PERGAMON

The International Journal of Biochemistry & Cell Biology 30 (1998) 209–215

The International Journal of ***Biochemistry & Cell Biology***

The decrease of liver LDL receptor mRNA during fasting is related to the decrease in serum T3

Anita M.G. van der Wal, Onno Bakker *, Wilmar M. Wiersinga

Department of Endocrinology, Academical Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

Received 31 July 1997; accepted 18 September 1997

Abstract

Fasting is associated with a reduction in serum T3 and T4 and a rise of plasma LDL cholesterol. We hypothesized that an hypothyroid-like condition induced by fasting is responsible for the rise in LDL cholesterol. We therefore examined the relation between changes in thyroid hormone and cholesterol metabolism in rats fasted for 0, 8, 12, 24 or 48 h. Fasting resulted in a decrease of liver 5′-deiodinase mRNA from 8 h (to 50%, $p < 0.05$, $n = 6$), of serum T3 from 12 h and of serum T4 at 48 h; serum TSH remained unchanged. Furthermore, plasma LDL cholesterol increased from 24 h onwards preceded by a decrease of liver LDL receptor mRNA which in turn is related to serum T3 ($r = 0.55$, $p < 0.05$, $n = 19$). Adding T3 at a concentration such that normal T3 levels are maintained during 48 h fasting, prevents the decrease in the LDL receptor mRNA. Fasting did not change hepatic HMG CoA reductase mRNA but decreased cholesterol 7α-hydroxylase mRNA, which however was not related to the decrease of serum T3. In conclusion: (1) Fasting induces a hypothyroid-like condition in which inhibition of hepatic conversion of T4 into T3 may be responsible for the decrease of serum T3. (2) Fasting induces an increase of plasma LDL cholesterol, apparently caused by a decrease of hepatic LDL receptor gene expression which is (partly) related to the fall in serum T3. (234).

Keywords: LDL receptor; Thyroid hormone; Fasting

1. Introduction

Fasting is associated with a reduction in serum T3 and T4, without changes in serum TSH [20]. This change in thyroid hormone metabolism, which also occurs during illness, is termed the sick euthyroid syndrome (SES) the pathogenesis of which is unclear. The changes are related to a decrease in thyroidal secretion and a decrease of type-I deiodinase activity in the liver [8, 21]. Furthermore, fasting is associated with a decrease in the maximal binding capacity of the thyroid hormone receptor (TR) in different tissues [5, 17]; it has also been reported that the SES is associated with changes in the TR isoform mRNA expression [26]. Fasting also induces a rise of plasma LDL cholesterol according to some but not all studies [6, 10, 12], with plasma total cholesterol remaining unchanged. Most of these changes are also found during hypothyroidism

* Corresponding author. Tel.: +31-20-5666071; fax: +31-20-6917682; e-mail: o.bakker@amc.uva.nl

1357-2725/98/$19.00
PII: S1357-2725(97)00120-9

and we therefore hypothesized that the hypothyroid-like condition induced by fasting is responsible for the rise in LDL cholesterol. To test this hypothesis we studied the effect of fasting on the relationship between serum thyroid hormones and liver mRNA's coding for 5′-deiodinase and for three key proteins in cholesterol metabolism: the two enzymes HMG CoA reductase (involved in cholesterol synthesis) and cholesterol 7α-hydroxylase (the rate limiting enzyme in cholesterol degradation) [13] and the LDL receptor, which mediates cholesterol endocytosis [4]. Thyroid hormone increases both cholesterol 7α-hydroxylase and HMG CoA reductase activities and the number of hepatic LDL receptors [4, 13, 23] and also their mRNAs [14, 28] (Ness and Lopez, 1995) [7]. Our results indicate that a decrease in the conversion of T4 into T3 could be responsible for the decrease in serum T3 during fasting. The increase in plasma LDL cholesterol is apparently caused by a decrease in hepatic LDL receptor mRNA levels, which are related to the decrease in serum T3. This conclusion is supported by the fact that the expression of the LDL receptor mRNA does not decrease when normal T3 levels are maintained during fasting.

2. Materials and methods

2.1. Animals

In the first experiment male Wistar rats (275–325 gram body weight) were fasted for 0, 8, 12, 24, or 48 h (six animals per group). Fasting was initiated such that sacrifice was at the same time point (9 a.m.) in order to minimize any diurnal effects. Animals were given water *ad libitum* and kept on a defined light cycle (light: 7 a.m.–7 p.m., dark 7 p.m.–7 a.m.). Blood was collected by heart puncture whereafter animals were sacrificed by exsanguination. Livers were excised and frozen in liquid nitrogen. In the second experiment animals (two per group) were fasted for 48 h while they received a supplementary dose of T3 (0.2 μg/100 g BW, i.p. at 12 h intervals) to maintain normal T3 levels. All experiments were approved by our local animal welfare committee.

2.2. Assays

Serum T3 and T4 were analyzed by an in-house radio-immuno-assay (RIA). TSH was measured by RIA using rat TSH antigen NIADDK-rRSH-I-8 for iodination, specific rat TSH antiserum NIADDK-anti-rTSH-S-5, and rat TSH reference preparation NIADDK-rTSH-RP-2 as standards (courtesy of National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, Bethesda, MD, USA). Free Fatty Acids were analyzed using a NEFA C kit (Wako Chemicals GmbH, Neuss, Germany). Plasma cholesterol and triglyceride concentrations were determined enzymatically using a Cobas Bio analyzer (Boehringer Mannheim, Germany). The amount of HDL cholesterol in plasma was determined by measuring cholesterol concentrations in the supernatants after precipitating the plasma samples with heparin/$MnCl_2$. LDL cholesterol was estimated by subtracting HDL cholesterol from the HDL + LDL fraction, which was measured in the supernatants after precipitating the plasma samples with 10% SDS in 0.15 M NaCl [25].

2.3. RNA isolation and quantification of the mRNA levels

Total rat liver RNA was prepared from frozen livers using 3 M LiCl/6 M urea (experiment 1) [1] or using TriPure isolation reagent (Boehringer Mannheim, Germany; experiment 2). Polyadenylated RNA was isolated using the polyATract mRNA isolation system of Promega (Madison, WI, USA). Digoxigenin–UTP labeled RNA probes were made using the RNA labeling kit of Boehringer Mannheim (Germany). The probes used were; cholesterol 7α-hydroxylase (clone p7α-11) [15], (kindly provided by Dr. M. Noshiro, Hiroshima, Japan); a 1.7 kb *Hin*dIII–*Bgl*II fragment of the human LDL receptor (containing exons 1–11) subcloned into pBluescript (derived from pLDLR3) [27]; a 1.1 kb *Hin*dIII fragment of the human HMG-CoA reductase cDNA (derived from pHRED-102) [11] (both generous gifts from Dr. D.W. Russel, Dallas, TX, USA); a full length rat C/EBP cDNA in

Table 1
Effect of fasting on body- and liver weight and on thyroid function tests

Fasting (h)	Fall in body weight (g)	Liver weight (g)	Serum T3 (nmol/l)	Serum T4 (nmol/l)	Serum TSH (ng/ml)
0	—	12.9 ± 0.3	0.97 ± 0.04	65 ± 2	1.8 ± 0.1
8	9.5 ± 1.6	10.9 ± 0.2**	0.92 ± 0.04	63 ± 4	1.6 ± 0.1
12	14.6 ± 2.7	9.8 ± 0.4**	0.84 ± 0.02*	65 ± 4	1.4 ± 0.1
24	18.8 ± 2.0	8.6 ± 0.9**	0.78 ± 0.04**	58 ± 3	1.7 ± 0.2
48	43.2 ± 7.2	7.3 ± 0.2**	0.55 ± 0.08**	39 ± 19**	1.5 ± 0.1

Values mean ± SD, $n = 6$.
* $p < 0.05$.
** $p < 0.01$ compared to 0 h fasting.

pBluesript [9, 23] (kindly provided by Dr. S.L. McKnight (Baltimore, MD, USA)) or β-actin (Boehringer Mannheim GmbH, Germany) were used as an internal control. The cDNA probe for rat 5′-deiodinase [2] was a gift from Dr P.R. Larsen (Boston, MA, USA). Hybridizations were carried out overnight at 68°C, following the manufacturer's protocol (Boehringer Mannheim Biochemica, Germany), except that HMG-CoA reductase was hybridized at 42°C. The digoxigenin-labeled probes were detected according to the Boehringer Mannheim protocol. Quantification of the mRNA levels was performed by scanning the autoradiographs using an XRS/3CX scanner and whole band analysis (version 2.4 1991) from Millipore Corporation (Ann Arbor, MI, USA).

2.4. Statistical analysis

Differences between fasted and non-fasted rats were analyzed using an unpaired *t*-test. Correlations were evaluated using Pearson correlation analysis.

3. Results

Fasting resulted in a decrease of serum T3 after 12 h; serum T4 decreased only after 48 h, whereas serum TSH remained constant (Table 1). Total plasma cholesterol did not change, HDL cholesterol was decreased after 48 h and LDL cholesterol levels increased from 24 h onwards; plasma triglycerides decreased, and serum free fatty acids increased (Table 2). Liver 5′-deiodi-

Table 2
Effect of fasting on plasma cholesterol, free fatty acid (FFA) and triglyceride (TG) levels

Fasting (h)	Cholesterol (mmol/l)	LDL cholesterol (mmol/l)	HDL cholesterol (mmol/l)	FFA (mEq/l)	TG (mmol/l)
0	1.85 ± 0.19	0.45 ± 0.23	0.72 ± 0.10	0.35 ± 0.06	1.66 ± 0.13
8	2.10 ± 0.25	0.40 ± 0.23	0.79 ± 0.09	0.50 ± 0.07**	1.95 ± 0.55*
12	1.65 ± 0.19	0.53 ± 0.14	0.82 ± 0.15	0.55 ± 0.05**	0.62 ± 0.08***
24	1.88 ± 0.32	0.72 ± 0.18**	0.77 ± 0.15	0.63 ± 0.06**	0.82 ± 0.08***
48	1.67 ± 0.32	0.81 ± 0.31**	0.59 ± 0.08*	0.57 ± 0.06**	0.56 ± 0.09***

Values mean ± SD, $n = 6$.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$ compared to 0 h fasting.

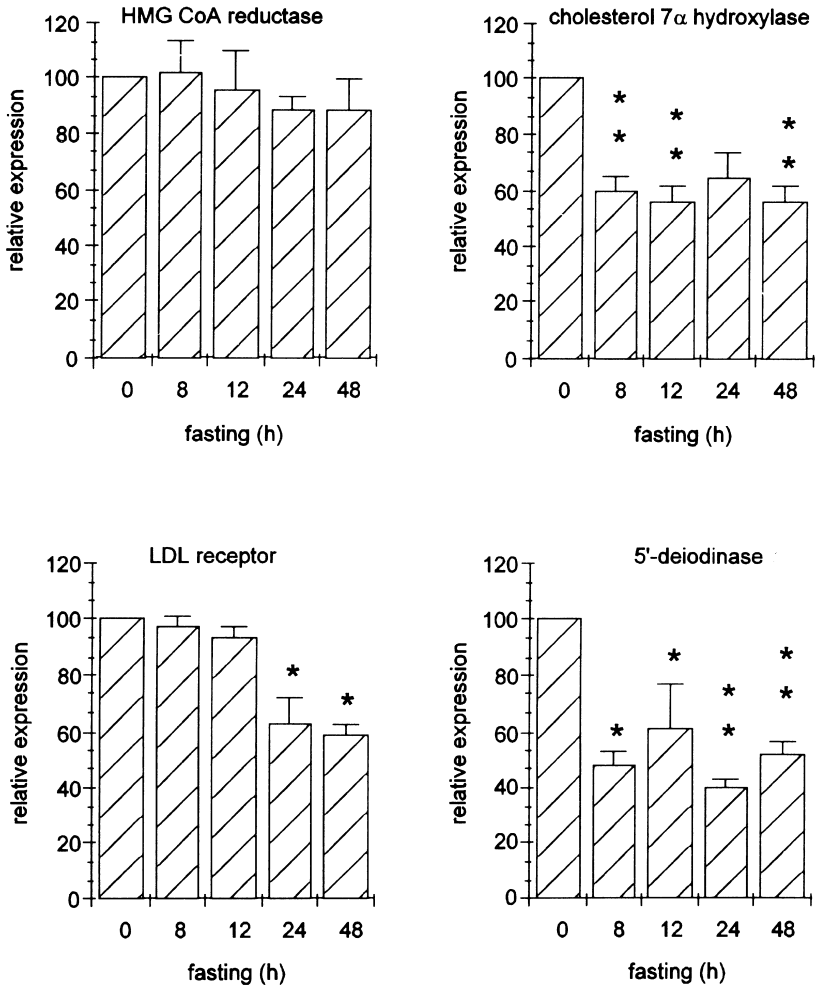

Fig. 1. Effect of fasting on the levels of the rat liver mRNA's of 5′-deiodinase, LDL receptor, HMG-CoA reductase and cholesterol 7α-hydroxylase expressed as percentage of non-fasting animals. Mean ± SEM, $n = 6$; *$p < 0.05$, **$p < 0.01$ vs 0 h.

nase mRNA decreased rapidly after 8 h (Fig. 1) followed by a decrease in serum T3 (Table 1). Liver mRNA encoding cholesterol 7α-hydroxylase decreased rapidly to 60% of baseline values at 8 h whereas the LDL receptor mRNA decreased from 24 h fasting onwards to 55% of control values (Fig. 1). Levels of liver mRNA coding for HMG-CoA reductase were not affected during the 48 h fast (Fig. 1). A relationship was observed between serum T3 and T4 concentrations and liver LDL receptor mRNA. 5′-Deiodinase mRNA expression was related to serum T3 but not to serum T4 (Table 3). Furthermore, we found a correlation between LDL serum levels and the LDL receptor mRNA (Table 3). In the second experiment we again found a 60% decrease in the expression of the LDL receptor mRNA in rats that fasted for 48 h. Interestingly, when these rats were given a T3 dose (0.2 μg/100 g BW, i.p. at 12 h intervals) to restore plasma T3 to normal levels during their 48 h fast (Table 4) no decrease in LDL receptor mRNA expression is seen (Table 4). That T3 was active in this case can be seen from both the lowered T4 (19 nmol/l) and undetectable plasma TSH levels (Table 4).

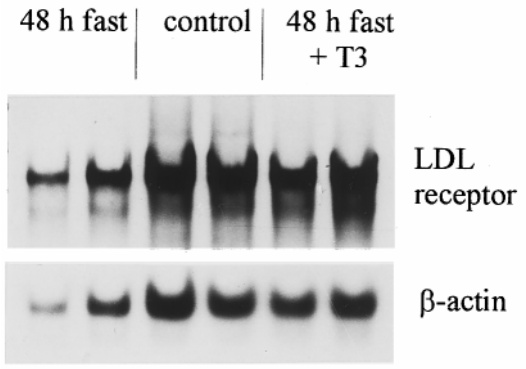

Fig. 2. Autoradiograph of a typical Northern blot. The blot was hybridized and signal was detected as described in Materials and Methods. 3 μg of polyA$^+$ RNA was applied per lane in duplicate. The lanes are marked as follows: 48 h fast, rats which fasted 48 h; control, rats which had access to food; 48 h fast + T3, rats which fasted 48 h during which their serum T3 levels were maintained euthyroid by 12 hourly i.p. injections of 0.2 μg T3/100 g b.w. Both the signal from the LDL receptor and the β-actin signal of the same blot are shown.

4. Discussion

In the present study we examined the relationship between changes in thyroid hormone and cholesterol metabolism during fasting. Fasting was associated with a decrease of serum T3 as reported before [20], which occurred from 12 h onwards. Interestingly, this was preceded by a decline in liver iodothyronine 5′-deiodinase mRNA which is similar to the observations made by Boelen *et al.* [3] in lipopolysaccharide-treated mice. It contrasts however with another study that reported a decrease of 5′-deiodinase mRNA following the drop in serum T3 [16]. These authors suggested impaired thyroidal secretion as the cause of the decline in serum T3, and viewed the decrease of liver 5′-deiodinase mRNA during food deprivation as secondary to the decrease of serum T3. Our results suggest the opposite, e.g. the decrease of serum T3 is likely the result of diminished T4 deiodination into T3.

Food deprivation did not affect plasma total cholesterol levels, but LDL cholesterol increased at 24 h whereas free fatty acids and triglycerides decreased. The levels of total, LDL and HDL cholesterol we measured in the rat are comparable to those reported earlier [24]. The changes in serum lipid profiles during fasting may even have implications for the energy metabolism of the cell as it was recently shown that the changes found in membrane lipid composition during hypothyroidism may influence a number of membrane-bound enzymes of the oxidative phosphorylation pathway [18, 19]. Concurrent with the plasma LDL cholesterol increase, liver LDL receptor mRNA was decreased. These results agree with recent data obtained in hypothyroid rats, which suggest that the mRNA level of the hepatic LDL receptor reflects the protein level and that the rise in plasma LDL cholesterol could be explained by decreased LDL cholesterol uptake in the liver due to a reduced number of LDL receptors (Ness and Lopez, 1995) [28, 7]. Similar observations were also made in experiments with rabbits in which fasting also induced a downregulation of liver LDL receptor mRNA followed by an increase of plasma LDL cholesterol [22]. The time course of events in our studies, the relationship between serum thyroid hormones and liver LDL receptor mRNA and the fact that LDL receptor mRNA expression is not lowered when normal T3 levels are maintained during fasting suggest that the decrease of LDL receptors is the result of the decrease in thyroid hormones during fasting. Indeed, gene expression of

Table 3
Correlations between serum T3, T4 and LDL and the mRNAs of cholesterol 7α-hydroxylase, HMG-CoA reductase, LDL-receptor and 5′-deiodinase

	Cholesterol 7α hydroxylase (n = 20)	HMG-CoA reductase (n = 20)	LDL-receptor (n = 19)	5′-deiodinase (n = 20)
Serum T3	r = 0.35, ns	r = 0.27, ns	r = 0.55, $p < 0.05$	r = 0.51, $p < 0.05$
Serum T4	r = 0.25, ns	r = 0.18, ns	r = 0.62, $p < 0.01$	r = 0.41, ns
Serum LDL	r = −0.36, ns	r = −0.28, ns	r = −0.55, $p < 0.05$	r = −0.25, ns

Table 4
Thyroid function tests and LDL receptor mRNA expression in fasted rats treated with T3 to maintain normal T3 levels during fasting

Group	T3[b] (nmol/l)	T4[b] (nmol/l)	TSH[b] (ng/ml)	LDLr mRNA[b] relative expression (%)
Control	1.3 (1.2–1.4)	55 (51–59)	1.0 (1.0–1.0)	100
48 h fast	0.9 (0.8–1.0)	30 (28–32)	1.1 (1.6–0.7)	40 (32–48)
48 h fast + T3	1.2 (1.2–1.2)	19 (17–21)	n.d.[a]	86 (79–92)

[a] n.d., not detectable.
[b] Mean of $n = 2$, individual values between brackets.

the LDL receptor is increased in thyroid hormone excess and decreased as a result of thyroid hormone deficiency [14]. Fasting is associated with a decrease in cholesterol 7α-hydroxylase enzyme activity [13] and in our study we also found a rapid decrease (within 8 h) of its mRNA during fasting. This is interesting in the light of the study of Ness *et al.* [14] who showed that cholesterol 7α-hydroxylase expression reacts rapidly and to low doses of T3, so any small decrease in T3 levels as a result of fasting may result in the quick response and decreased expression we observed. We found no relation however between cholesterol 7α-hydroxylase mRNA levels and serum T3 or T4 levels. Finally the absence of clear changes in HMG-CoA reductase mRNA in our studies was somewhat surprising, as HMG CoA reductase enzyme activity decreases during fasting [13]. An explanation for this could be the fact that HMG CoA reductase mRNA responds rather slowly to exogenously added T3 in a hypophysectomized rat (maximum stimulation after 72 h [14]) and therefore could also be slow to react to the decreased T3 during fasting.

A complex picture thus emerges of the relationship between thyroid hormone and cholesterol metabolism during fasting. Hepatic LDL receptor mRNA appears to be downregulated as a result of the decrease of serum T3 (and T4) although regulation by a factor which controls both cannot be ruled out by our experiments. Cholesterol 7α-hydroxylase mRNA decreases independent of the changes in serum thyroid hormones and HMG CoA reductase mRNA remains unchanged despite the fact that gene expression of these two proteins is (partly) T3 dependent. The change in plasma LDL cholesterol levels during fasting thus appears to be the result of a decreased LDL receptor expression.

Acknowledgements

We would like to thank our colleagues of the Laboratory of Endocrinology for measuring thyroid hormones and the members of the Department of Endocrinology for helpful discussions.

References

[1] F.M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seichman, J.A. Smith, K. Struhl, Current protocols in molecular biology, John Wiley and Sons, New York, 1987.
[2] M.J. Berry, L. Banu, P.R. Larsen, Type I iodothyronine deiodinse is a selenocysteine-containing enzyme, Nature 349 (1991) 438–440.
[3] A. Boelen, M.C. Platvoet-ter Schiphorst, O. Bakker, W.M. Wiersinga, The role of cytokines in the lipopolysaccharide-induced sick euthyroid syndrome, J. Endocrinol. 146 (1995) 475–483.
[4] M.S. Brown, P.T. Kovanen, J.L. Goldstein, Regulation of plasma cholesterol by lipoprotein receptors, Science 212 (1981) 628–635.
[5] K.D. Burman, Y. Lukes, F.D. Wright, L. Wartofsky, Reduction in hepatic triiodothyronine binding capacity induced by fasting, Endocrinology 101 (1977) 1331–1334.
[6] B.P. Daggy, A. Bensadoun, Enrichment of apolipoprotein B-48 in the LDL density class following *in vivo* inhibition of hepatic lipase, Biochim. Biophys. Acta 877 (1986) 252–261.
[7] N. Hoogerbrugge, H. Jansen, B. Staels, L.T. Kloet, J.C. Birkenhager, Growth hormone normalizes low-density

lipoprotein receptor gene expression in hypothyroid rats, Metabolism 45 (1996) 680–685.
[8] W.B. Kinlaw, H.L. Schwartz, J.H. Oppenheimer, Decreased serum triiodothyronine in starving rats is due primarily to diminished thyroidal secretion of thyroxine, J. Clin. Invest. 75 (1985) 1238–1241.
[9] W.H. Landschulz, P.F. Johnson, E.Y. Adashi, B.J. McKnight, S.L. McKnight, Isolation of a recombinant copy of the gene encoding C/EBP, Gen. Dev. 2 (1988) 786–800.
[10] S. Lanza-Jacoby, S.H. Wong, A. Tabares, D. Baer, T. Schneider, Disturbances in the composition of plasma lipoproteins during gram-negative sepsis in the rat, Biochim. Biophys. Acta 1124 (1992) 233–240.
[11] K.L. Luskey, B. Stevens, Human 3-hydroxy-3-methylglutaryl coenzyme A reductase, J. Biol. Chem. 260 (1985) 10271–10277.
[12] M. Maislos, N. Khamaysi, A. Assali, Y. Abou-Rabiah, I. Zvili, S. Shany, Marked increase in plasma high-density-lipoprotein cholesterol after prolonged fasting during Ramadan, Am. J. Nutr. 57 (1993) 640–642.
[13] D. Mayer, The circadian rythm of synthesis and catabolism of cholesterol, Arch. Toxicol. 36 (1976) 267–276.
[14] G.C. Ness, L.C. Pendleton, Y.C. Li, J.Y.L. Chiang, Effect of thyroid hormone on hepatic cholesterol 7α hydroxylase, LDL receptor, HMG-CoA reductase, farnesyl pyrophosphate synthetase and apolipoprotein A-I mRNA levels in hypophysectomized rats, Biochem. Biophys. Res. Comm. 172 (1990) 1150–1156.
[15] M. Noshiro, M. Nishimoto, K. Morohashi, K. Okuda, FEBS Lett. 257 (1989) 97–100.
[16] B. O'Mara, W. Dittrich, T.J. Lauterio, Pretranslational regulation of type-I 5′-deiodinase by thyroid hormones and in fasted and diabetic rats, Endocrinology 133 (1993) 1715–1723.
[17] J.H. Oppenheimer, H.L. Schwartz, Factors determining the level of activity of 3,5,5′-triiodothyronine-responsive hepatic enzymes in the starved rat, Endocrinology 107 (1980) 1460–1468.
[18] G. Paradies, F.M. Ruggiero, P. Dinoi, The influence of hypothyroidism on the transport of phosphate and on the lipid composition in rat-liver mitochondria, Biochim. Biophys. Acta 1070 (1991) 180–186.
[19] G. Paradies, F.M. Ruggiero, P. Dinoi, G. Petrsillo, E. Quagliariello, Decreased cytochrome oxidase activity and changes in phospholipids in heart mitochondria from hypothyroid rats, Arch. Biochem, Biophys. 307 (1993) 91–95.
[20] G.I. Portnay, J.T. O'Brian, J. Bush, A.G. Vagenakis, F. Arky, R.A. Arky, S.H. Ingbar, L.E. Braverman, The effect of starvation on the concentrations and binding of thyroxine and triiodothyronine in serum and on the response to TRH, J. Clin. Endocrinol. Metab. 39 (1974) 191–194.
[21] S. Santini, I.J. Chopra, A radioimmunoassay of rat type I iodothyronine 5′-monodeiodinase, Endocrinology 131 (1992) 2521–2526.
[22] H. Shimano, H. Aburatani, N. Mori, S. Ishibashi, T. Mokuno, H. Mokuno, M. Kawakami, Y. Akanuma, F. Murase, T. Murase, N. Yamada, Down-regulation of hepatic LDL receptor protein and mRNA in fasted rabbits, J. Biochem. 104 (1988) 712–716.
[23] B. Staels, A. Van Tol, L. Chan, H. Will, G. Verhoeven, J. Auwerx, Alterations in thyroid status modulate apolipoprotein, hepatic triglyceride lipase, and low density lipoprotein receptor in rats, Endocrinology 127 (1990) 1144–1152.
[24] T.E. Strandberg, R.S. Tilvis, T.A. Miettinen, Diurnal variation of plasma methyl sterols and cholesterol in the rat: relation to hepatic cholesterol synthesis, Lipids 19 (1984) 202–205.
[25] G.R. Warnick, J.J. Albers, A comprehensive evaluation of the heparin–manganese precipitation procedure for estimating high density lipoprotein cholesterol, J. Lipid Res. 19 (1978) 65–76.
[26] G.R. Williams, J.A. Franklyn, J.M. Neuberger, M.C. Sheppard, Thyroid hormone receptor expression in the sick euthyroid syndrome, Lancet 2 (1989) 1477–1481.
[27] T. Yamamoto, C.G. Davis, M.S. Brown, W.J. Schneider, M.L. Casey, J.L. Goldstein, D.W. Russel, The human LDL receptor: a cystein-rich protein with multiple Alu sequences in its mRNA, Cell 39 (1984) 27–38.
[28] G.C. Ness, D Lopez, Transcriptional regulation of rat hepatic low-density lipoprotein receptor and cholesterol 7α hydroxylase by thryroid hormone, Arch. biochem. Biophys. 323 (1995) 404–408.