# Homocysteine Thiolactone-Induced Hyperhomocysteinemia Does Not Alter Concentrations of Cholesterol and SREBP-2 Target Gene mRNAs in Rats

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**The present rat study was conducted to test whether hyperhomocysteinemia induced by dietary homocysteine (Hcy) alters the cholesterol concentration in plasma and tissue and the gene expression of genes involved in cholesterol biosynthesis and uptake. Therefore, rats were fed 100 or 200 mg Hcy per kilogram body mass per day (Hcy100 group and Hcy200 group, respectively) as DL-homocysteine thiolactone, or an Hcy-free diet, which served as control, over 14 days. Rats from the Hcy100 group and the Hcy200 group had higher plasma Hcy concentrations (34.4 ± 4.6 and 69.4 ± 11.5 μ*M*, respectively) than rats fed an Hcy-free diet (9.5 ± 1.7 μ*M*). The concentration of Hcy in liver was 2.6 and 3.8 times higher, and in small intestine was 2.6 and 5.1 times higher, in the Hcy100 group and the Hcy200 group, respectively, than in control rats ($P < 0.05$). The concentrations of cholesterol in plasma, lipoproteins, liver, and small intestine and the relative mRNA concentrations of sterol regulatory element-binding protein 2 (SREBP-2), 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, and low-density lipoprotein (LDL) receptor in liver and small intestine were not influenced by DL-homocysteine thiolactone supplementation. In conclusion, in view of the experimental conditions used here, increased plasma and tissue concentrations of Hcy do not alter cholesterol metabolism of liver and intestine.** Exp Biol Med 232:81–87, 2007

**Key words:** homocysteine; cholesterol; gene expression; liver; small intestine; rat

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Received May 16, 2006.
Accepted June 17, 2006.

1535-3702/07/2321-0081$15.00
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Homocysteine (Hcy) is an intermediary product in methionine metabolism that can be either converted to cysteine by transsulfuration or methylated in a folate- and vitamin $B_{12}$–dependent reaction to form methionine. Hyperhomocysteinemia is considered an independent risk factor for vascular disorders resulting from atherosclerosis (1–3). Several potential mechanisms for homocysteine-induced atherosclerosis have been proposed. These include, for example, decreased bioavailability and synthesis of endothelium-derived nitric oxide (4), the vascular production of superoxide (5), increased proliferation of smooth muscle cells (6), enhanced coagulability (7), and protein modification (8). Additionally, it has been suggested that Hcy also may contribute to atherosclerosis by lipid dysregulation. A few studies have found a positive correlation between the plasma concentrations of Hcy and the plasma concentrations of cholesterol in hyperhomocysteinemic patients (9, 10) and in experimental animals (11–14).

Different dietary animal models of hyperhomocysteinemia have been performed to study possible Hcy-mediated effects on lipid metabolism. Those animal models include the oral administration of a high-methionine diet for 2 to 10 weeks (12–14) or excessive methionine combined with inadequate amounts of folate, which lead to increased plasma Hcy concentrations (12). The findings from those experiments show that dietary intervention with excessive methionine stimulates genes involved in cholesterol biosynthesis, such as the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, and the low-density lipoprotein (LDL) receptor, which is responsible for the uptake of LDL from plasma into cells *via* an activation of the sterol regulatory element-binding protein-2 (SREBP-2; Refs. 12–14), a transcription factor involved in gene expression of HMG-CoA reductase and LDL receptor (15–17). Hcy generated from excessive amounts of methionine has been suggested as the factor responsible for the alteration of cholesterol levels and SREBP-2 activation (12, 13).

However, results from other studies propose a direct effect of methionine on cholesterol metabolism, without any involvement of Hcy (18–21). For example, rabbits that were fed a diet enriched in methionine and lysine developed hypercholesterolemia within 3.5 weeks, although Hcy concentration was not increased (21). Additionally, another study has shown that a methionine-induced stimulation of *N*-methylation of phosphatidylethanolamine (PE) to form phosphatidylcholine (PC) could be responsible for the hypercholesterolemia which was observed in rats fed high-methionine diets for 2 weeks (20). Both research groups proposed that hypercholesterolemia induced by high dietary methionine concentrations might be due to a disturbed hepatic phospholipid metabolism via *S*-adenosylmethionine (SAM; Refs. 20, 21), the active form of methionine that reflects dietary methionine level (20, 22).

Based on those different findings, there is considerable uncertainty regarding whether Hcy plays a causal role in hypercholesterolemia. To shed more light onto the role of homocysteine in cholesterol metabolism we performed a study in which we administered rats a diet supplemented with homocysteine as DL-homocysteine thiolactone. If the action of excessive methionine on cholesterol metabolism is mediated primarily by homocysteine, one would expect that hyperhomocysteinemia induced by an administration of dietary homocysteine also alters the concentration of cholesterol and the expression of genes involved in cholesterol synthesis, as observed with a high-methionine or folate-deficient diet. For that purpose rats were fed either a diet containing two different amounts of homocysteine as DL-homocysteine thiolactone over 14 days or an Hcy-free diet serving as control. The dietary administration of DL-homocysteine thiolactone has been shown to be a successful experimental approach to increase the Hcy concentrations in plasma and liver and to study Hcy-induced atherogenic processes (23, 24). The concentrations of dietary homocysteine used for our study were chosen to increase the plasma and liver concentrations of Hcy to extents similar to those observed in animal models of hyperhomocysteinemia (12, 13) and to induce hyperhomocysteinemia within clinical relevant ranges. To assess the Hcy-increasing effect of exogenous Hcy, we determined the Hcy concentrations in plasma, liver, and small intestine and the ratio of SAM to *S*-adenosylhomocysteine (SAH) in liver, which is important for a wide variety of methylation reactions (25). Besides the concentration of cholesterol in plasma, liver, and small intestine, we determined the relative concentrations of mRNA coding for SREBP-2 and its target genes, such as HMG-CoA reductase and the LDL receptor in liver and small intestine.

## Materials and Methods

**Animals.** Male 5-month-old Sprague-Dawley rats ($n = 30$) supplied by Charles River (Sulzfeld, Germany) with an initial body weight (±SD) of 464 ± 28 g were assigned randomly to three groups of 10 rats each. The rats of the Hcy100 group were fed daily Hcy as DL-homocysteine thiolactone (Sigma-Aldrich, Taufkirchen, Germany), which corresponded to an intake of about 100 mg Hcy per kg body mass; the rats of the Hcy200 group received a diet that corresponded to a daily intake of about 200 mg Hcy per kg body mass. The control group received a diet without homocysteine. The rats were kept individually in macrolon cages in a room maintained at a temperature of 23°C and 50%–60% relative humidity with lighting from 0600 to 1800 h. The experimental procedures described followed established guidelines for the care and handling of laboratory animals and were approved by the council of Saxony-Anhalt, Germany.

**Experimental Diets.** All rats were fed a semi-synthetic diet according to the AIN-93M diet formulated for maintenance of adult rats (26), which contained the following ingredients (g/kg): cornstarch, 633; casein, 140; sucrose, 100; soybean oil, 40; cellulose, 40; mineral mixture, 35; vitamin mixture, 9; DL-methionine, 2.0; and choline chloride, 1.0. Vitamins and minerals were supplemented according to recommendations from the American Institute of Nutrition for rat diets (26). Folate, vitamin $B_{12}$, and vitamin $B_6$, which are involved in one-carbon metabolism, were added in amounts of 2 mg, 25 μg, and 6 mg, respectively, per kilogram diet. DL-homocysteine thiolactone was included in the diet at the expense of cellulose. The diet for rats from the Hcy100 group contained 2.8 g/kg DL-homocysteine thiolactone, and the diet for rats from the Hcy200 group contained 5.6 g/kg DL-homocysteine thiolactone. To avoid differences in food intake, each rat received 17 g food per day. This food amount was close to the energetic requirement for maintenance for adult rats (27). Intakes of 17-g diets containing 2.8 and 5.6 g/kg DL-homocysteine thiolactone correspond to daily intakes of approximately 100 and 200 mg Hcy per kilogram body mass, respectively, for each rat from the Hcy100 and Hcy200 group. During the whole experiment, all rats were fed equal amounts of diet daily. Diets were administered once daily at 0800 h. Water was freely available from nipple drinkers. The experimental diets were fed for 14 days.

**Sample Collection.** After a feeding period of 14 days the rats were killed by decapitation under light anesthesia with diethyl ether. Rats were not fasted before killing, because food deprivation leads to a significant downregulation of the genes involved in cholesterol metabolism (28) that were to be measured in this study. Plasma was separated from heparinized whole blood by centrifugation at 1880 *g* for 10 mins at 4°C. Livers were excised, weighed, and immediately snap frozen in liquid nitrogen. Samples of liver for total RNA isolation were stored at −80°C. The whole small intestine was carefully rinsed with ice-cold saline and opened lengthwise. Enterocytes of the small intestine were isolated by scraping (29). Enterocytes that were scraped off the gut mucosa were then mixed, weighed, and snap frozen in liquid nitrogen. For

total RNA isolation, aliquots of the enterocytes were stored at −80°C.

**Plasma Lipoprotein Isolation.** Plasma lipoproteins were separated by stepwise ultracentrifugation (Mikro-Ultrazentrifuge; Sorvall Products, Bad Homburg, Germany) at 900,000 *g* at 4°C for 1.5 hrs. The lipoprotein fractions were collected on the basis of their densities (ρ; kg/l) as described previously for rats (30): LDL: 1.006 kg/l $<$ ρ $<$ 1.063 kg/l; and HDL: ρ $>$ 1.063 kg/l. Plasma densities were adjusted with sodium chloride and potassium bromide. The lipoprotein fractions used for cholesterol analysis were removed by suction.

**Cholesterol Analysis.** Lipids from liver and small intestine were extracted with a mixture of *n*-hexane and isopropanol (3:2 [v/v]; Ref. 31). The concentration of cholesterol in liver and small intestine was determined in aliquots of the lipid extracts after evaporating the solvent mixture and dissolving the lipids in a mixture of Triton X-100 and chloroform (1:1 [v/v]; Ref. 32). Concentrations of total cholesterol in plasma and lipoprotein fractions, liver, and small intestine were determined using an enzymatic reagent kit (catalog no. 1.14830; VWR International, Darmstadt, Germany).

**Analyses of Hcy, SAM, and SAH.** Total Hcy (free Hcy, D- and L-enantiomers, Hcy disulfides, and Hcy-cysteine mixed disulfides) was determined by high-performance liquid chromatography (HPLC; Ref. 33). For measurement of Hcy concentrations in liver and small intestine, aliquots of the tissue were thawed and homogenized in ice-cold phosphate-buffered saline. The concentrations of SAM and SAH in the liver were measured by HPLC (34). Samples of frozen liver were thawed and homogenized in 0.5 *M* ice-cold perchloric acid, and the homogenates were centrifuged at 16,000 *g* for 20 mins at 4°C. The resulting supernatants were applied to an HPLC column (Hypersil ODS 250 × 4 $mm^2$, 5 μm; Agilent Technologies, Waldbronn, Germany). The mobile phase was a 100-m*M* potassium dihydrogen phosphate solution containing 10 m*M* sodium heptane sulphonate and 3% (v/v) methanol. The flow rate was 1.5 ml/min, and the elution was monitored at 254 nm.

**Gene Expression Analysis.** For analysis of gene expression, total RNA was extracted from frozen liver samples using Trizol reagent (Invitrogen, Karlsruhe, Germany). RNA was quantified by $A_{260}$, and its integrity was verified by agarose gel electrophoresis using ethidium bromide for visualization. Total RNA, oligo dT primer (Operon, Köln, Germany) and RevertAid M-MuLV reverse transcriptase (MBI Fermentas, St. Leon-Rot, Germany) were used for cDNA synthesis (Mastercycler Personal; Eppendorf, Hamburg, Germany). The concentration of cDNA was analyzed by real-time detection polymerase chain reaction (PCR; DNA Engine Opticon System; MJ Research Inc., Waltham, MA). The expression signal of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as internal control for normalization, because the expression of GAPDH that was obtained as the value of cycle threshold (C(T)) was not influenced by dietary treatment. PCR was carried out in a final volume of 20 μl reaction mixture containing 500 μ*M* dNTP (Roth, Karlsruhe, Germany); 3.5 m*M* $MgCl_2$, 1.25 U Taq DNA polymerase, and 4 μl of 5× buffer (all from Promega, Mannheim, Germany); 0.5 μl of 10× Sybr Green I (Sigma-Aldrich, Taufkirchen, Germany); 2 μl first-strand cDNA; and 1.34 μl primer mix. The primer sequences were as follows: GAPDH (EC 1.2.1.12), up: 5′-GCA-TGG-CCT-TCC-GTG-TTC-C-3′, low: 5′-GGG-TGG-TCC-AGG-GTT-TCT-TAC-TC-3′; SREBP-2, up: 5′-CCG-GTA-ATG-ATG-GGC-CAA-GAG-AAA-G-3′, low: 5′-AGG-CCG-GGG-GAG-ACA-TCA-GAA-G-3′; HMG-CoA reductase (EC 1.1.1.34), up: 5′-AAG-GGG-CGT-GCA-AAG-ACA-ATC-3′, low: 5′-ATA-CGG-CAC-GGA-AAG-AAC-CAT-AGT-3′; LDL receptor, up: 5′-AGA-ACT-GCG-GGG-CCG-AAG-ACA-C-3′, low: 5′-AAA-CCG-CTG-GGA-CAT-AGG-CAC-TCA-3′. The DNA of GAPDH, SREBP-2, HMG-CoA reductase, and LDL receptor was amplified in cycles of 20 secs denaturation at 95°C, 30 secs annealing at primer-specific temperatures, and 40 secs elongation at 72°C. Fluorescence was measured at 72°C. A final melting curve guaranteed the authenticity of the target product.

**Statistical Analysis.** Means of the three groups were compared by the Fisher's multiple range test using Minitab Statistical Software (Minitab, State College, PA). Values in the text are means ± SD. Means were considered significantly different at $P < 0.05$.

## Results

**Growth and Liver Weight.** All rats with diet-induced hyperhomocysteinemia appeared normal. Final body weights of rats were not influenced by the dietary treatment and were similar to the body weights at the beginning of the experiment, because the amounts of diets offered to rats were close to the energetic requirement for maintenance (control group: 470 ± 27 g; Hcy100 group: 473 ± 20 g; Hcy200 group: 475 ± 33 g; $n = 10$). Body weight gains within the 14 days of experiment did not differ between the three groups of rats (control group: 7.0 ± 3.8 g; Hcy100 group: 10.3 ± 7.7 g; Hcy200 group: 10.1 ± 5.5 g; $n = 10$). The relative liver weights were not different between the three groups of rats (control group: 2.92 ± 0.22 g/100 g of body wt; Hcy100 group: 2.96 ± 0.20 g/100 g of body wt; Hcy200 group: 2.84 ± 0.20 g/100 g of body wt; $n = 10$).

**Hcy, SAM, and SAH Concentrations.** Rats from the Hcy100 and the Hcy200 groups had markedly higher concentrations of total Hcy in plasma, liver, and small intestine than rats fed the Hcy-free control diet (Fig. 1). There was a dose-dependent increase of the plasma and tissue Hcy concentrations in the rats. The concentration of SAM in liver was not different between the three groups of

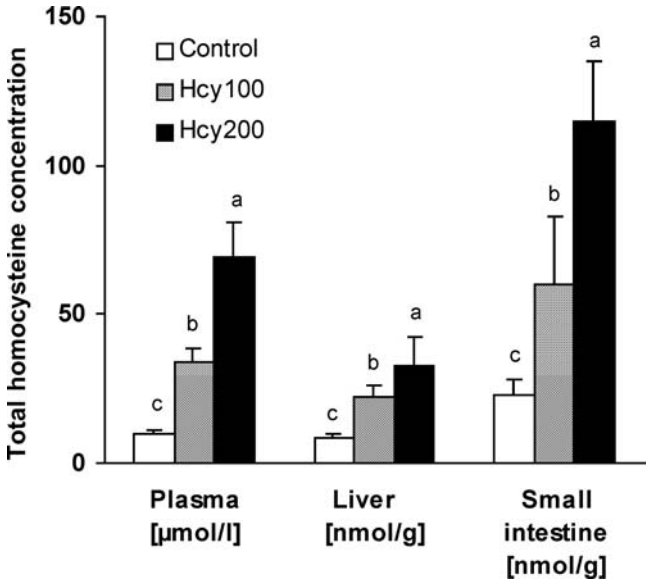


**Figure 1.** Concentration of total Hcy in plasma, liver, and small intestine of rats with a mean dietary intake of either 0 (control), 100 (Hcy100), or 200 (Hcy200) mg Hcy per kg body mass per day. Each bar represents means ± SD, $n = 10$. Bars that are not indicated with the same superscript are significantly different by Fisher's multiple range test, $P < 0.05$.

rats (Table 1). The concentration of SAH and the ratio of SAH to SAM in liver were significantly higher in the Hcy200 group than in the control group. Rats from the Hcy100 group had intermediate values of SAH and ratio of SAH to SAM that were not different from those of the control group and the Hcy200 group.

**Cholesterol Concentrations.** The concentration of cholesterol in plasma, LDL, HDL, liver, and small intestine did not differ between the rats fed the Hcy-supplemented diets and the rats fed the control diet without Hcy (Table 2).

**Relative mRNA Concentrations.** The relative mRNA concentrations of SREBP-2, HMG-CoA reductase, and LDL receptor in liver and small intestine also were not altered by the administration of dietary Hcy compared with the control rats (Fig. 2).

## Discussion

The findings from this rat experiment show that exogenously administered Hcy as DL-homocysteine thiolactone, which led to markedly increased concentrations of Hcy in plasma, liver, and small intestine, did not alter the cholesterol concentrations in plasma and tissues and the expression of the key genes involved in cholesterol biosynthesis and uptake. As plasma and liver cholesterol concentrations were not influenced, we assume that excretion of cholesterol *via* bile acids also was not altered by dietary DL-homocysteine thiolactone. The failure of Hcy to affect cholesterol concentration and the mRNA coding for SREBP-2 target genes is in contrast to previous findings with hyperhomocysteinemic rats (12, 13). Since hyperhomocysteinemia in those studies was not induced by the administration of dietary homocysteine but by an excess of dietary methionine, we suggest that the cholesterol dysregulation observed in those rodents (12, 13) was not driven by Hcy itself but by another factor related to excessive dietary methionine, inadequate amounts of folate, or other experimental conditions. Factors such as age of the animals, duration of the experiment, overnight fasting, or dietary factors other than methionine could be responsible for the discrepancies observed. Although it is quite unusual to use commercially available diets that contained undefined ingredients for nutrition studies as in the studies of Werstuck *et al*. (12) and Woo *et al*. (13), these authors suggest that Hcy is responsible for the activation of SREBP-2, a transcription factor that plays a critical role in the homeostatic regulation of cholesterol in the cell and is responsible for the observed cholesterol accumulation in the hyperhomocysteinemic rodents (12, 13). SREBP-2 is synthesized as inactive precursor bound to the endoplasmatic reticulum membranes, and activation of this membrane-bound transcription factor involves a two-step proteolytic cascade through which the SREBP-2 molecule is released from the endoplasmic reticulum membrane and obtains its mature form in the Golgi complex as a transcription factor. The active SREBP-2 enters the nucleus and binds to promoter or enhancer regions of genes involved in cholesterol homeostasis, such as LDL receptor and enzymes involved in sterol biosynthesis (35). Under the experimental conditions used in our study the relative mRNA concentration of SREBP-2 of animals fed DL-homocysteine thiolactone was not different from that in the control group. Additionally, we suggest that nuclear concentrations of SREBP-2 remained unaffected by dietary Hcy treatment, because the relative concentrations of mRNA coding for the SREBP-2 target genes such as

**Table 1.** Concentrations of SAM and SAH and the Ratio of SAH to SAM in Liver of Rats with a Mean Intake of Either 0 (Control), 100 (Hcy100), or 200 (Hcy200) mg Hcy per kg Body Mass per Day[a]

| Groups | Control | Hcy100 | Hcy200 |
|---|---|---|---|
| SAM (nmol/g) | 193 ± 25 | 197 ± 27 | 181 ± 15 |
| SAH (nmol/g) | 37.6 ± 9.4[c] | 45.7 ± 8.5[b,c] | 48.7 ± 10.8[b] |
| Ratio of SAH to SAM (nmol/nmol) | 0.20 ± 0.05[c] | 0.23 ± 0.04[b,c] | 0.27 ± 0.06[b] |

[a] Data are expressed as means ± SD, $n = 10$.
[b,c] Values in a row not sharing the same superscript letter are significantly different by Fisher's multiple range test at $P < 0.05$.

**Table 2.** Concentration of Total Cholesterol in Plasma, Lipoproteins, Liver, and Small Intestine of Rats with a Mean Intake of Either 0 (Control), 100 (Hcy100), or 200 (Hcy200) mg Hcy per kg Body Mass per Day[a]

| Groups | Control | Hcy100 | Hcy200 |
|---|---|---|---|
| Plasma (m*M*) | 2.06 ± 0.34 | 1.98 ± 0.36 | 1.84 ± 0.33 |
| LDL (m*M*) | 0.72 ± 0.18 | 0.64 ± 0.18 | 0.56 ± 0.13 |
| HDL (m*M*) | 0.81 ± 0.09 | 0.78 ± 0.14 | 0.68 ± 0.15 |
| Liver (μmol/g) | 11.5 ± 1.0 | 11.4 ± 0.7 | 11.4 ± 1.0 |
| Small intestine (μmol/g) | 14.6 ± 1.4 | 12.8 ± 2.7 | 14.0 ± 2.4 |

[a] Data are presented as mean ± SD, $n = 10$. No statistically significant differences ($P < 0.05$) in any parameter were observed between the three groups of rats.

HMG-CoA reductase and the LDL receptor were not significantly different from those of the control group. The failure of Hcy to affect the mRNA coding for SREBP-2 target genes such as HMG-CoA reductase and the LDL receptor in liver and small intestine confirms that Hcy by itself does not dysregulate the cholesterol metabolism, although the Hcy concentrations in plasma, liver, and small intestine of the Hcy-treated rats in our experiment were within pathophysiologic ranges already.

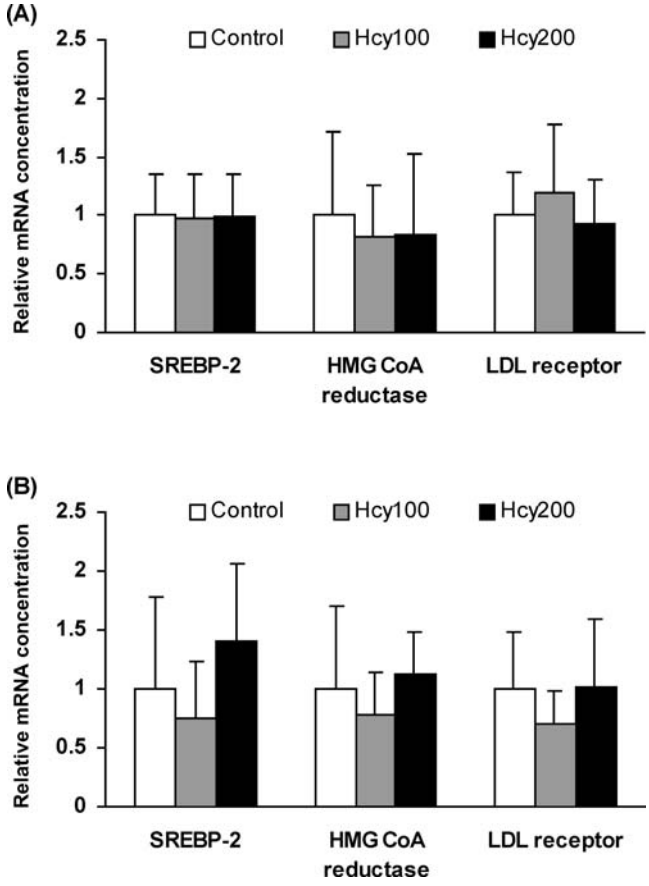


**Figure 2.** Relative mRNA concentrations of SREBP-2, HMG-CoA reductase, and LDL receptor in liver (A) and small intestine (B) of rats with a mean intake of either 0 (control), 100 (Hcy100), or 200 (Hcy200) mg Hcy per kg body mass per day. Concentrations were related to the reference gene GAPDH. Values of the control group were set 1. Each bar represents mean ± SD, $n = 10$. There were no differences in the relative mRNA concentrations of the genes measured in liver and small intestine between the three groups of rats.

The dietary administration of DL-homocysteine thiolactone has been used already as a successful dietary model of hyperhomocysteinemia in a few previous studies (23, 24). Findings from our study show that plasma Hcy concentration dose dependently increased within 2 weeks as dietary Hcy rose. Additionally, the increase of circulating Hcy was accompanied by an increase of the concentrations of cellular Hcy in hepatocytes and enterocytes, and the concentration of cellular SAH in the Hcy200 group. Therefore, data from the present study indicate that the orally administered Hcy as DL-homocysteine thiolactone is an efficient model to induce hyperhomocysteinemia and to increase cellular levels of Hcy, and further show that Hcy as DL-homocysteine thiolactone is absorbed and transported efficiently through the bloodstream into solid tissues. However, analysis of Hcy in plasma, liver, and small intestine by HPLC did not differentiate between the D- and L-homocysteine. From a few studies it is known that the effects of Hcy on endothelial cells, such as chemokine expression (36) and formation of reactive oxygene species (37), could only be observed with L- but not D-homocysteine. Assuming the fact that D-homocysteine does not induce any effects, the remaining L-homocysteine concentrations in the animals fed DL-homocysteine thiolactone are suggested as being high enough and being within ranges in which Hcy had a hypercholesterolemic effect as observed in other rodent studies (12, 13). Although we observed an alteration of the hepatic SAH concentration in the Hcy200 group compared with the control group, the concentration of SAM was not influenced by dietary DL-homocysteine thiolactone. Assuming that the hepatic SAM concentration, which is suggested as playing a critical role in the formation of PC from PE and in turn for regulation of cholesterol metabolism, is responsible for the hypercholesterolemia observed in studies with high-methionine diets (20, 22), it could be possible that the failure of Hcy to affect cholesterol concentration in our study is due to the fact that SAM concentration in liver remained unchanged.

In addition to the rat experiments, there also exist data from cell culture studies in which the effects of Hcy on primary hepatocytes and HepG2 cells were tested on

cholesterol synthesis by use of free L-Hcy or DL-Hcy in the incubation medium (12, 13). Consistent with their *in vivo* findings, both research groups have found that the intracellular cholesterol concentration increased when the cells were incubated with a medium that contains increasing concentrations of Hcy from 1 to 5 m*M*. However, one must not forget that these Hcy concentrations are 10- to 50-fold higher than the plasma Hcy concentrations observed in severe hyperhomocystemic patients with rare genetic disorders (38). Therefore, it is questionable whether the use of such extremely high concentrations of Hcy is an appropriate *in vitro* model to verify possible effects observed *in vivo*. However, the plasma Hcy concentrations induced in the present rat study were within clinical relevant ranges. Apart from the fact that complete deficiencies of cystathionine β-synthase or 5,10-methylenetetrahydrofolate reductase are associated with plasma Hcy concentrations greater than 100 μ*M* (39), moderately increased concentrations of plasma homocysteine ranging from 15 to 60 μ*M* are quite prevalent in the general population (38) and can be caused by renal impairment (40); folate and vitamin $B_6$ deficiencies, particularly in high-methionine states (41, 42); certain medications such as fibrates or anticonvulsives (43); or common polymorphisms in the methylene tetrahydrofolate reductase gene (44).

In conclusion, this study shows that hyperhomocysteinemia produced by feeding of DL-homocysteine thiolactone does not influence expression of genes involved in cholesterol homeostasis and also does not alter the concentration of cholesterol in plasma, circulating lipoproteins, liver, and intestine.


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