CMLS, Cell. Mol. Life Sci. 59 (2002) 540–545
1420-682X/02/030540-06 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Research Article

Thyroid hormone controls carnitine status through modifications of γ-butyrobetaine hydroxylase activity and gene expression

S. Galland, B. Georges, F. Le Borgne, G. Conductier, J. Viana Dias and J. Demarquoy*

Université de Bourgogne, Faculté des Sciences Gabriel, 6 boulevard Gabriel, 21000 Dijon (France),
Fax: + 33 380 39 63 30, e-mail: jdemarqu@u-bourgogne.fr

Received 23 October 2001; received after revision 11 January 2002; accepted 15 January 2002

Abstract. The carnitine system plays a key role in β-oxidation of long-chain fatty acids by permitting their transport into the mitochondrial matrix. The effects of hypothyroidism and hyperthyroidism were studied on γ-butyrobetaine hydroxylase (BBH), the enzyme responsible for carnitine biosynthesis in the rat. In rat liver, BBH activity was decreased in the hypothyroid state and increased in hyperthyroid animals. The modifications in BBH activity correlated with changes in the enzyme Vmax values. These changes were shown to be related to hepatic BBH mRNA abundance. Thyroid hormones are known to interact with lipid metabolism, in particular by increasing long-chain fatty acid oxidation through activation of carnitine-dependent fatty acid import into mitochondria. Our study showed that thyroid hormones also increased carnitine bioavailability.

Key words. Carnitine; fatty acid metabolism; thyroid hormone; gene expression; γ-butyrobetaine hydroxylase.

Thyroid hormones are known to interact profoundly with fatty acid oxidation [1]. In mammals, fatty acid oxidation takes place in mitochondria. The major site of control of fatty acid oxidation is the system that transports cytosolic-activated fatty acids through mitochondrial membranes to the matrix [2]. This system involves three enzymes: carnitine palmitoyltransferase I (CPT-I), carnitine-acylcarnitine translocase and the CPT-II, all of which require carnitine as a substrate [3]. Thus, carnitine is strictly necessary for fatty acid transport into mitochondria.

Carnitine present in human and animal tissues is derived either from dietary items [4, 5] or from a biosynthetic pathway [6, 7]. In the rat, carnitine biosynthesis essentially takes place in the liver and involves five enzymatic reactions with methionine and lysine as early precursors [8, 9]. The last reaction is the hydroxylation of γ-butyrobetaine to carnitine. This reaction is catalyzed by γ-butyrobetaine hydroxylase (BBH; E.C. 1.14.11.1) [10]. BBH is a cytosolic enzyme that, in rat, is mainly present in the liver [11].

The oxidation of long-chain fatty acids is dramatically increased in the hyperthyroid state [12]. The thyroid hormone T3 stimulates intramitochondrial carnitine-dependent fatty acid import and oxidation by its action on CPTs and translocase activities [13, 14].

To date, there has been no report on the effect of the thyroid state on carnitine levels and biosynthesis. We previously reported the molecular cloning of rat BBH cDNA and also reported that BBH gene expression might be under the control of the nature of the diet as well as several hormones involved in control of the metabolism [15]. In this study, we report the effect of thyroid hormone on carnitine parameters and BBH activity and gene expression in the rat.

* Corresponding author.

Materials and methods

Reagents

Chemicals or drugs were purchased from Sigma (France) and were of analytical grade. Restriction and modifying enzymes were purchased from either Sigma or Promega (France). [^{32}P]dCTP was purchased from NEN (France). [^{3}H]acetyl-coenzyme A was obtained from Amersham (UK).

Animals

All studies were performed on adult male Wistar rats (Dépré, France) weighing between 200–220 g. French and European guidelines and laws were followed for the care and treatment of the rats. Animals were divided in three groups of four animals placed into individual cages. The first group was made hypothyroid by including 0.02% 6-propyl-2-thiouracil (PTU) in the drinking water for 3 weeks [16]. The PTU water was made fresh daily and kept in amber bottles. The thyroid status of the animals was checked as in Pamplona et al. [17] and the plasma T3 level was found to be 0.14 ± 0.02 ng/ml. In the second group, rats were made hyperthyroid (T3 serum concentration 2.69 ± 0.18 ng/ml) by an intraperitoneal injection (30 µg/100 g body weight) of thyroid hormone (T3; 3,3′,5′-triiodo-L-thyronine) for 6 days [18]. The third group of control animals (T3 serum level 0.45 ± 0.07 ng/ml) comprised mock-treated animals receiving a normal diet. All animals had free access to water and food (AO4; UAR, France). For further analysis, animals were sacrificed by cervical dislocation and tissues or organs were removed and immediately placed into ice-cold homogeneization buffer containing 300 mM sucrose, 1 mM EGTA in 50 mM Tris-HCl, pH 7.5.

Cytosolic fractions

Organs were minced, washed three times in the same ice-cold homogenization buffer, briefly dried and weighed. They were homogenized in this buffer with six strokes of a loose-fitting Teflon pestle at 200 rpm in an ice-cold Elvehjem homogenizer. The homogenate was centrifuged at 13,000 g for 30 min at 4 °C. The resulting supernatant was subsequently centrifuged at 100,000 g for 1 h at 4 °C. The final supernatant representing the cytosolic fraction was used to determine carnitine and BBH activity.

BBH activity and carnitine determination in tissues

BBH activity measurement and carnitine determination were done on cytosolic fractions. BBH activity was measured as described by Galland et al. [11]. Carnitine formed during the BBH-catalyzed reaction or contained in organs or tissues was determined as described using the method of McGarry and Foster [19] modified by Galland et al. [11]. Enzyme activity was expressed in pmol carnitine formed per minute and per milligram of protein.

Protein concentration determination

Protein concentration was estimated using the BCA procedure (BioRad, France) with bovine serum albumin as a standard.

Kinetic studies

These were performed on cytosolic fractions. Incubations were carried out as described above except that varying concentrations of γ-butyrobetaine were used.

Statistical analysis

Results are expressed as mean values ± SE. Statistical analysis of the data was done using the Student's t test (Statistica).

Isolation of RNA and Northern analysis

Total RNA from rat tissues was isolated as described in Chomczynski and Sacchi [20] and electrophoresed on a 1.2% agarose formaldehyde gel and transferred onto a nylon membrane (Hybond N; Amersham, France). The membrane was hybridized with [^{32}P]dCTP-labelled BBH probe at 42 °C overnight and washed in 2 × SSC, 0.1% SDS at room temperature twice for 15 min, then in 0.2 × SSC, 0.1% SDS at 65 °C for 15 min. The blots were exposed to Kodak X-omat AR films for 24–96 h with intensifying screens at –80 °C.

Quantitation of Northerns was done after scanning the blots using the BioRad Quantity One hardware and software. The 1.8-kb hybridizing material was quantitated and normalized against actin [15].

Probe for northern analysis

A full-length BBH cDNA clone was digested by *Eco*RI and *Hin*dIII and electrophoresed on a 1% agarose gel. The resulting fragment was isolated, electroeluted and precipitated with sodium acetate 3 M (pH 5.2) and ethanol (100%) and labelled with [^{32}P]dCTP using the Prime a Gene labelling system (Promega).

Results and discussion

Carnitine levels in several organs of euthyroid, hypothyroid and hyperthyroid rats

As shown in figure 1, in rat liver, the carnitine level was dramatically decreased in the hypothyroid state (–36.2%), while in the hyperthyroid state, the amount of hepatic carnitine was increased by a factor of 2.14. The protein content of liver cytosolic fractions remained unchanged by these treatments (105.7 ± 6.1, 109.4 ± 2.4 and 105.5 ± 5.8 mg protein/g liver in eu-, hypo- and hyperthyroid rats, respectively). Thus, the level of hepatic carnitine was modified by changes in thyroid hormone status, while the carnitine concentration remained unchanged in the kidney and skeletal muscle cells. The heart

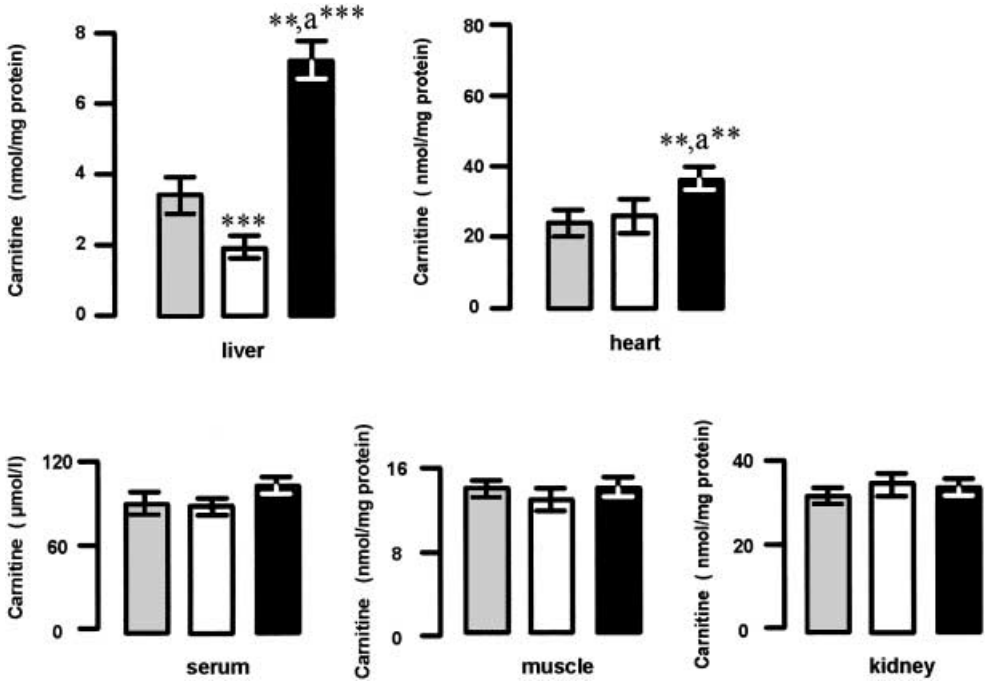

Figure 1. Carnitine levels in several organs of euthyroid, hypothyroid and hyperthyroid rats. Rats were made hypothyroid and hyperthyroid as indicated in Materials and methods. Carnitine levels were determined as described in the text from euthyroid (stippled bars), hypothyroid (open bars) and hyperthyroid (filled bars) rats in liver, heart, serum, skeletal muscle and kidney. Carnitine content is expressed in nmol/mg protein or in µmol/l. Values are the mean ± SE (n = 4). Asterisks denote significant differences between the hypo- or hyperthyroid group and euthyroid group. Asterisks marked a** or a*** compare the hyper- to hypothyroid group (** $p < 0.01$, *** $p < 0.005$).

carnitine level was significantly increased in the hyperthyroid state (+49%). These data suggested that thyroid hormone controlled the hepatic conversion of γ-butyrobetaine to carnitine but did not alter parameters related to intracellular transport of carnitine in kidney or skeletal muscle. On the other hand, the cardiac level of carnitine was increased by T3 treatment. The level of carnitine in the serum was the same in eu- and hypothyroid rats (97 ± 16 vs 93 ± 13 µM). Hyperthyroid rats exhibited a higher level of carnitine (111 ± 12 µM) even if this increase was not statistically different. Previous work with mice and rats showed a decreased carnitine content in some organs, especially in skeletal muscle [21, 22]. Pande and Parvin [23] observed that, in rats, dietary thyroxine increased the carnitine content in the liver and serum while carnitine levels in heart, skeletal muscle and the urinary excretion of carnitine were not affected. Thyroxine treatment has also been reported to enhance carnitine in hearts of guinea pigs [24]. In fact, because of differences in dose and duration of thyroid hormone treatment and in animal species, a direct comparison of these divergent results seems to be difficult. In our work, we have clearly shown that carnitine levels in the liver and the heart were increased in hyperthyroid rats while the carnitine content remained unchanged in the other tested organs, particularly in skeletal muscle.

Moreover, in T3-treated animals, we observed a significant increase in heart weight and augmented cardiac frequency (data not shown). In rats, induced hyperthyroidism was previously reported to result in a cardiovascular hyperdynamic state with tachycardia [25]. The cardiac cells in hyperthyroid rats had a significantly larger diameter and more mitochondria than those of control rats. The activities of cardiac enzymes involved in energy utilization showed a marked increase, particularly those participating in fatty acid metabolism [26]. This suggests that, in hyperthyroid rat hearts, both energy production and utilization systems are enhanced to meet the added workload. Thus, fatty acids, through their increased oxidative metabolism, contributed to this energy supply. Since fatty acid oxidation depends on the presence of carnitine, the increased level of carnitine in hyperthyroid hearts may support the increasing rate of fatty acid oxidation.

Because the heart cannot synthesize carnitine de novo, the increased cardiac concentration of carnitine we observed was probably due to enhanced carnitine uptake by cardiomyocytes. Compared to the data obtained in other tissues and especially in skeletal muscle cells, this also suggested the presence of a specific carnitine transporter in heart cells that differs from that in the skeletal muscle or kidney cells.

Increased urinary excretion of carnitine in hyperthyroidism has been shown in rats and humans. Hyperthyroidism is known to increase carnitine-dependent fatty acid import into mitochondria and the oxidation pathway with the production of the acylcarnitine moiety and elevation of total urinary carnitine excretion, reflecting an increased urinary acylcarnitine/free carnitine ratio. The observed increased carnitine content may compensate the enhanced carnitine utilization in tissues (especially in liver and heart) with a resulting augmentation of its excretion.

BBH activity in rat liver

In rat, BBH activity is only present in the liver [15]. We found that this enzymatic activity was dependent on the level of thyroid hormones. In the euthyroid state (control animals), BBH activity was found to be 208 pmol carnitine formed/min per milligram protein. In the hypothyroid state, this activity was significantly decrease (–38%) and in hyperthyroid animals, this activity was increased (+58%, fig. 2). These changes appeared to be in the same range as the modification in carnitine levels observed in tissues after T3 treatment.

BBH is an enzyme known to exhibit allosteric regulation. We determined if the modifications in BBH activity due to T3 treatment were associated with changes in the velocity (Vmax) or the efficiency (Km) of this reaction. Using Michaelis-Menten equations, these two parameters were determined for BBH in control rat liver cytosol and in hypothyroid and hyperthyroid rat liver cytosols. As shown in figure 3, compared to control rats, hypothyroid rats exhibited a decreased Vmax value (–40%). In hyperthyroid rats, the Vmax value for the BBH reaction was increased by 52%. In both cases, the Km for carnitine remained unchanged. Our data suggested that thyroid hormone interacted with BBH and γ-butyrobetaine conversion to carnitine by increasing the total amount of BBH present in the liver and not by modifying the affinity of the reaction.

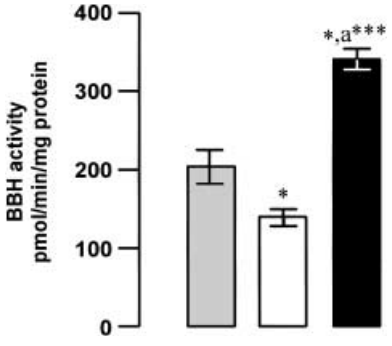

Figure 2. Effects of hyperthyroidism and hypothyroidism on hepatic BBH activity. Rats were made hypothyroid and hyperthyroid and BBH activity was determined as described in Materials and methods. BBH activity was measured in liver from euthyroid (stippled bar), hypothyroid (open bar) and hyperthyroid (filled bar) rats. BBH activity is expressed in pmol carnitine formed/min per milligram protein. Values are the mean ± SE (n = 4). Asterisks denote significant differences between the hypo- or hyperthyroid group and the euthyroid group. The asterisk marked a*** compares the hyper- to hypothyroid group (* $p < 0.05$, *** $p < 0.005$).

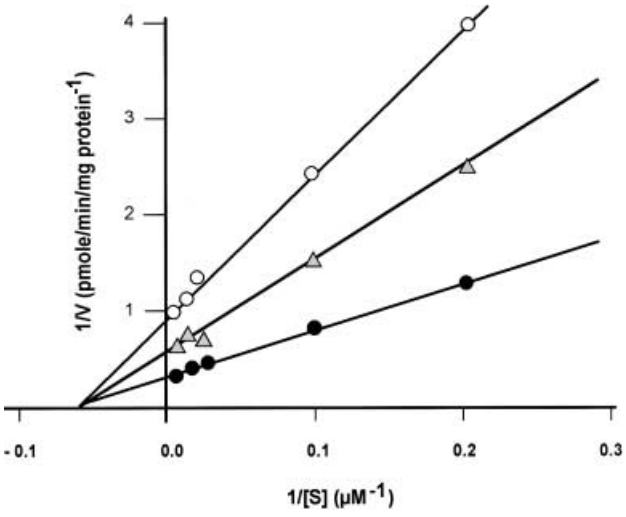

Figure 3. Effects of hyperthyroidism and hypothyroidism on BBH kinetic parameters. BBH activity was measured with varying concentrations of γ-butyrobetaine (as described in Materials and methods) in liver cytosolic fractions from euthyroid (stippled triangles), hypothyroid (open circles) and hyperthyroid (closed circles) rats. Michaelis constant and Vmax value determination was done on the Lineweaver-Burk plot. Velocity (V) was expressed in pmol carnitine formed/min per milligram protein and [S] was the concentration of γ-butyrobetaine in the reaction system.

BBH mRNA levels in euthyroid, hypothyroid and hyperthyroid rats

BBH mRNA levels were measured by Northern analysis in the liver of control and treated animals. After normalization, our data (figure 4) showed that BBH mRNA levels were modified according to the thyroid status of the animal. In hypothyroid animals, the amount of BBH mRNA was decreased by 33% compared to euthyroid animals. In contrast, the level of BBH mRNA showed a 1.7-fold increase in hyperthyroid animals. This suggested that the increase in BBH activity was related to a transcriptional effect of thyroid hormones, as it has been described for many proteins.

Fatty acid oxidation is a major source of energy for skeletal muscle and cardiac cells, and carnitine plays a major role in this process. Our results suggest that the carnitine level increased in response to an augmentation in BBH activity. Thyroid hormones regulate carnitine bioavailability through an increase in the BBH mRNA level. These hormones have been shown to act on the regulation of hepatic function and especially on lipid metabolism by a similar mechanism, i.e. alteration of the transcriptional activity of genes involved in lipid metabolism such as CPT-I and translocase [27]. The activity of CPT-I was increased three- to fourfold in liver mitochondria from hy-

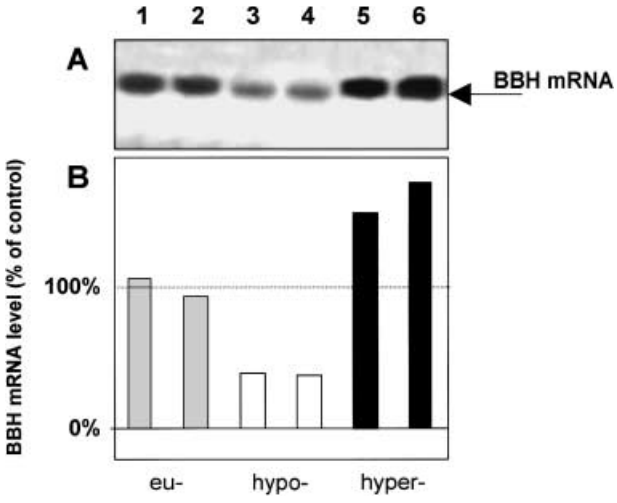

Figure 4. BBH mRNA levels in euthyroid, hypothyroid and hyperthyroid rats. BBH mRNA levels were measured by Northern analysis in the liver of control and treated animals. (*A*) Total liver RNA was extracted as described in Materials and methods. Total RNA (25 µg) from euthyroid (lanes 1, 2), hypothyroid (lanes 3, 4) and hyperthyroid (lanes 5, 6) rats was electrophoresed, blotted and hybridized with a [^{32}P]-labelled BBH probe and visualized by autoradiography. The same blot was reprobed with the β-actin cDNA control. Similar observations were made in three separate experiments. (*B*) The relative abundance of hybridizing materials from densitometry of autoradiograms was normalized against actin and plotted. The arbitrary value of 100% was given to the amount detected in euthyroid rat liver.

perthyroid rats compared with hypothyroid rats [28]. This was found to be related to an increase in the carnitine-dependent oxidation of activated long-chain fatty acids [28]. Here, we found a 2.5-fold increase in BBH activity between hypothyroid and hyperthyroid states.

The T3 stimulatory effect on CPT I was related to a decrease in the sensitivity of this enzyme to malonylCoA and to a rise in mRNA abundance [29]. Thyroid hormones have also been reported to increase carnitine-acylcarnitine translocase activity in rat heart. Kinetic analysis of the carnitine-acylcarnitine exchange reaction showed that the thyroid state affects the Vmax of this process, while having no effect on the Km value. This exchange reaction was stimulated in heart mitochondria by 36% in hyperthyroid rats and decreased by 39% in hypothyroid rats [14, 27]. These effects all favor fatty acid oxidation in enhancing the key carnitine-dependent import of fatty acids into mitochondria.

An increase in intracellular carnitine content has been shown to lead to a rise in fatty acid oxidation [30] by increasing the transfer of acyl moities onto carnitine and by reducing sensitivity of CPT-I to its natural inhibitor, malonyl-CoA [31]. Our data showed that thyroid hormones also act by increasing carnitine bioavailability through a stimulation of the last step of its biosynthesis in the liver and probably its import into heart cells.

Taken together, these data suggest that thyroid hormones, by increasing BBH mRNA abundance and subsequently BBH activity in rat liver, induce a marked increase in bioavailable carnitine in this organ. Increased levels of carnitine have been shown to stimulate fatty acid oxidation through mechanisms involving increased CPT-I activity. Thus thyroid hormones may increase fatty acid oxidation by enhancing carnitine bioavailability.

Interestingly, carnitine may act as a peripheral inhibitor of thyroid hormone uptake [32]. This inhibitory effect may be part of a retro-control loop between thyroid hormone and carnitine. In their study, Benvenga et al. [32] also showed that high doses of T3 inhibit radioactive carnitine uptake by two cell lines, hepatocytes (HepG2) and neurons (NB 41A3). These results may appear to contradict ours but may be explained by methodological differences between the two studies. Benvenga et al. [32] used tumor-derived cell lines and not whole animals. They found that thyroid hormones inhibit the uptake of radiolabelled carnitine into these cells with high doses of L-T3 or L-T4 (1–10 µM), i.e. 250- to 2500-fold the L-T3 dose used in our study. Moreover, they compared cell uptake of carnitine between cells loaded with 1 or 10 µM of thyroid hormone and cells without thyroid hormone (and consequently not in a euthyroid status as were our control rats). Benvenga et al. [32] observed the effect of a high dose of thyroid hormone on carnitine uptake in a very short interval (2 h). This effect is not discussed but could result from a direct cell membrane action of thyroid hormone on the carnitine transporter (a mechanism that is not mentioned by the authors), probably in a non-physiological manner considering the L-T3 and L-T4 doses used. In our study, thyroid hormone treatment was long term (6 days), avoiding transitory variations due to a massive supply of L-T3 and permitting observation of more stable effects.

Acknowledgements. This work was supported by the Région Bourgogne, the Fondation pour la Recherche Médicale and the Ministère de la Recherche et de l'Enseignement Supérieur.

1 Pucci E., Chiovato L. and Pinchera A. (2000) Thyroid and lipid metabolism. Int. J. Obes. Relat. Metab. Disord. **24 (Suppl 2):** S109–S112
2 McGarry J. D. and Brown N. F. (1997) The mitochondrial carnitine palmitoyltransferase system: from concept to molecular analysis. Eur. J. Biochem. **244:** 1–14
3 Kerner J. and Hoppel C. (2000) Fatty acid import into mitochondria. Biochim. Biophys. Acta **1486:** 1–17
4 Mitchell M. E. (1978) Carnitine metabolism in human subjects. I. Normal metabolism. Am. J. Clin. Nutr. **31:** 293–306
5 Krajcovicova-Kudlackova M., Simoncic R., Bederova A., Babinska K. and Beder I. (2000) Correlation of carnitine levels to methionine and lysine intake. Physiol. Res. **49:** 399–402
6 Bremer J. (1983) Carnitine – metabolism and functions. Physiol. Rev. **63:** 1420–1480
7 Rebouche C. J. and Seim H. (1998) Carnitine metabolism and its regulation in microorganisms and mammals. Annu. Rev. Nutr. **18:** 39–61

8 Rebouche C. J. (1982) Sites and regulation of carnitine biosynthesis in mammals. Fed. Proc. **41:** 2848–2852
9 Carter A. L., Abney T. O. and Lapp D. F. (1995) Biosynthesis and metabolism of carnitine. J. Child. Neurol. **10 (Suppl 2):** S3–S7
10 Lindstedt G. (1967) Hydroxylation of gamma-butyrobetaine to carnitine in rat liver. Biochemistry **6:** 1271–1282
11 Galland S., Le Borgne F., Guyonnet D., Clouet P. and Demarquoy J. (1998) Purification and characterization of the rat liver gamma-butyrobetaine hydroxylase. Mol. Cell. Biochem. **178:** 163–168
12 Heimberg M., Olubadewo J. O. and Wilcox H. G. (1985) Plasma lipoproteins and regulation of hepatic metabolism of fatty acids in altered thyroid states. Endocr. Rev. **6:** 590–607
13 Jansen M. S., Cook G. A., Song S. and Park E. A. (2000) Thyroid hormone regulates carnitine palmitoyltransferase Ialpha gene expression through elements in the promoter and first intron. J. Biol. Chem. **275:** 34989–34997
14 Paradies G., Ruggiero F. M., Petrosillo G. and Quagliariello E. (1996) Stimulation of carnitine acylcarnitine translocase activity in heart mitochondria from hyperthyroid rats. FEBS Lett. **397:** 260–262
15 Galland S., Le Borgne F., Bouchard F., Georges B., Clouet P., Grand-Jean F. et al. (1999) Molecular cloning and characterization of the cDNA encoding the rat liver gamma-butyrobetaine hydroxylase. Biochim. Biophys. Acta. **1441:** 85–92
16 Claustre J., Balende C. and Pujol J. F. (1996) Influence of the thyroid hormone status on tyrosine hydroxylase in central and peripheral catecholaminergic structures. Neurochem. Int. **28:** 277–281
17 Pamplona R., Portero-Otin M., Ruiz C., Bellmunt M. J., Requena J. R., Thorpe S. R. et al. (1999) Thyroid status modulates glycoxidative and lipoxidative modification of tissue proteins. Free Radic. Biol. Med. **27:** 901–910
18 McAllister R. M., Luther K. L. and Pfeifer P. C. (2000) Thyroid status and response to endothelin-1 in rat arterial vessels. Am. J. Physiol. Endocrinol. Metab. **279:** E252–E258
19 McGarry J. D. and Foster D. W. (1976) An improved and simplified radioisotopic assay for the determination of free and esterified carnitine. J. Lipid. Res. **17:** 277–281
20 Chomczynski P. and Sacchi N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162:** 156–159
21 Cederblad G. and Engstrom G. (1978) Effect of thyroxine treatment on carnitine levels in mice. Acta. Pharmacol. Toxicol. **43:** 1–5
22 Suzuki M., Tokuyama K. and Yamane A. (1983) Carnitine metabolism in thyroid hormone treated rats and mice. J. Nutr. Sci. Vitaminol. **29:** 413–428
23 Pande S. V. and Parvin R. (1980) Clofibrate enhancement of mitochondrial carnitine transport system of rat liver and augmentation of liver carnitine and gamma-butyrobetaine hydroxylase activity by thyroxine. Biochim. Biophys. Acta. **617:** 363–370
24 Bressler R. and Wittels B. (1966) The effect of thyroxine on lipid and carbohydrate metabolism in the heart. J. Clin. Invest. **45:** 1326–1333
25 Tanaka T., Morita H., Koide H., Kawamura K. and Takatsu T. (1985) Biochemical and morphological study of cardiac hypertrophy. Effects of thyroxine on enzyme activities in the rat myocardium. Basic Res. Cardiol. **80:** 165–174
26 Sugden M. C., Priestman D. A., Orfali K. A. and Holness M. J. (1999) Hyperthyroidism facilitates cardiac fatty acid oxidation through altered regulation of cardiac carnitine palmitoyltransferase: studies in vivo and with cardiac myocytes. Horm. Metab. Res. **31:** 300–306
27 Paradies G., Ruggiero F. M., Petrosillo G. and Quagliariello E. (1997) Alterations in carnitine-acylcarnitine translocase activity and in phospholipid composition in heart mitochondria from hypothyroid rats. Biochim. Biophys. Acta. **1362:** 193–200
28 Stakkestad J. A. and Bremer J. (1983) The outer carnitine palmitoyltransferase and regulation of fatty acid metabolism in rat liver in different thyroid states. Biochim. Biophys. Acta. **750:** 244–252
29 Mynatt R. L., Park E. A., Thorngate F. E., Das H. K. and Cook G. A. (1994) Changes in carnitine palmitoyltransferase-I mRNA abundance produced by hyperthyroidism and hypothyroidism parallel changes in activity. Biochem. Biophys. Res. Commun. **201:** 932–937
30 Clouet P., Sempore G., Tsoko M., Gresti J., Demarquoy J., Niot I. et al. (1996) Effect of short- and long-term treatments by a low level of dietary L-carnitine on parameters related to fatty acid oxidation in Wistar rat. Biochim. Biophys. Acta. **1299:** 191–197
31 Bird M. I. and Saggerson E. D. (1985) Interacting effects of L-carnitine and malonyl-CoA on rat liver carnitine palmitoyltransferase. Biochem. J. **230:** 161–167
32 Benvenga S., Lakshmanan M. and Trimarchi F. (2000) Carnitine is a naturally occurring inhibitor of thyroid hormone nuclear uptake. Thyroid **10:** 1043–1050