Table 1: Experiments included in the study.

species	age	sex	tissue	Control sample	Tester sample (hormone, time, dose)
rat	3 months	M	liver	c.i. of vehicle	c.i. of bGH, 1 week, 5 μg/h
rat	2 years	M	liver	c.i. of vehicle	c.i. of hGH, 3 weeks, 0.34 μg/g body weight/day
rat	2 months	M	hepatocytes	medium	bGH added to medium, 24 hours, 100 ng/ml
rat	3 months	M/F	liver	male	female
rat	3 months	M	liver	vehicle	17-α-ethinylestradiol, 1 day, 5 mg/kg body weight
mouse	3 months	M	liver	hypothyroid	injection of T3 & TT4, 2 hours, 5 μg T3 + 5 μg T4

Expression profiles from six independent studies regarding gender differences and hormonal regulation of hepatic gene expression were included in the study. bGH = bovine growth hormone, hGH = human growth hormone, T3 = triiodothyronine, T4 = thyroxine, c.i. = continuous infusion, hepatocytes = primary rat hepatocytes. All rat experiments used Sprague Dawley rat strain. The mice strain used to study thyroid hormone actions was a hybrid of 129/Sv X C57Bl76J.

Table 2: The primers, amplicon sizes and annealing temperatures used for gene expression measurements by real-time PCR.

gene	left primer	right primer	size	temp
FAT/CD36	GCAACAACAAGGCCAGGTAT	TGTGGCTGAGCAGAAAGAGA	200	54
Ppp3ca	GCAGGCTGGAAGAAAGTGTC	AAGGCCCACAAATACAGCAC	200	54
Hsd11b1	TTTTGCAGAGCGATTTGTTG	TGCTCAGGACCACATAGCTG	200	54
Phyh	TACGTGGAGTGCTTCACTGG	CCATTGTTCCTGTCGATGTG	200	54
Srebp1a	GCGCCATGGACGAGCTG	TTGGCACCTGGGCTGCT	200	57
Srebp1c	GGAGCCATGGATTGCACATT	GCTTCCAGAGAGGAGGCCAG	200	54
Srebp2	CCCTTGACTTCCTTGCTGCA	GCGTGAGTGTGGGCGAATC	200	54

The expression of phytanoyl-CoA hydroxylase (Phyh), hydroxysteroid 11-beta dehydrogenase 1 (Hsd11b1), the catalytic subunit of protein phosphatase 3, alpha isoform (Ppp3ca), fatty acid translocase/cd36 antigen (FAT/CD36) and sterol regulatory element-binding proteins (SREBPs) 1a, 1c and 2 were measured using the primers shown in the Table. To allow comparison between samples, the expression data for each of the genes was normalized to the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which was always run in parallel.

effects on gene expression after *in vivo* or *in vitro* GH treatment were confirmed for some genes using quantitative real-time PCR (Tables 2 and 3).

As shown in figure 1b, the correlation between GH treatment and thyroid hormone or estrogen treatment in young males was rather low. This is not surprising since both hormones have distinct liver functions not always overlapping those of GH. Differences in expression can also arise from the choice of treatment duration, dose and mode of hormone treatment and this could result in the underestimation of commonly regulated genes. This ambiguity can only be resolved by measuring more expression profiles in experiments specifically designed to study hormonal interactions.

GH and estrogen contribute to gender differences in hepatic gene expression

When we compared the gender-related expression differences to the rest of the experiments (Figure 1c, Table 4), it was evident that as many as 48% of the female-enriched transcripts were also up-regulated by continuous GH infu-

Table 3: Comparisons of GH response in liver and in primary hepatocytes

Genes	in vivo		hepatocytes	
	Array	RT-PCR	Array	RT-PCR
Phyh	0.34	0.6	0.54	0.6
Hsd11β1	0.31	0.28	0.63	0.5
Ppp3ca	1.33	1.43	1.48	1.33
CD36	2.39	2.78	1.26	2.07

Microarray and RT-PCR expression measurements for phytanoyl-CoA hydroxylase (Phyh), hydroxysteroid 11-beta dehydrogenase 1 (Hsd11β1), the catalytic subunit of protein phosphatase 3, alpha isoform (Ppp3cα) and fatty acid translocase/CD36 antigen (FAT/CD36). The real-time PCR results were normalized to GAPDH. The ratios between treated and untreated samples *in vivo* (young male rats treated with bGH or vehicle for 1 week via minipumps, n = 4 per group), and the ratios between GH-treated (24 hours) and untreated primary hepatocytes are shown.