Endocr (2008) 33:196–204
DOI 10.1007/s12020-008-9067-9

ORIGINAL PAPER

Effects of the synthetic liver X receptor agonist T0901317 on the growth hormone and thyroid hormone axes in male rats

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Received: 14 March 2008 / Accepted: 30 April 2008 / Published online: 13 May 2008

Abstract Liver X receptors (LXRs), activated by oxysterols, play an important role in the regulation of lipid and glucose metabolism, which is also markedly dependent on thyroid hormone and growth hormone (GH) status. Here, we investigated how a 1-week exposure to the synthetic LXR agonist T0901317 affected GH secretion and thyroid hormone status in male rats. While the pulse frequency of GH secretion was marginally affected there was a highly significant decrease in the triiodo-L-thyronine/thyroxine (T3/T4) ratio in plasma. This effect was associated with decreased expression of deiodinase 1 (DIO1) and 2 (DIO2) mRNA in the liver and thyroid gland, respectively. Expression of sterol regulatory element binding protein-1c (SREBP-1c), the hallmark of stimulated lipogenesis, was markedly increased in both thyroid and pituitary implying that protracted pharmacological LXR activation may promote lipid accumulation in these endocrine tissues. These findings suggest that attention must be given to pituitary hormone dependent axes when developing therapeutic strategies based on agonism of the LXRs, e.g. for treatment of atherosclerosis.

Keywords LXR · Liver · Thyroid · Pituitary · GH · T3 · T4 · Rat

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Introduction

The liver X receptors LXRα (NR1H3) and LXRβ (NR1H2) are members of the nuclear receptor gene family of transcription factors that play a major role in the regulation of cellular metabolism [1, 2]. Studies in animals, in particular the phenotypic characterization of LXR deficient mice [3–5], and the identification of oxidized cholesterol derivatives, the oxysterols, as endogenous LXR ligands [6] have confirmed that these receptors have a critical role in regulating lipid and glucose whole-body metabolism [7–9]. The possibility that LXRs also act as sensors of glucose has recently been put forward [10]. LXRα is expressed most highly in metabolically active tissues such as liver, adipose tissue, intestine, and macrophages, whereas LXRβ is expressed ubiquitously. In addition, both LXR isoforms are expressed in the pituitary gland [11, 12]. Treatment of mice with synthetic LXR agonists reduces the development of atherosclerosis, probably by enhancing reverse cholesterol transport and by reducing inflammation [13, 14]. However, LXR agonists also have undesirable effects such as increased synthesis of fatty acids and triglycerides, eventually leading to hepatic steatosis and hypertriglyceridemia [9, 13, 15, 16]. Despite this, the LXRs remain as potential targets for intervention in human metabolic disease.

LXRs mediate their gene regulatory effects through the obligatory formation of heterodimers with retinoid X receptors (RXR) that bind to specific DNA response elements in target genes. Target genes include ATP-binding cassette (ABC)-transporters and sterol regulatory element binding protein 1c (SREBP-1c) that regulates several lipogenic genes, including fatty acid synthase (FAS) [14, 17].

Pituitary hormones and hormones secreted by peripheral endocrine organs in response to hypothalamo-pituitary activation are also fundamental in regulating multiple

homeostatic functions, including lipid and glucose metabolism. Both the thyroid hormones [18–20] and growth hormone (GH) [21–24] are important determinants of lipid and glucose metabolism as well as of energy expenditure and body fat mass. Yet, whether LXR agonists influence these endocrine axes is essentially unknown.

GH secretion is markedly episodic and displays a clear sex difference in the patterns of secretion [25], which in turn has differential and sometimes opposite effects on GH regulated gene expression and functions [26, 27]. Interestingly, hepatic gene expression profiling in mice treated with the synthetic LXR agonist T0901317 [28] indicates that LXR activation regulates genes whose expression is also regulated by the sexually dimorphic secretion of GH, e.g. the GH receptor, major urinary proteins, and androgen regulated vas deferens protein.

In this study we have tested the hypothesis that LXR activation regulates the activity of the hypothalamo-pituitary axes, in particular those exerting profound metabolic control. Therefore, we have characterized the effect of a 1-week exposure to T0901317 on GH secretion, and circulating levels of thyroid stimulating hormone (TSH), thyroid and steroid hormones in male rats. GH secretion was marginally affected while pharmacological LXR activation interfered significantly with thyroid hormone homeostasis.

Results

Experiment 1: effect of the synthetic LXR agonist T0901317 on the parameters of pulsatile growth hormone secretion in male rats

Inclusion of the LXR agonist T0901317 in the diet had no significant effect on either food intake or body weight gain (Table 1). At the end of the treatment period proportionate liver weights were elevated by 14% ($P < 0.01$; Table 1) in T0901317-treated rats. Conversely, T0901317 treatment resulted in a 40% reduction in proportionate retroperitoneal fat weights ($P < 0.05$; Table 1), without affecting epididymal fat weights (Table 1). As expected, T0901317 treatment resulted in a 2.5-fold elevation in circulating triglycerides ($P < 0.01$; Table 1).

Rats fed the standard laboratory chow displayed episodic GH secretory profiles characteristic of male rats [25] (Fig. 1), with 6–9 bursts of GH secretion during the 24 h sampling period, some of which comprised multiple peaks. Treatment with T0901317 had no significant effect on total secretory output (AUC), median GH concentrations, or pulse height (Table 2). However, T0901317 treatment resulted in a small but significant (20%) reduction in peak number and frequency. Mean baseline secretion (OC_5) in T0901317-treated rats was less than 50% of that in rats on standard diet, but given the degree of variation these means were not significantly different ($P = 0.1945$; Table 2). Measurement of circulating IGF-1 in terminal blood samples was not affected by treatment with T0901317 (194 ± 32 nM (standard diet) vs. 209 ± 25 nM (T0901317); $P = 0.441$).

Experiment 2: effect of the synthetic LXR agonist T0901317 on baseline growth hormone secretion in male rats

Since the baseline values determined in experiment 1 were near the limits of detection for the assay, a more rigorous assessment of the effects of T0901317-treatment on baseline GH secretion was performed in a second experiment in which a delayed addition assay protocol was used in conjunction with larger sample volumes collected over a shorter sampling period.

Table 1 Effect of dietary supplementation with T0901317 on physical body parameters in male rats

Variable	Standard diet	T0901317	*P*
Experiment 1 (*n*=)	4	6	
Cumulative food intake (g)	152.1 ± 8.7	145.6 ± 5.2	0.507
Δ Body weight (g)	29.1 ± 5.0	25.5 ± 4.2	0.599
Liver weight (%BW)	3.69 ± 0.08	4.19 ± 0.10	**0.008**
Retroperitoneal fat weight (%BW)	0.084 ± 0.005	0.053 ± 0.007	**0.011**
Epididymal fat weight (%BW)	0.292 ± 0.009	0.284 ± 0.017	0.744
Plasma triglyceride (nM)	0.25 ± 0.05	0.63 ± 0.07	**0.004**
Experiment 2 (*n*=)	12	11	
Cumulative food intake (g)	173.8 ± 5.0	175.0 ± 12.3	0.926
Δ Body weight (g)	26.4 ± 2.9	25.8 ± 2.0	0.857
Liver weight (%BW)	3.56 ± 0.08	4.05 ± 0.11	**0.002**
Retroperitoneal fat weight (%BW)	0.093 ± 0.010	0.070 ± 0.006	0.076
Epididymal fat weight (%BW)	0.206 ± 0.012	0.217 ± 0.014	0.560
Plasma triglyceride (nM)	0.27 ± 0.02	0.70 ± 0.10	**0.0002**

Cumulative food intake, body weight gain, proportionate liver, retroperitoneal and epididymal fat weights, and circulating triglycerides were measured in male rats in experiments 1 and 2. Values shown are mean ± SEM, with statistical comparisons performed by Student's *t*-test

P-values in bold indicate significant differences

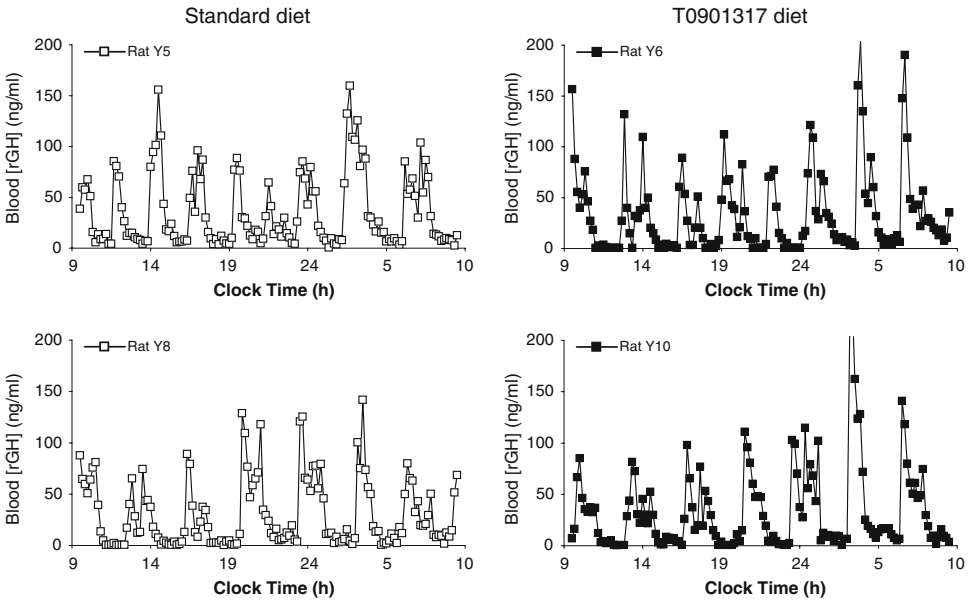

Fig. 1 Effect of the synthetic LXR agonist T0901317 on the parameters of pulsatile growth hormone secretion in male rats. Representative 24 h rGH profiles from male rats maintained on standard diet (open symbols) or diet containing T0901317 (closed symbols) and subjected to automated blood sampling. Statistical analysis of the parameters of secretion is shown in table 2

Table 2 Analysis of the parameters of GH secretion in male rats treated with T0901317

Variable	Standard diet ($n = 4$)	T0901317 ($n = 6$)	P
Area under curve (ng/ml min)	826.3 ± 53.7	726.8 ± 52.6	0.239
Baseline (OC_5) (ng/ml)	2.45 ± 0.93	1.04 ± 0.54	0.195
Median (OC_{50}) (ng/ml)	17.05 ± 2.42	13.83 ± 1.96	0.331
Pulse height (OC_{95}) (ng/ml)	105.59 ± 2.85	113.82 ± 9.82	0.528
Pulse number (pulses/24 h)	21.25 ± 0.75	17.5 ± 0.92	**0.020**
Pulse frequency (pulses/h)	0.89 ± 0.03	0.73 ± 0.04	**0.020**
Pulse height (9 highest) (ng/ml)	111.50 ± 10.47	109.77 ± 8.69	0.902

Total secretory output was determined by quantification of area under curve, with distribution analysis used to determine baseline (OC_5), median (OC_{50}), and peak (OC_{95}) concentrations and PULSAR analysis used to quantify pulse number, frequency, and height (obtained from the nine highest peaks). Values shown are mean ± SEM, with statistical comparisons performed by Student's *t*-test

P-values in bold indicate significant differences

Table 3 Analysis of the parameters of baseline GH secretion in male rats treated with T0901317

Variable	Standard diet ($n = 5$)	T0901317 ($n = 6$)	P
Area under curve (ng/ml min)	187.7 ± 50.3	204.2 ± 21.5	0.755
Baseline (OC_5) (ng/ml)	0.202 ± 0.082	0.226 ± 0.035	0.781
High baseline (OC_{10}) (ng/ml)	0.259 ± 0.087	0.296 ± 0.040	0.694
Median (OC_{50}) (ng/ml)	1.425 ± 0.287	2.430 ± 0.429	0.096

Total secretory output was estimated by quantification of area under curve, with distribution analysis used to determine baseline (OC_5), high baseline (OC_{10}), and median (OC_{50}) concentrations. Values shown are mean ± SEM, with statistical comparisons performed by Student's *t*-test

As in experiment 1, T0901317-treatment had no effect on either cumulative food intake, or body weight gain (Table 1). Similarly, treatment with T0901317 increased circulating triglycerides 2.5-fold and proportionate liver weights by 13% ($P < 0.001$ and $P < 0.01$ respectively; Table 1). Proportionate retroperitoneal fat weights following T0901317 treatment were 75% of that in rats maintained on the standard diet ($P = 0.076$), but proportionate epididymal fat weight was unaffected (Table 1).

Using this protocol, neither baseline (OC_5), high baseline (OC_{10}), nor median (OC_{50}) circulating GH concentrations were affected by the inclusion of T0901317 in the diet (Fig. 2, Table 3).

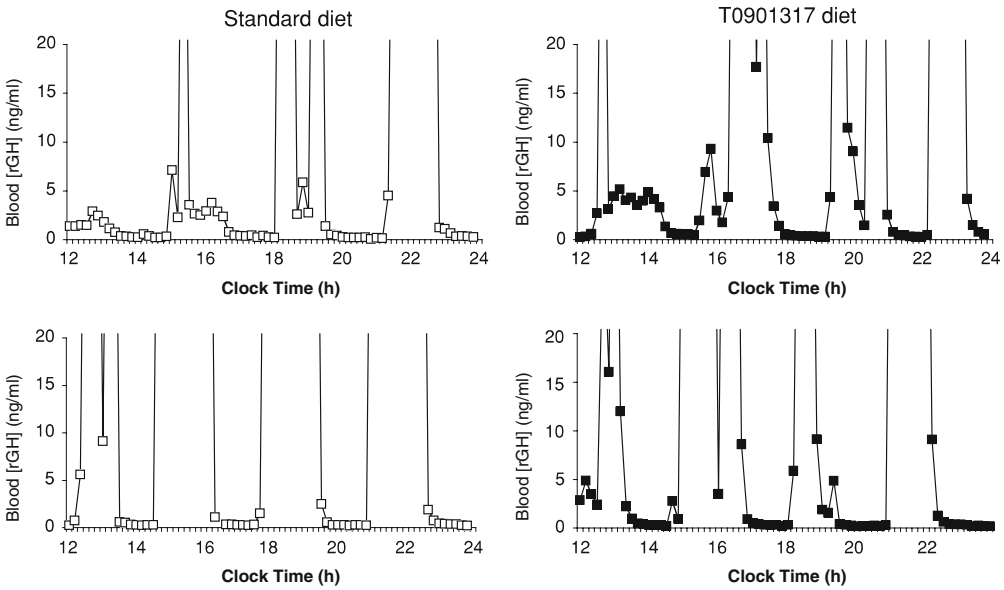

Fig. 2 Effect of the synthetic LXR agonist T0901317 on baseline growth hormone secretion in male rats. Representative 12 h rGH profiles from male rats maintained on standard diet (open symbols) or diet containing T0901317 (closed symbols) and subjected to automated blood sampling with increased sample volume and delayed addition RIA to enable rigorous analysis of baseline secretion. Statistical analysis of the parameters of secretion is shown in table 3

Table 4 Effect of the synthetic LXR agonist T0901317 on circulating metabolic and endocrine factors in male rats

Plasma variable	Standard diet	T0901317	*P*
Experiments 1 and 2 (*n*=)	16	17	
Triglyceride (nM)	0.26 ± 0.01	0.68 ± 0.07	**<0.0001**
Cholesterol (nM)	2.27 ± 0.08	2.28 ± 0.08	0.946
NEFA (mM)	0.16 ± 0.02	0.22 ± 0.02	**0.037**
Glucose (mM)	10.1 ± 0.2	9.5 ± 0.2	**0.015**
Insulin (nM)	0.12 ± 0.02	0.11 ± 0.01	0.674
Corticosterone (ng/ml)	359 ± 42	301 ± 28	0.250
Testosterone (nM)	5.3 ± 0.8	3.3 ± 0.4	**0.024**

Circulating triglycerides, cholesterol, non-esterified fatty acids (NEFA), glucose, insulin, corticosterone, and testosterone were measured in plasma in male rats fed standard diet or T0901317. Values shown are mean ± SEM of pooled data from experiments 1 and 2, with statistical comparisons performed by Student's *t*-test

P-values in bold indicate significant differences

Effect of the synthetic LXR agonist T0901317 on circulating metabolic and endocrine factors in male rats

In addition to increased levels of circulating triglycerides by the T0901317 feeding, the level of non-esterified fatty acids (NEFA) was significantly increased ($P < 0.05$; Table 4) and there was a small decrease in glucose levels ($P < 0.05$; Table 4). Corticosterone levels were unchanged by the T0901317 diet, while testosterone levels were decreased ($P < 0.05$; Table 4).

Effect of the synthetic LXR agonist T0901317 on the thyroid hormone axis in male rats

As shown in Table 5, plasma T3 levels were decreased by the T0901317-treatment ($P < 0.01$), and T4 levels were 115% of that in animals maintained on a standard diet ($P = 0.089$). When expressed as a T3/T4 ratio, T0901317 treatment resulted in a highly significant decrease of the ratio in plasma ($P < 0.001$). In contrast, the plasma level of TSH was not changed by T0901317 treatment.

Effect of the synthetic LXR agonist T0901317 on mRNA levels of genes involved in thyroid hormone homeostasis and lipogenesis

The decreased ratio of T3/T4 in plasma without an effect on the TSH level following LXR activation by T0901317 made us measure the mRNA levels of the types 1 and 2 iodothyronine deiodinases (DIO1 and DIO2, respectively) being responsible for conversion of T4 to T3 [29]. The T0901317-diet reduced the level of DIO1 mRNA by 28% in liver ($P < 0.05$; Table 6) but did not affect the expression of DIO1 in pituitary, thyroid, or retroperitoneal (RP) fat. DIO2 mRNA was not detected in liver samples, not changed in samples from the pituitary gland or RP fat, but was decreased by 62% in the thyroid ($P < 0.05$; Table 6). Transport of thyroid hormones across the plasma membrane is mediated by specific transporters [30]. The expression of monocarboxylate transporter 8 (MCT8, also known as SLC16A2), a specific transporter of thyroid hormones [31], was unchanged in liver, pituitary, and thyroid by the T0901317 diet.

Expression of the master regulator of fatty acid synthesis SREBP-1c [17] was markedly induced by T0901317 not only in liver and RP fat, but also in pituitary and thyroid (Table 6). Significantly increased mRNA levels of FAS in the pituitary accompanied the induced expression of SREBP-1c. In the thyroid FAS mRNA expression

Table 5 Effect of dietary supplementation with T0901317 on the thyroid hormone axis

Plasma variable	Standard diet	T0901317	*P*
Experiments 1 and 2 (n=)	16	17	
TSH (ng/ml)	9.1 ± 1.4	8.9 ± 1.0	0.900
T3 (nM)	0.88 ± 0.02	0.78 ± 0.03	**0.006**
T4 (nM)	39.1 ± 0.8	44.2 ± 1.9	0.089
T3/T4 (ratio × 10^2)	2.3 ± 0.05	1.9 ± 0.07	**0.0001**

Circulating thyroid hormone levels (T3 and T4) and TSH levels were measured in male rats maintained on standard diet or T0901317. Values shown are mean ± SEM of pooled data from experiments 1 and 2, with statistical comparisons performed by Student's *t*-test

P-values in bold indicate significant differences

following T0901317 treatment was 127% of that with standard diet; this was not statistically significant ($P = 0.453$).

Discussion

Endocrine axes govern growth and reproductive functions and are intimately associated with regulation of metabolism and vice versa. This study demonstrates that pharmacological stimulation of LXR in male rats interferes with the thyroid hormone status and, to a lesser extent, with the pattern of GH secretion. Importantly, increased expression of SREBP-1c was demonstrated in the pituitary and thyroid gland, suggesting that LXR activation also influences de novo lipogenesis in these endocrine glands. As previously reported [17, 32], liver weight and hepatic expression of SREBP-1c increased. Moreover, plasma levels of triglycerides and NEFA increased in line with other reports using higher doses [16, 33]. Of note is the observation that LXR activation had no effect on epididymal fat weight but reduced the weight of retroperitoneal fat. LXR activation is mainly associated with increased SREBP-1c expression and increased lipogenesis in white adipose tissue (WAT). T0901317 treatment increases SREBP-1c expression also in brown adipose tissue but, in addition, decreases 11β hydroxy steroid dehydrogenase (11β HSD) and uncoupling protein 1 (UCP-1) expression in this fat depot but not in epididymal fat [28]. Thus, it might be speculated that also the two WAT depots, retroperitoneal and epididymal fat, respond differently and that the reduced retroperitoneal fat pad size could be due to increased lipolysis, uncoupling and/or decreased synthesis of triglycerides.

Following T0901317 treatment we observed a small but significant reduction of glucose levels. Reduced plasma levels of glucose following LXR stimulation have previously been demonstrated in murine models of type 2 diabetes but not in wild type mice [7, 8, 34]. Similarly, in contrast to studies performed in mice [35, 36], plasma

Table 6 Gene regulatory effects of dietary supplementation with T0901317 in male rats

Tissue	Gene	Standard diet	T0901317	Fold change	*P*
Liver (*n*=)		15	16		
	SREBP-1c	0.82 ± 0.10	18.5 ± 4.1	21	**0.0002**
	DIO1	0.64 ± 0.05	0.46 ± 0.04	0.7	**0.0124**
	DIO3	11.6 ± 5.83	8.27 ± 3.63	0.7	0.6238
	MCT8	0.71 ± 0.08	0.97 ± 0.14	1.4	0.1268
Pituitary (*n*=)		15	16		
	SREBP-1c	1.65 ± 0.12	9.66 ± 1.32	5.9	**0.0002**
	FAS	1.07 ± 0.04	1.60 ± 0.12	1.5	**0.0003**
	DIO1	1.05 ± 0.07	1.05 ± 0.10	1	1.0000
	DIO2	1.64 ± 0.17	1.58 ± 0.20	1	0.850
	MCT8	1.37 ± 0.09	1.45 ± 0.12	1.1	0.6037
Thyroid (*n*=)		12	10		
	SREBP-1c	3.59 ± 1.05	86.9 ± 17.6	24	**<0.0001**
	FAS	2.90 ± 0.74	3.69 ± 0.70	1.3	0.4528
	DIO1	0.95 ± 0.24	0.91 ± 0.16	1	0.8976
	DIO2	2.75 ± 0.57	1.05 ± 0.18	0.4	**0.0164**
	DIO3	2.16 ± 0.75	2.28 ± 0.54	1	0.893
	MCT8	1.02 ± 0.28	0.92 ± 0.16	0.9	0.7827
RP fat (*n*=)		12	11		
	SREBP-1c	1.16 ± 0.23	13.9 ± 3.1	12	**0.0003**
	DIO1	0.80 ± 0.16	1.04 ± 0.38	1.3	0.553
	DIO2	2.14 ± 0.57	3.1 ± 0.62	1.4	0.264

qPCR was used to measure relative mRNA levels of SREBP-1c, DIO1, DIO2, DIO3, FAS, and MCT8 in samples from liver, pituitary, thyroid, and retroperitoneal (RP) fat. Values shown are mean ± SEM of pooled data from experiments 1 and 2 (liver and pituitary) or data from experiment 2 (thyroid and RP fat), with statistical comparisons performed by Student's *t*-test

P-values in bold indicate significant differences

corticosterone in the rat was not increased in response to the T0901317 treatment. Direct stimulatory effects on genes encoding adrenal steroidogenic enzymes have been demonstrated; both pharmacological activation of LXRs by T0901317 and deficiency in LXRα in mice increase plasma corticosterone [35–37]. In this context, we cannot exclude that the dose of T0901317 employed in this study was insufficient to increase circulating corticosterone level nor that the adrenal axis in these two rodent models respond differently to LXR activation.

From studies in LXR deficient mice it is clear that the two LXR paralogs are pivotal for normal testicular structure and functioning and involve effects at the pituitary level [38, 39]. We found that testosterone levels were decreased by the T0901317 diet, corroborating that interference with LXR-regulated pathways impairs testicular function. Another possibility is that the reduced level of testosterone was due to increased hepatic metabolism of testosterone similar to hepatic estrogen deactivation following activation of LXR [40] or that the level of sex hormone binding globulin (SHBG) was affected.

Since GH secretion is markedly episodic and sexually dimorphic it is vital to employ serial blood sampling to analyze GH secretion. Except for an indication of a minor reduction in the number and frequency of GH peaks, our analysis revealed no effect of T0901317 on GH secretion in male rats. The mechanism by which pulse frequency is reduced and the potential physiological significance of this phenomenon remain unclear. The observed reduction in pulse frequency is unlikely to affect male-specific and GH-regulated hepatic variables because these variables can be reproduced in GH deficient rats by single daily injections of GH [41]. In addition, we did not detect increased basal GH levels characteristic of female rats which we have previously shown to feminize GH-regulated hepatic variables [41].

The thyroid secretes T3 and T4 in a ratio that reflects the ratio present in the thyroid gland. In the rat, about 40% of T3 production is accounted for by thyroid secretion with extrathyroidal tissues contributing the remaining 60% by converting T4 to T3 ([29] and references therein). The unchanged level of TSH together with the significant decrease of T3 and the suggested increase of T4 levels in T0901317 treated rats implied that less T4 was converted to T3 or that T3 inactivation and/or degradation was enhanced. Since LXRs regulate transcription of target genes, we measured the mRNA levels of the deiodinase genes responsible for thyroid hormone homeostasis. We did not detect any difference in expression of DIO3 in liver or thyroid by the T0901317 treatment that could explain the lower level of circulating T3; DIO3 catalyzes the conversion to biologically inactive thyroid hormones [42]. The two pathways for activation of T4 to T3, catalyzed by the isozymes DIO1 and DIO2, have been demonstrated to be equally important in the euthyroid condition [43]. The expression and regulation of these enzymes are both species and tissue specific; in accordance with published data, essentially no expression of DIO2 was detected in rat liver [44]. DIO1 in the liver has been suggested to contribute to the control of circulating levels of T3 via both activation and inactivation effects; however, genetic inactivation of hepatic DIO1 in mice does not change the level of thyroid hormones in plasma [29, 45, 46]. T0901317 reduced the hepatic expression of DIO1 in this study, a finding previously reported in a genome wide expression analysis in mouse liver [28]. Both thyroid hormone receptors (TR) and LXR convey transcriptional regulation via so-called direct repeat 4 (DR4) elements, i.e. a repeat of the consensus sequence AGGTCA separated by 4 nucleotides, and a functional DR4 element has been characterized in the human DIO1 promoter [47]. The fact that hepatic expression of DIO1 is induced by T3 [48, 49], while LXR activation led to reduced expression suggests differential recruitment of cofactors. Moreover, the LXR-mediated regulation of DIO1 appeared tissue specific in that the levels were unchanged in the other tissues examined.

Surprisingly, DIO2 expression in the thyroid was markedly suppressed by the T0901317-diet. DIO2 regulates the intracellular concentration of T3 and its expression is down-regulated by T3 [50]. The marked down-regulation of DIO2 in the thyroid could theoretically be the underlying cause of the reduced T3/T4 ratio in plasma. However, expression of DIO2 mRNA in the rat thyroid is very low, and furthermore, DIO2 activity is undetectable in thyroid of adult rats [44]. We observed 100-fold lower expression of DIO2 than of DIO1 mRNA in the thyroid (data not shown). Interestingly, an association between LXR and DIO2 has previously been observed; in LXR-deficient mice maintained on a 'Western' diet, DIO2 mRNA and activity are increased. This change was, however, not accompanied by changes in plasma T3, T4, or TSH [51]. To this end, our data may suggest that the reduced expression of DIO1 in the liver contributed to the reduced level of T3 in plasma in response to the T0901317-diet. Although the mechanisms remain elusive, it is clear that pharmacological LXR activation affected thyroid hormone homeostasis.

From studies on other organs, it is obvious that interfering with LXR pathways affects lipid homeostasis and causes organ dysfunction. It has been shown that LXR agonist treatment of mice affects the hypothalamo-pituitary-adrenal (HPA) axis [36], supposedly by independent actions on the pituitary and the adrenal gland [37], and LXRs are implicated in testicular and oocyte functioning [38, 52]. Increased expression of SREBP-1c in response to LXR activation is at least in part behind increased lipid

storage. The marked increase in SREBP-1c expression and the parallel increase in FAS mRNA in the pituitary by the T0901317-diet suggest that de novo lipogenesis might occur in this organ. Although oil red O staining of pituitary sections did not reveal any lipid droplets in this study (data not shown), it is reasonable to assume that more prolonged activation of LXR will result in unwanted lipid accumulation in the pituitary. This would eventually have detrimental effects on the whole endocrine system and therefore jeopardize anti-atherosclerotic therapies based on LXR targeting.

Materials and methods

Animal experiments

The animal procedures described conformed to the institutional and national ethical guidelines for in vivo experiments in rodents and were specifically sanctioned by local ethical review at Cardiff University.

Experiment 1: effect of the synthetic LXR agonist T0901317 on the parameters of pulsatile growth hormone secretion in male rats

Male Sprague Dawley rats (weighing 215.5–228.6 g; Harlan UK Limited, Bicester, Oxon, UK) were placed in metabolic cages for 3 days prior to the replacement of standard laboratory rat chow (Cardiff) with either standard rodent chow (R3; Lactamine AB, Vadstena, Sweden) or the same food prepared with 0.005% (w/w) *N*-(2,2,2-Trifluoroethy [k1]l)-*N*-[4-[2,2,2-trifluoro-1-hydroxy-1(trifluoromethyl) ethyl]phenyl]-benzenesulfonamide (T0901317) (Cayman Chemical Company, Tallinn, Estonia); equivalent to a daily dose of 4-5 mg/kg body weight. This dose has previously been shown to reverse the protective effect of LXRα on the lipid profile in mice [53]. After 2–3 days maintenance on these revised diets, the rats were prepared with single-bore jugular vein cannulae under halothane anesthesia. During the next 48–72 h, during which the rats continued to receive the revised diets, cannula patency was maintained with an intermittent infusion of saline as previously described [54]. On the seventh day of treatment with the revised diets, the rats were subjected to automated serial blood sampling in which 100 µl samples of 1:5 whole blood were collected from each rat every 10 min for 24 h (commencing at 09:30 h). Circulating GH concentrations were determined in diluted whole blood by radioimmunoassay (RIA), as described below. Body weight and food intake were monitored daily throughout the treatment period. At the end of the sampling period the rats were re-anesthetized and killed by decapitation. Trunk blood was collected, centrifuged, and separated plasma stored at −20°C for subsequent determination of hormones and metabolic factors. In addition, liver, retroperitoneal fat and epididymal fat were dissected and weighed, sub-samples being snap frozen in liquid nitrogen for subsequent mRNA analysis.

Experiment 2: effect of the synthetic LXR agonist T0901317 on baseline growth hormone secretion in male rats

In order to determine whether exposure to T0901317 increases the baseline secretion of GH, a second cohort of male Sprague Dawley rats (weighing 215.2–243.9 g; Harlan UK Limited, Bicester, Oxon, UK) were given either standard R3 rodent chow or T0901317-containing chow and prepared with single-bore jugular vein cannulae as described above. On the seventh day of treatment with the revised diets, the rats were subjected to automated serial blood sampling in which 200 µl samples of 1:5 whole blood were collected from each rat every 10 min for 12 h (commencing at 12:30 h). Circulating GH concentrations were determined in diluted whole blood using a delayed addition RIA protocol, as described below. Body weight and food intake were monitored daily throughout the treatment period. At the end of the sampling period the rats were re-anesthetized and killed by decapitation, with trunk blood, liver, retroperitoneal fat, and epididymal fat samples collected as described above. In addition, pituitary and thyroids were dissected whole and snap frozen in liquid nitrogen for subsequent mRNA analysis.

Analysis of blood and plasma samples

Blood rGH concentrations in experiment 1 were determined by a simultaneous antibody/iodinated-rGH addition RIA protocol, with the results expressed in terms of the reference preparation RP-2, using the reagents supplied by NIDDK (intra-assay variation 1.2%; sensitivity 0.25 ng/ml). Blood rGH concentrations in experiment 2 were determined using identical assay reagents in a protocol in which the standards/samples were preincubated with the anti-rGH antibody for 24 h prior to the addition of iodinated-rGH (intra-assay variation 1.2%; sensitivity 0.063 ng/ml).

Plasma triglycerides (Tg) and total cholesterol levels were measured with enzymatic colorimetric assays (CHOD-PAP kits, Roche Diagnostics GmbH, Mannheim, Germany). Insulin was measured using a RIA method (Linco Research Inc., Missouri, USA). Insulin like growth factor 1 (IGF1) was analyzed with RIA DSL-2900 (Diagnostic Systems Inc., Webster, TX). Non-esterified fatty

acid (NEFA) levels were determined using NEFA C Assay kit (Wako Chemicals GmbH, Neuss, Germany). Glucose was measured using a photometric assay (ABX Diagnostics-Parch Euromedicine, France). Corticosterone was measured using a RIA kit (Amersham Life Science, Amersham International, UK; RPA 548). Total testosterone was determined using Coat-A-Count RIAs (Diagnostic Products Corporation, Los Angeles, CA). Total plasma thyroxine (T4) and triiodothyronine (T3) were determined using RIA, Coat-A-Count (Diagnostic Products Corporation). TSH was measured using the rTSH Biotrak Assay System (Amersham Bioscience, Uppsala, Sweden).

RNA analysis

Total RNA was isolated using the RNeasy kit (Qiagen). RNA, 100–500 ng, was reverse-transcribed using the SuperScript II reverse transcriptase kit (Invitrogen). Quantitative real time PCR (qPCR) was performed using the Power SYBR Green master mix (ABI) and amplified in an ABI Prism 7500 Sequence detector. Primers were designed using Primer Express software and span over exon boundaries; primer sequences are available on request. Amplification of specific transcripts was confirmed by dissociation curve analysis and further checked by agarose gel electrophoresis. We calculated relative changes by the comparative method using 18 S as the reference gene using controls as calibrators.

Statistical analysis

Total secretory output for GH was determined by calculation of area under curve (AUC) using GraphPad Prism (San Diego, CA). The profiles of circulating rGH were assessed by distribution analysis [55] with peak height, median and baseline secretion being determined by the calculation of the observed concentrations at 95%, 50%, and 5%, respectively. In the second study, a more rigorous analysis of baseline secretion was performed by the additional calculation of high baseline (observed concentration at 10%). In addition, rGH secretory profiles were also subjected to PULSAR analysis with the cut-off parameters set to give a false positive error rate of 5% [G(1) = 3.98; G(2) = 2.40; G(3) = 1.68; G(4) = 1.24; G(5) = 0.93] [56] with single point peaks excluded. With PULSAR-identified peaks, pulse height was determined by calculating the mean peak height of the nine highest peaks in each 24-h profile.

All data shown are mean ± SEM, with statistical comparisons performed by Student's t-tests.

Acknowledgments We thank Bo Carlsson, KaroBio AB, Sweden, for help with analysis of TSH. We also thank Anders Elmgren and co-workers at AstraZeneca R&D Mölndal, Sweden, for kind help with plasma assays. This research was funded by AstraZeneca R&D, Mölndal, Sweden, the Swedish Science Council (AM) and the Biotechnology and Bioscience Research Council (UK) (Grants 72/S11914 & BB/C505032) to TW & JSD.

References

1. M. Robinson-Rechavi, H. Escriva Garcia, V. Laudet, J. Cell Sci. **116**, 585–586 (2003)
2. A.I. Shulman, D.J. Mangelsdorf, N. Engl. J. Med. **353**, 604–615 (2005)
3. S. Alberti, G. Schuster, P. Parini, D. Feltkamp, U. Diczfalusy, M. Rudling, B. Angelin, I. Bjorkhem, S. Pettersson, J.A. Gustafsson, J. Clin. Invest. **107**, 565–573 (2001)
4. D.J. Peet, S.D. Turley, W. Ma, B.A. Janowski, J.M. Lobaccaro, R.E. Hammer, D.J. Mangelsdorf, Cell **93**, 693–704 (1998)
5. G.U. Schuster, L. Johansson, S. Kietz, T.M. Stulnig, P. Parini, J.K. Gustafsson, Biochem. Biophys. Res. Commun. **348**, 176–182 (2006)
6. J.M. Lehmann, S.A. Kliewer, L.B. Moore, T.A. Smith-Oliver, B.B. Oliver, J.L. Su, S.S. Sundseth, D.A. Winegar, D.E. Blanchard, T.A. Spencer, T.M. Willson, J. Biol. Chem. **272**, 3137–3140 (1997)
7. G. Cao, Y. Liang, C.L. Broderick, B.A. Oldham, T.P. Beyer, R.J. Schmidt, Y. Zhang, K.R. Stayrook, C. Suen, K.A. Otto, A.R. Miller, J. Dai, P. Foxworthy, H. Gao, T.P. Ryan, X.C. Jiang, T.P. Burris, P.I. Eacho, G.J. Etgen, J. Biol. Chem. **278**, 1131–1136 (2003)
8. B.A. Laffitte, L.C. Chao, J. Li, R. Walczak, S. Hummasti, S.B. Joseph, A. Castrillo, D.C. Wilpitz, D.J. Mangelsdorf, J.L. Collins, E. Saez, P. Tontonoz, Proc. Natl. Acad. Sci. USA **100**, 5419–5424 (2003)
9. E.G. Lund, L.B. Peterson, A.D. Adams, M.H. Lam, C.A. Burton, J. Chin, Q. Guo, S. Huang, M. Latham, J.C. Lopez, J.G. Menke, D.P. Milot, L.J. Mitnaul, S.E. Rex-Rabe, R.L. Rosa, J.Y. Tian, S.D. Wright, C.P. Sparrow, Biochem. Pharmacol. **71**, 453–463 (2006)
10. N. Mitro, P.A. Mak, L. Vargas, C. Godio, E. Hampton, V. Molteni, A. Kreusch, E. Saez, Nature **445**, 219–223 (2006)
11. R. Apfel, D. Benbrook, E. Lernhardt, M.A. Ortiz, G. Salbert, M. Pfahl, Mol. Cell Biol. **14**, 7025–7035 (1994)
12. M. Teboul, E. Enmark, Q. Li, A.C. Wikstrom, M. Pelto-Huikko, J.A. Gustafsson, Proc. Natl. Acad. Sci. U. S. A. **92**, 2096–2100 (1995)
13. S.B. Joseph, E. McKilligin, L. Pei, M.A. Watson, A.R. Collins, B.A. Laffitte, M. Chen, G. Noh, J. Goodman, G.N. Hagger, J. Tran, T.K. Tippin, X. Wang, A.J. Lusis, W.A. Hsueh, R.E. Law, J.L. Collins, T.M. Willson, P. Tontonoz Proc. Natl. Acad. Sci. U. S. A. **99**, 7604–7609 (2002)
14. J.J. Repa, K.E. Berge, C. Pomajzl, J.A. Richardson, H. Hobbs, D.J. Mangelsdorf, J. Biol. Chem. **277**, 18793–18800 (2002)
15. J.J. Repa, D.J. Mangelsdorf, Annu. Rev. Cell Dev. Biol. **16**, 459–481 (2000)
16. J.R. Schultz, H. Tu, A. Luk, J.J. Repa, J.C. Medina, L. Li, S. Schwendner, S. Wang, M. Thoolen, D.J. Mangelsdorf, K.D. Lustig, B. Shan. Genes Dev. **14**, 2831–2838 (2000)
17. J.J. Repa, G. Liang, J. Ou, Y. Bashmakov, J.M. Lobaccaro, I. Shimomura, B. Shan, M.S. Brown, J.L. Goldstein, D.J. Mangelsdorf Genes Dev. **14**, 2819–2830 (2000)
18. A. Lanni, M. Moreno, A. Lombardi, P. de Lange, F. Goglia, J. Endocrinol. Invest. **24**, 897–913 (2001)
19. A. Roos, S.J. Bakker, T.P. Links, R.O. Gans, B.H. Wolffenbuttel, J. Clin. Endocrinol. Metab. **92**, 491–496 (2007)

20. P.M. Yen, Physiol. Rev. **81**, 1097–1142 (2001)
21. H. Attallah, A.L. Friedlander, A.R. Hoffman, Growth Horm. IGF Res. **16**(Suppl A), S62–S67 (2006)
22. M.B. Davidson, Endocr. Rev. **8**, 115–131 (1987)
23. R.M. Luque, R.D. Kineman, Endocrinology **147**, 2754–2763 (2006)
24. N. Moller, J. Gjedsted, L. Gormsen, J. Fuglsang, C. Djurhuus, Growth Horm. IGF Res. **13**(Suppl A), S18–S21 (2003)
25. S. Eden, Endocrinology **105**, 555–560 (1979)
26. A. Mode, J.A. Gustafsson, Drug Metab. Rev. **38**, 197–207 (2006)
27. A. Mode, E. Wiersma-Larsson, A. Strom, P.G. Zaphiropoulos, J.A. Gustafsson, J. Endocrinol. **120**, 311–317 (1989)
28. T.M. Stulnig, K.R. Steffensen, H. Gao, M. Reimers, K. Dahlman-Wright, G.U. Schuster, J.A. Gustafsson, Mol. Pharmacol. **62**, 1299–1305 (2002)
29. A.C. Bianco, D. Salvatore, B. Gereben, M.J. Berry, P.R. Larsen, Endocr. Rev. **23**, 38–89 (2002)
30. J. Jansen, E.C. Friesema, C. Milici, T.J. Visser, Thyroid **15**, 757–768 (2005)
31. E.C. Friesema, S. Ganguly, A. Abdalla, J.E. Manning Fox, A.P. Halestrap, T.J. Visser, J. Biol. Chem. **278**, 40128–40135 (2003)
32. J.W. Chisholm, J. Hong, S.A. Mills, R.M. Lawn, J. Lipid. Res. **44**, 2039–2048 (2003)
33. A. Grefhorst, B.M. Elzinga, P.J. Voshol, T. Plosch, T. Kok, V.W. Bloks, F.H. van der Sluijs, L.M. Havekes, J.A. Romijn, H.J. Verkade, Kuipers F., J. Biol. Chem. **277**, 34182–34190 (2002)
34. A. Grefhorst, T.H. van Dijk, A. Hammer, F.H. van der Sluijs, R. Havinga, L.M. Havekes, J.A. Romijn, P.H. Groot, D.J. Reijngoud, F. Kuipers, Am. J. Physiol. Endocrinol. Metab. **289**, E829–E838 (2005)
35. C.L. Cummins, D.H. Volle, Y. Zhang, J.G. McDonald, B. Sion, A.M. Lefrancois-Martinez, F. Caira, G. Veyssiere, D.J. Mangelsdorf, J.M. Lobaccaro, J. Clin. Invest. **116**, 1902–1912 (2006)
36. K.R. Steffensen, S.Y. Neo, T.M. Stulnig, V.B. Vega, S.S. Rahman, G.U. Schuster, J.A. Gustafsson, E.T. Liu, J. Mol. Endocrinol. **33**, 609–622 (2004)
37. M. Nilsson, T.M. Stulnig, C.Y. Lin, A.L. Yeo, P. Nowotny, E.T. Liu, K.R. Steffensen, Mol. Endocrinol. **21**, 126–137 (2007)
38. K.M. Robertson, G.U. Schuster, K.R. Steffensen, O. Hovatta, S. Meaney, K. Hultenby, L.C. Johansson, K. Svechnikov, O. Soder, J.A. Gustafsson, Endocrinology **146**, 2519–2530 (2005)
39. D.H. Volle, K. Mouzat, R. Duggavathi, B. Siddeek, P. Dechelotte, B. Sion, G. Veyssiere, M. Benahmed, J.M. Lobaccaro, Mol. Endocrinol. **21**, 1014–1027 (2007)
40. H. Gong, P. Guo, Y. Zhai, J. Zhou, H. Uppal, M.J. Jarzynka, W. Song, S. Cheng, W. Xie, Mol. Endocrinol. **21**, 1781–1790 (2007)
41. C. Legraverend, A. Mode, S. Westin, A. Strom, H. Eguchi, P.G. Zaphiropoulos, J.A. Gustafsson, Mol. Endocrinol. **6**, 259–266 (1992)
42. Hernandez, A., Thyroid **15**, 865–874 (2005)
43. T.T. Nguyen, F. Chapa, J.J. DiStefano III, Endocrinology **139**, 4626–4633 (1998)
44. B. Gereben, D. Salvatore, J.W. Harney, H.M. Tu, P.R. Larsen, Mol. Endocrinol. **15**, 112–124 (2001)
45. Kohrle, J., Methods Enzymol. **347**, 125–167 (2002)
46. F. Streckfuss, I. Hamann, L. Schomburg, M. Michaelis, R. Sapin, M.O. Klein, J. Kohrle, U. Schweizer, Biochem. Biophys. Res. Commun. **337**, 739–745 (2005)
47. C.Y. Zhang, S. Kim, J.W. Harney, P.R. Larsen, Endocrinology **139**, 1156–1163 (1998)
48. M.J. Berry, A.L. Kates, P.R. Larsen, Mol. Endocrinol. **4**, 743–748 (1990)
49. L.L. Amma, A. Campos-Barros, Z. Wang, B. Vennstrom, D. Forrest, Mol. Endocrinol. **15**, 467–475 (2001)
50. L.A. Burmeister, J. Pachucki, D.L. St Germain, Endocrinology **138**, 5231–5237 (1997)
51. N.Y. Kalaany, K.C. Gauthier, A.M. Zavacki, P.P.A. Mammen, T. Kitazume, J.A. Peterson, J.D. Hoerton, D.J. Garry, A.C. Bianco, D.J. Mangelsdorf, Cell Metab. **1**, 231–244 (2005)
52. K.R. Steffensen, K. Robertson, J.A. Gustafsson, C.Y. Andersen, Mol. Cell. Endocrinol. **256**, 9–16 (2006)
53. M. Lehrke, C. Lebhertz, S.C. Millington, H.-P. Guan, J. Millar, D.J. Rader, J.M. Wilson, M.A. Lazar, Cell Metab. **1**, 297–308 (2005)
54. J.S. Davies, D.A. Carter, T. Wells, Endocrinology **145**, 2950–2958 (2004)
55. D.R. Matthews, P.C. Hindmarsh, P.J. Pringle, C.G. Brook, Clin. Endocrinol (Oxf). **35**, 245–252 (1991)
56. G.R. Merriam, K.W. Wachter, Am. J. Physiol. **243**, E310–E318 (1982)