *Y*, respectively. To assess the significance of this correlation, we calculated the distribution of correlations for a large number of quasi-randomly paired samples. This model was "random" in the sense that 30 pairs of comparisons were paired arbitrarily (we did not mix samples from different tissue types) to create 95 fold change files. We then exhaustively paired all 95 of these samples, ensuring that we were always looking at four different samples each time. This resulted in 3,692 sets for which we computed correlation coefficients. We looked at the distribution of the absolute value of the correlation coefficient. To calculate the *P* value, we used the formula $P = [(N_{\#} > 0.738) + 1]/[(N_{\text{total}}) + 1]$, where $N_{\#}$ is the number of pairs with $\rho > 0.738$ (four in our case), and N_{total} is the total number of pairs.

Supplemental Note 1

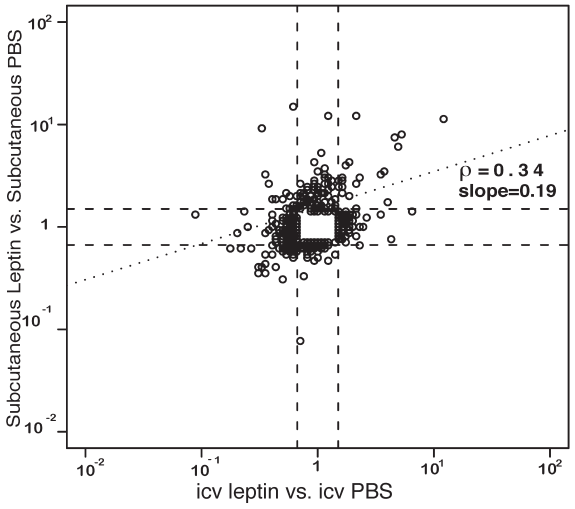
For both methods, we first calculated the ratio of gene expression between icv leptin versus icv PBS treatment and then subcutaneous leptin versus subcutaneous PBS treatment. It was necessary to establish the ratio of gene expression in the icv leptin– and subcutaneous leptin–treated samples relative to their own controls, because icv and subcutaneous pumps each have distinct background effects. In the first method, the standard "detection *P* values" and "change *P* values" from the Affymetrix software were used to identify leptin-regulated genes with an absolute fold change cutoff of 1.5 relative to controls. From this, we compiled two distinct lists of leptin-regulated genes: one from the icv leptin treatment and another from the subcutaneous leptin treatment (each relative to its own control; see Supplementary Tables 3, 4, 5, and 6). Expression of 185 genes changed following both leptin administration protocols (i.e., icv and subcutaneous).

Supplemental Note 2

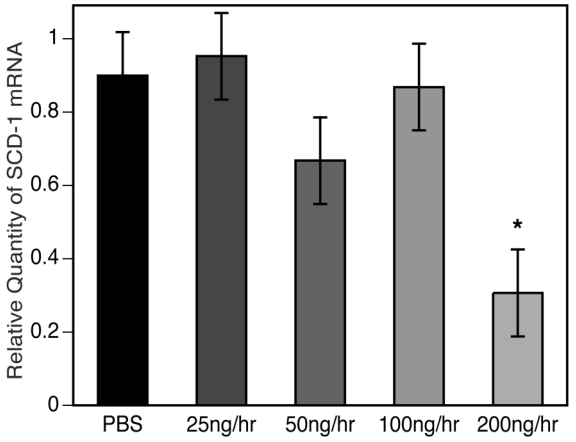
Even though 0.897 is an intrinsically high correlation coefficient, we further evaluated the significance of this correlation by analyzing the patterns of all genes that changed in abundance after either treatment modality. In order to establish the statistical significance of this correlation, we calculated a background correlation from 30 microarray experiments previously performed in our laboratory (see Supplementary Table 7) and matched them into a total of 3,692 pairs. Initially, we looked for genes that were similarly regulated in both samples in the pairwise comparison (i.e., the intersection set) but found that only about 10% of the 3,692 pairs had a number of genes comparable to the 185 identified in icv versus subcutaneous leptin treatment. Because there were an insufficient number of genes in the intersection set for most of the 3,692 pairs, we modified the analysis by computing the correlation of genes in the union of the lists of regulated genes. There were 379 genes in the union set of icv leptin versus PBS and subcutaneous leptin versus PBS treatment.

Supplemental Note 3

A list of the RNA sources from the top 25 pairwise comparisons can be found in Supplementary Table 8 online. The comparisons producing equivalent correlations to that of icv versus subcutaneous leptin were invariably between very similar samples, such as between a WT liver and liver from a leptin-treated aP2-nSREBP-1c mouse. Leptin is known to normalize the liver phenotype of these animals (9). Another strong similarity was between *ob/ob* and PBS-treated aP2-nSREBP-1c liver. The livers from these animals are both steatotic, and this comparison is essentially between two leptin-deficient livers. Other pairwise analysis with high correlations included comparisons between untreated or PBS-treated tissue or comparisons between treatments with subtherapeutic doses of leptin (i.e., low doses that have no biologic effect).



Supplemental Figure 1
Computational analyses of the muscle transcription profile after icv and subcutaneous leptin treatment. A pairwise comparison of icv and subcutaneous leptin gene expression is shown for genes regulated by either treatment (the union set). ρ denotes the correlation value.



Supplemental Figure 2
Regulation of SCD-1 mRNA levels by several doses of peripheral leptin. TaqMan real-time PCR of liver RNA samples from aP2-nSREBP-1c transgenic mice treated with PBS, 25 ng/h, 50 ng/h, 100 ng/h, and 200 ng/h peripheral leptin using primers and probe specific for SCD-1. Error bars indicate the SE; $n = 3$ for 25 ng/h leptin, and $n = 4$ for 0 ng/h, 50 ng/h, 100 ng/h, and 200 ng/h leptin. *$P < 0.001$, 200 ng/h leptin vs. 0 ng/h.