# LIPOPROTEIN METABOLISM IN HYPOTHYROIDISM

## THE CONTRIBUTION OF GROWTH HORMONE

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# LIPOPROTEIN METABOLISM IN HYPOTHYROIDISM

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LIPOPROTEINE METABOLISME TIJDENS HYPOTHYREOIDIE

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*"It is what we think we know already that often prevents us from learning"*
Claude Bernard 1878

Aan Peter en Pieter

## TABLE OF CONTENTS : page

**List of abbreviations** 8

**Chapter 1** Introduction 9

1.1 General introduction. 11
1.2 Plasma lipoprotein metabolism. 11
1.3 1.3.1 Lipid metabolism and thyroid function. 13
1.3.2 Plasma lipid concentrations in hypothyroids.
1.3.3 Lipoprotein metabolism in hypothyroidism.
1.4 Growth hormone activity during hypothyroidism. 16
1.5 Effects of thyroid hormone substitution on lipid metabolism in hypophysectomized rats. 17
1.6 Impact of growth hormone on plasma lipoprotein concentrations. 18
1.7 Growth hormone and atherosclerosis. 20
1.8 Scope of the thesis. 21

**Chapter 2** Relationship between insulin-like growth factor-I and low-density lipoprotein cholesterol levels in primary hypothyroidism in women. 29

**Chapter 3** Growth hormone and thyroxine affect lipoprotein metabolism in hypothyroid and hypophysectomized rats. 41

**Chapter 4** Interaction of growth hormone activity and thyroid hormone in the lipoprotein metabolism of hypothyroid rats. 53

**Chapter 5** Growth hormone enhances mRNA and activity of the low density lipoprotein-receptor in the liver of hypothyroid rats. 69

**Chapter 6** Insulin-like growth factor-I stimulates the LDL receptor activity in HepG2 cells. 83

**Chapter 7** Growth hormone normalizes hepatic lipase mRNA levels and activity in hypothyroid rat liver. **95**

**Chapter 8** Growth hormone decreases plasma cholesterol independent from thyroid hormone activity. **103**

**General discussion and conclusions** **117**

**Summary** **129**

**Samenvatting** **135**

**Dankwoord** **141**

**Curriculum vitae** **143**

## List of abbreviations

| | |
|---|---|
| FFA | Free fatty acids |
| GH | Growth hormone |
| HDL | High-density lipoprotein |
| HepG2 | Human hepatoma cell line |
| HL | Hepatic Lipase |
| IDL | Intermediate-density lipoprotein |
| IGF-I | Insulin-like growth factor-I |
| LDL | Low-density lipoprotein |
| LPL | Lipoprotein lipase |
| mRNA | Messenger Ribonucleic acid |
| $T_3$ | 3,3',5-Triiodo-thyronine |
| $T_4$ | Thyroxine |
| TC | Total serum cholesterol |
| TG | Triglyceride |
| TH | Thyroid hormone |
| TSH | Thyroid stimulating hormone |
| VLDL | Very low-density lipoprotein |

Chapter 1

# INTRODUCTION

## 1.1 GENERAL INTRODUCTION.

Supported by the firmly established connection between elevated serum cholesterol levels and increased risk of coronary heart disease, both physicians and patients are striving for better cholesterol control (1). A better understanding of the mechanisms of cholesterol metabolism will provide insight in the means of lowering cholesterol levels. The regulation of cholesterol metabolism is multifactorial, with diet and hormones playing important roles. The influence of hormones on lipid metabolism is of major clinical interest, because abnormalities of various hormone secretions may induce lipoprotein aberrations which possibly account for the atherogenic risk accompanying common medical disorders such as obesity, diabetes mellitus and hypothyroidism. Despite a large number of data from clinical, epidemiological, animal and autopsy studies linking hypothyroidism to the development of atherosclerotic disease, the precise relationship between the two and the pathogenetic mechanisms involved are poorly understood (2). This thesis will focus on the influence of the hormonal changes that occur during hypothyroidism on lipoprotein metabolism. Before describing what is known about the lipid metabolism in hypothyroidism, the metabolism of relevant lipoproteins in the normal, euthyroid state will be reviewed briefly.

## 1.2 PLASMA LIPOPROTEIN METABOLISM.

Most pathways of plasma lipid transport originate in or converge upon the liver, making that organ central to the lipoprotein metabolism (3; Figure 1.1). In the fasted state, very low-density lipoprotein (VLDL) is the main plasma carrier of triglycerides (TG). It is continuously produced and secreted by the liver. After synthesis and excretion, the particle is metabolized by lipoprotein lipase (LPL), an enzyme predominantly present at the surface of endothelial cells, in muscle and adipose tissue. LPL catalyzes the extrahepatic elimination of TG from the plasma (4); it breaks down the TG content of VLDL and produces intermediate-density lipoprotein (IDL)(5). During this process, the VLDL surface components, phospholipids and apoproteins, are transferred to high-density lipoprotein (HDL). IDL can be removed from the plasma by two

pathways, it may either be removed by the liver, especially larger VLDL particles are taken up directly after delipidation (6), or further converted to low-density lipoprotein (LDL). Hepatic Lipase (HL) is thought to mediate this latter process (7,8). The synthesis of large TG enriched VLDL is promoted by high carbohydrate intake and alcohol (9). Cholesterol feeding produces a situation with cholesterol-ester enriched small VLDL-particles which are preferably converted through IDL to LDL (10). LDL is the major cholesterol transporting lipoprotein in plasma. Elevated plasma LDL-cholesterol levels are associated with the development of premature atherosclerosis. A central role in the hepatic clearance of lipoproteins from the blood stream is played by the LDL-receptor (11). It is not only responsible for the uptake of LDL, but may contribute to the clearance of other lipoproteins that contain apolipoprotein (apo) B-100, like IDL. The activity of LDL-receptors in hepatocytes is, among others, regulated by the cellular content of cholesterol (12).

HDL's form a heterogeneous population of particles, derived from many sources. Part of the plasma HDL originates from surface remnants released during lipolysis of VLDL and chylomicrons. An inverse relationship between the plasma concentration of HDL and TG is well established (13,14). Another amount of HDL is produced as nascent-particles of intestinal or liver origin. Once the nascent HDL-particles reach the circulation, they accumulate cholesterol from peripheral tissues. In humans a major function of most lipoproteins is to deliver lipids to cells, but the main metabolic role of HDL is the acceptance of excess cholesterol from peripheral cells (15). A high concentration of plasma HDL-cholesterol is thought to be favorable, and related to regression of atherosclerosis. The major protein in HDL is apo A-I: it performs many roles, like being a cofactor for the plasma cholesterol esterifying enzyme lecithin:cholesterol acyl transferase (LCAT). LCAT activity is involved in the acceptance of cholesterol by HDL, for instance from peripheral tissue (9). Once $HDL_3$ is converted to $HDL_2$-like particles by lipolysis of VLDL, LCAT stimulates the formation of mature $HDL_2$. Cholesteryl-esters can be transferred and exchanged between lipoproteins; this process is catalyzed by cholesteryl-ester transfer protein (CETP) (16). CETP facilitates the transfer of the ester from HDL into less dense VLDL and LDL in exchange for triglycerides, to convert $HDL_2$ to $HDL_3$-like particles. The delivery of cholesterol from $HDL_2$ to the liver has been suggested to be

modulated by HL. The mechanism by which HL is involved in HDL metabolism is not exactly known. Like LPL, the enzyme hydrolyses TG and phospholipids, but appears to be more effective towards phospholipids. It has been proposed that HL modulates the flux of unesterified cholesterol between HDL and tissue by affecting the phospholipid-free cholesterol ratio in the particle (8). A strong negative correlation exists between HL-activity and HDL-levels. Absence of HL-activity is associated with HDL levels, especially HDL2.

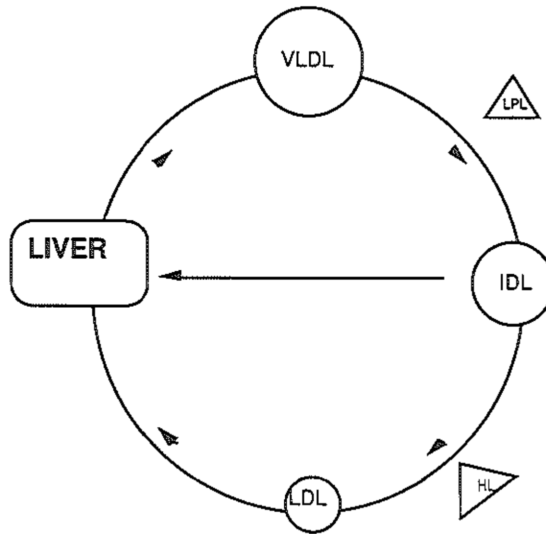


**Figure 1.1:** Metabolism of VLDL and LDL and the role of hepatic lipase (HL) and lipoprotein lipase (LPL) under normal conditions.

## 1.3.1 LIPID METABOLISM AND THYROID FUNCTION.

It has been recognized for almost 70 years that hypothyroidism influences cholesterol metabolism (17-19). Plasma lipoproteins represent one of the most sensitive target systems for thyroid hormones: alterations in lipoprotein metabolism are an indicator of thyroid function. Hypercholesterolemia is typically found in individuals with hypothyroidism. Conversely hypochol-

esterolemia is associated with hyperthyroidism. The hypercholesterolemia of hypothyroidism can be an important risk factor for atherosclerosis.

### 1.3.2 PLASMA LIPOPROTEIN CONCENTRATIONS IN HYPOTHYROIDS.

The dyslipoproteinemia of hypothyroid patients is characterized by an increased plasma concentration of total cholesterol, because of an increase in the concentration of plasma LDL-cholesterol (20-22; Figure 1.2). The major apolipoprotein in LDL, apo B-100, is also markedly increased (22,23). Less uniform are the effects of hypothyroidism on plasma HDL concentration. In man HDL levels have been reported to be either increased (24), normal (20) or even decreased (25). Apo A-I, the main apolipoprotein in HDL, is reported to be increased in the hypothyroid state, while the effects on apo A-II, an other protein of HDL are less clear (22,23). In hypothyroid rats, both LDL and HDL are increased (26); in these animals apo A-I mRNA levels in the liver can be enhanced by thyroxin, while liver apo A-II mRNA will decrease (28). Plasma TG levels are normal or only moderately elevated in most hypothyroid patients (27). In human plasma apo E is mainly associated with VLDL and HDL. Apo E is reported not to change in the hypothyroid patients (28) (neither is liver apo E mRNA influenced by alteration in thyroid status in the rat, 29). The hyperlipoproteinemia of hypothyroidism is reversible by restoration of the euthyroid state (30).

### 1.3.3 LIPOPROTEIN METABOLISM IN HYPOTHYROIDISM.

The actions of thyroid hormone, or a deficiency of this hormone, on the liver are likely to be of central importance to its effects on lipoprotein metabolism. In patients with primary hypothyroidism the hypercholesterolemia is mainly caused by a decreased catabolism of LDL (20): a diminished removal of $^{131}$I-labeled LDL was shown to exist in these patients (31,32; Figure 1.2). Studies in humans as well as *in vitro* experiments indicate that the reduced fractional catabolic rate of LDL results from a modulation of the expression of the LDL-receptor in the liver (33-35). This organ possesses over seventy percent of the

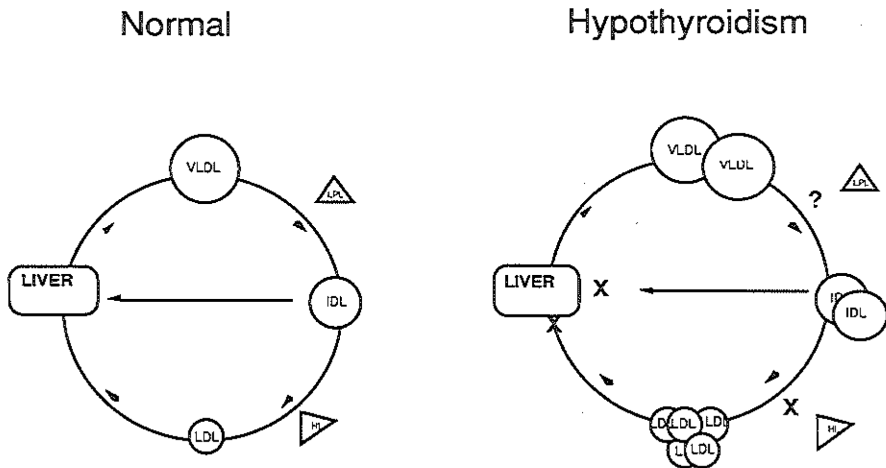


**Figure 1.2:** Lipoprotein metabolism affected by hypothyroidism. During hypothyroidism both the activity of hepatic lipase (HL) and the activity of the LDL-receptor are decreased (indicated by X). This results in an increase in both IDL and LDL. The effect of hypothyroidism on lipoprotein lipase (LPL) activity is uncertain.

body's LDL-receptor. In rats, replacement therapy with thyroxin increases LDL-receptor activity, in parallel with an increase in LDL-receptor mRNA (36).

The majority of body cholesterol synthesis occurs in the liver. It is decreased in hypothyroid patients (20). In hypothyroid rats a decreased activity of HMG-CoA reductase was found (37). As HMG-CoA reductase catalyses the rate limiting reaction in cholesterol synthesis, the decreased activity of this enzyme might be responsible for the low cholesterol production rate in hypothyroidism. Nonetheless, obese hypothyroid subjects show elevated synthesis rates of cholesterol (20), which is probably due to a habitual excessive intake of calories.

The effects of thyroid hormone deficiency on the concentration of plasma LDL-cholesterol are well recognized. In contrast, there are conflicting reports about the influence of the hypothyroid state on the metabolism of plasma triglycerides. Non-obese hypothyroid patients generally have normal levels of TG, while obese patients often have hypertriglyceridemia (38). In these patients synthesis of VLDL by the liver appears to be increased (38). The

proposed mechanism is a lessened peripheral utilization of FFA and glucose, shunting them to TG synthesis. A relationship between TG concentration and severity of hypothyroidism has been suggested (39), but is not always confirmed (40). Perfused livers from hypothyroid rats, exhibit an increased rate of TG secretion, when compared to euthyroid rats (41). Another mechanism whereby hypothyroidism might increase plasma TG, is a decreased LPL-activity. However, the results from studies on LPL-activity during hypothyroidism are as conflicting as TG concentrations: some find a decreased activity (27,41), others do not see any influence of either thyroid hormone deficiency or substitution (38,42).
In contrast to these conflicting results on LPL, the activity of HL is generally reported to be decreased in patients as well as in rats with hypothyroidism (41-44; Figure 1.2). As HL catalyzes the interconversion of HDL-subclasses, hypothyroidism influences the catabolism of $HDL_2$- to $HDL_3$-particles (41,43,45). In hypothyroids, with a decreased HL-activity, $HDL_3$ is little affected in amount and cholesterol content, while $HDL_2$ fluctuates inversely with thyroid hormone concentration (44,46,47). Another result of the decreased HL-activity in hypothyroids is an increase in the plasma concentration of IDL (48,49) and small VLDL indicating a defective conversion of VLDL to LDL (50).
Next to these effects of hypothyroidism on cholesterol homeostasis in the liver, some extra hepatic effects of hypothyroidism are reported, e.g. a relatively high absorption of cholesterol in the intestine of patients with hypothyroidism, which will add to the hypercholesterolemia of hypothyroidism (20). However in hypothyroid rats the percentage of cholesterol that was absorbed in the intestine was not changed (51). The activities of LCAT (52,53) and CETP (23) have also been reported to be moderately decreased in severe hypothyroidism.

## 1.4 GROWTH HORMONE ACTIVITY DURING HYPOTHYROIDISM.

GH secretion is dependent on thyroid hormone availability. In hypothyroid patients and in hypothyroid rats, the secretion of GH is decreased, both spontaneously (54,55), and in response to stimuli (56-58). In rats it was shown

that the decreased GH secretion is associated with a marked reduction in hypothalamic content of GH-releasing hormone (55) and a reduced synthesis of GH in the pituitary gland (59-61). This decrease in GH synthesis results in a diminished GH content of the hypophysis (62). Experiments with pituitary tumor cells show that thyroid hormone stimulates GH synthesis at the level of GH gene transcription (63,64). This effect of thyroid hormone is mediated via the thyroid hormone receptor, in combination with cell specific response elements which stimulate GH gene expression (65). Both production and secretion of GH are restored to normal by thyroid hormone replacement *in vivo* (66-68).

Many effects of GH, like stimulation of growth, are mediated by Insulin-like Growth Factor I (IGF-I), previously called somatomedin-c (69). The IGF-I concentration in plasma shows a strong (but not an absolute) dependence on GH; e.g it also responds to thyroid hormone and nutritional status (70). In hypothyroid men plasma IGF-I concentrations are diminished (71), as the result of a decreased GH secretion (54). Evidently thyroid hormones are required for secretion and activity of GH. This means that in hypothyroid subjects both thyroid hormone and GH are decreased and both hormones will show their effects when the euthyroid state is restored.

## 1.5 EFFECTS OF THYROID HORMONE SUBSTITUTION ON LIPID METABOLISM IN HYPOPHYSECTOMIZED RATS.

Thyroid hormone substitution treatment in primary hypothyroidism restores at least thyroid hormone- and GH-activity. Observation of the effects of thyroid hormone substitution in hypophysectomized rats will help to understand the effects of thyroid hormone in cholesterol metabolism, without the intermingling with GH effects.

Early in the seventies, it was reported that in hypophysectomized rats, daily treatment with 2 mg of thyroid extract could not prevent hypercholesterolemia, but did lessen it by comparison with that observed in the untreated hypophysectomized rats (72). This suggests that more than thyroid hormone is necessary to normalize cholesterol metabolism of hypophysectomized rats. $T_3$ replacement studies were done in these rats, to study the effect of thyroid

hormone on detailed parts of cholesterol metabolism (73,74). The increase of LDL-cholesterol by hypophysectomy was significantly rectified but the normal level could not be maintained, whilst the decreased HDL level was not at all affected by thyroid hormones (73). Administration of $T_3$ to the hypophysectomized rats resulted in an increase in hepatic LDL-receptor mRNA levels (74); this increase was observed within 24 hours and needed a high doses of $T_3$ (25 µg $T_3$ per 100 g body weight). Quantitatively, bile acids represent the major fate of cholesterol. Cholesterol 7 alpha-hydroxylase catalyses the rate limiting reaction in the synthesis of bile acids. The effects of $T_3$ on the expression of 7 alpha-hydroxylase gene was studied. It was found that a physiological dose of $T_3$ (0.25 µg per 100 g body weight) increased 7 alpha-hydroxylase mRNA very rapidly, consistent with a primary effect on the transcription of the encoding gene (75). Because cholesterol is a substrate for bile acid synthesis, thyroid hormone thus enhances the utilization of cholesterol. The number of LDL-receptors is subject to a negative feedback regulation by the intracellular pool of free cholesterol. A decrease in intracellular cholesterol levels, will relieve the inhibition and induce transcription leading to increased amounts of mRNA for the LDL-receptor. This will increase the uptake of circulating LDL-cholesterol by the LDL-receptor and decrease the concentration of plasma LDL-cholesterol. The effects of $T_3$ on the LDL-receptor might partly be explained by this process.
High (unphysiological) doses $T_3$ for 4 days were needed to stimulate HMG-CoA reductase mRNA levels (74). The high doses and long duration time needed for this reaction to $T_3$ substitution, suggest that this effect represents a compensatory response to cholesterol depletion.

## 1.6 IMPACT OF GROWTH HORMONE ON PLASMA LIPOPROTEIN CONCENTRATIONS.

The first studies suggesting that GH is important in the regulation of cholesterol metabolism were presented by Byers et al (72,76). The administration of GH alone to thyroidectomized rats appeared essentially to be as effective as a thyroid extract in preventing the development of hypercholesterolemia. Some years later the involvement of GH in cholesterol metabolism in the human situation was shown. In hypercholesterolemic adults

supraphysiological doses of GH, acutely lowered the plasma cholesterol concentration (77). That GH may decrease plasma cholesterol concentration is supported by the finding in patients with acromegaly, which show a lowered plasma cholesterol concentration (78). In addition, in adults with GH deficiency, Merimee described increased cholesterol levels (79,80). In a long-term (6 month) experiment it was recently shown that during GH substitution in GH deficient adults, the concentration of total plasma cholesterol decreased to values lower than in the placebo group (81). On the other hand, growth hormone deficient children show either normal (82) or slightly increased (83) cholesterol levels. Contradictory effects of GH on serum lipid concentrations have been reported in studies in which physiological doses GH were given to GH deficient children (82-87). In these children plasma cholesterol has been reported to either decrease (84-87) or not to change (82,83). Overall, most authors conclude that GH lowers the concentration of plasma cholesterol in man. This decrease in the concentration of plasma cholesterol by GH consists of a reduction of both HDL and LDL (85). Different mechanisms may be proposed for the decrease in LDL cholesterol. First, a direct stimulating effect of GH-activity on the LDL-receptor activity was suggested, as IGF-I increases the number of LDL-receptors and augments the maximal rate of LDL internalization and degradation in swine granulosa cells (88). Secondly GH is proposed to stimulate the LDL-receptor activity, due to an increased use of cholesterol (89). GH was shown to induce an increased activity of 7 alpha-hydroxylase (90), this enzyme catalyses the rate limiting step in bile acid synthesis.

Although TG levels in GH deficient children and adults are either normal or increased (80,82,83), hypertriglyceridemia occurs in acromegaly (78) and after GH treatment of healthy controls (91). There are two plausible explanations for the induction of an increased TG concentration by GH. The first one is a decrease in LPL-activity. In acromegaly patients a decreased LPL-activity has been found (92) and GH replacement therapy of GH deficient children (93) and healthy volunteers (94) causes a reduction in the LPL-activity. A decreased LPL-activity will contribute to an increased plasma VLDL concentration. Especially $VLDL_2$, suggested to be a VLDL remnant, appears to rise (91). This latter effect can also be attributed to a lowered HL-activity. An other effect of GH on TG metabolism is the induction of an

enhanced lipolysis in adipose tissue (95,96), thereby producing increased plasma FFA concentrations. Increased FFA concentrations can induce triglyceride synthesis in the liver (97), and stimulate VLDL synthesis.
In humans, the concentration of plasma HDL cholesterol is reported to be either reduced or not influenced by GH replacement (85,93). HDL metabolism is complex with LPL and HL playing important roles. Both enzymes are found to decrease in acromegaly and during treatment with GH of normal adults or GH-deficient children (92-94). In hypophysectomized male rats HDL was not changed by intermittent GH-treatment, mimicking the male secretory pattern, while a continuous infusion with GH, mimicking the female secretory pattern, induced an increase of the concentration of plasma HDL cholesterol (98) suggesting an effect of GH on HDL metabolism. Also *in vitro* experiments support this contribution of GH to HDL metabolism, as a decreased HDL-receptor activity was shown after incubation of fibroblasts with IGF-I (99).

## 1.7 GROWTH HORMONE AND ATHEROSCLEROSIS.

Life expectancy is shortened in patients with hypopituitarism. Substitution of hydrocortisone, thyroid hormone and sex hormone does not normalize the life expectancy which on the average is lowered, because of vascular disorders. GH deficiency might explain the premature death from vascular disorders in hypopituitarism (100). On the other hand GH-activity has been linked to the development of diabetic micro- and macro-angiopathy (101,102). Also acromegalic patients (with high GH concentrations) show early atherosclerosis (78). The mechanism behind these effects of GH is not precisely known, though insulin resistance is thought to add to it. Both deficiency and excess of GH appear to be associated with the development of premature vascular disease, a better understanding of the contribution of GH in lipid metabolism might help to prevent these effects.

## 1.8 SCOPE OF THE THESIS.

Current data suggest a role for GH in the regulation of lipoprotein metabolism. In hypothyroidism not only the secretion of thyroid hormone, but also of GH is decreased. Generally the effects on plasma lipids seen in hypothyroid individuals are considered to be a consequence of decreased thyroid hormone levels. More then twenty years ago evidence was found that treatment of hypothyroid rats with GH in supraphysiologic doses affects plasma lipid concentrations, but whether a lack of GH (activity) is involved in the pathophysiology of lipid metabolism in hypothyroidism can not be answered from the present literature. The first aim was to investigate whether the changes occurring in lipid metabolism during hypothyroidism, merely result from a lack of thyroid hormone or are also attributable to a deficiency in GH-activity. In hypothyroid women the relationship between GH-activity (IGF-I) and plasma lipoproteins was evaluated (**Chapter 2**). The effects of a physiological dose of GH on lipoprotein concentrations during the hypothyroid status were studied in hypothyroid and hypophysectomized rats, as reported in **Chapter 3**. Furthermore, the effects on IGF-I and lipoproteins of substitution of hypothyroid rats with varying doses of thyroid hormone were studied (**Chapter 4**).
The second aim was to investigate the mechanism by which GH can affect lipid metabolism, especially during hypothyroidism. The major cause of hypercholesterolemia in hypothyroidism is a decreased LDL catabolism in the liver. Hypothyroid rats were treated with GH and the effects of the hypothyroid status and GH treatment on liver cell LDL-receptor mRNA, LDL receptor expression and HMG-CoA reductase was studied, as described in **Chapter 5**. In **Chapter 6** the effects of IGF-I, GH and $T_3$ on the expression and function of the LDL-receptor in a human hepatoma derived cell line (HepG2) are described.
Besides the effects of hypothyroidism on LDL metabolism, there are many conflicting reports considering the influence of thyroid status on HDL metabolism. However, data considering HL-activity, a key enzyme in HDL metabolism, uniformly show a decreased activity during hypothyroidism. HL is known to be controlled by several hormones, but it is not known whether HL-activity is regulated by GH. In **Chapter 3 and 4** the effect of the hypo-

thyroid status and of treatment with GH on HL-activity in liver tissue of hypothyroid rats is described. In **Chapter 7** effects of GH replacement in rats on HL mRNA levels and HL-activity in liver and in post heparin plasma are reported. The effects of GH substitution on serum LDL cholesterol and HL-activity in post-heparin plasma of patients with GH-deficiency were studied in relation to circulating thyroid hormone concentration as described in **Chapter 8.**

In the final chapter, **Chapter 9**, the information obtained in this thesis will be discussed.

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## Chapter 2

# RELATIONSHIP BETWEEN INSULIN-LIKE GROWTH FACTOR-I AND LOW-DENSITY LIPOPROTEIN CHOLESTEROL LEVELS IN PRIMARY HYPOTHYROIDISM IN WOMEN

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## ABSTRACT

The effect of insulin-like growth factor-I (IGF-I) on the disturbance of lipid metabolism during primary hypothyroidism was studied in 12 women with primary hypothyroidism. Significant increases in both low-density lipoprotein (LDL) cholesterol and intermediate-density lipoprotein (IDL) cholesterol were seen. Lipoprotein concentrations reverted to normal after substitution with thyroxine ($T_4$) until the euthyroid state was reached. A decrease in IGF-I of 65 % ($p<0.005$) was seen in hypothyroid patients and this was inversely correlated ($r=-0.75; p<0.01$) with the concentration of LDL-cholesterol. Multivariant regression analysis of LDL-cholesterol against IGF-I and free $T_4$ showed that IGF-I determines the concentration of LDL-cholesterol instead of free $T_4$.
Our data suggest that in hypothyroidism IGF-I is a determinant of the concentration of LDL-cholesterol. In addition, hypothyroidism can influence plasma lipoprotein metabolism by lowering the activity of the salt-resistant lipase (liver lipase).

## INTRODUCTION.

Hypothyroidism is often accompanied by hyperlipoproteinaemia. Studies of the different lipoprotein classes revealed an increase predominantly of low-density lipoprotein (LDL) cholesterol (Abrams and Grundy, 1981; Muls et al., 1985; Hylander and Rosenqvist, 1982; Valdemarsson, 1982). Sometimes, however, an increase in plasma concentrations of triglyceride (TG) and accumulation of intermediate-density lipoprotein (IDL) cholesterol is also found (Nikkilä and Kekki, 1972; Rossner and Rosenqvist, 1974), while only minor changes in concentrations of high-density lipoprotein (HDL) cholesterol have been reported (Abrams and Grundy, 1981; Valdemarsson, 1982; Lithell et al.,1981; Lisch et al.,1982). Lipoprotein changes revert to normal after substitution with thyroxine ($T_4$) (Hylander and Rosenqvist, 1982). The mechanism of these changes in lipoprotein metabolism in hypothyroidism is not fully understood. It has been suggested that the decreases in lipoprotein lipase-activity (LPL) and/or salt resistent liver lipase-activity (HL) are major contributors to the typical lipoprotein profiles in patients with thyroid

hypofunction (Valdemarsson, 1982), although a decrease of LPL is not always found (Abrams et al.,1970; Kraus et al.,1974). In addition, or alternatively, impairment of lipoprotein receptor binding may account for the change in lipoprotein concentration (Thompson et al.,1981; Scarabottolo et al.,1986). Generally the effects on plasma lipid metabolism are considered to be due to lowered thyroid hormone levels, but Byers et al. (1970) suggested that, in thyroidectomized rats, the chief cause of hypercholesterolaemia might be the effect of hypothyroidism on the secretion of growth hormone (GH). The administration of GH to these thyroidectomized rats was essentially as effective in preventing the occurrence of hypercholesterolaemia as a thyroid extract. These data suggested a relationship between hypothyroidism, GH-activity and hypercholesterolaemia. It is not known in what way GH or insulin-like growth factor-I (IGF-I) influences lipoprotein metabolism. In the present study we analyzed the relationship between IGF-I and various parameters of in hypothyroidism.

## MATERIALS AND METHODS.

### Patients.

The 12 women with primary hypothyroidism who participated in the study gave their informed consent. They were aged between 18 and 74 year, and the diagnosis was based on clinical grounds in combination with an increased level of thyroid-stimulating hormone (TSH) and a subnormal concentration of free $T_4$ (less than 8.5 pmol/l). A group of healthy euthyreoid women aged 21-43 yr (n=10) served as controls. Drugs known to influence serum lipids, e.g. beta-blockers or diuretics, were not administered. None of the patients had clinical or biochemical evidence of diabetes mellitus, liver or kidney disease, nor had any one of them primary hyperlipoproteinaemia before entering the study. Diet was not estimated. The study was approved by the Ethical Committee of the University Hospital Dykzigt, Rotterdam, The Netherlands.

### Sample collection

Two blood samples were obtained between 08.00 and 08.30 h from each subject after overnight fasting (10 h). The first blood sample was collected on ice in tubes containing potassium-EDTA (6.75 mg). After taking the first

blood sample a heparin bolus (50 IU/kg bodyweight; Tromboliquine; Organon, Oss, The Netherlands) was given i.v. and 20 min later a blood sample to measure lipase activities was taken from the contralateral arm and put into a tube containing heparin (143 U lithium heparin). Plasma was stored at -20 °C until analysis. Blood tests were repeated on the first six patients which entered the study after substitution with L-$T_4$ to euthyroidism.

**Analytical Methods**

Plasma concentrations of triglyceride and cholesterol were determined by enzymatic methods (Boehringer-test kit combination). Activities of HL and LPL were measured with the immunochemical method previously described (Huttunen et al, 1975; Jansen et al., 1980; Nozaki et al., 1986). Enzyme activity was expressed in mU. (1 mU represents the release of 1 nmol fatty acid from the substrate/min) Lipoprotein fractions were separated after 24 hour ultracentrifugation of the plasma for 24 h at 200 000g at 4 °C using a SW-41 rotor in a Beckmann L5-50 ultracentrifuge. Apo E isoforms were determined by isoelectric focusing of very low-density lipoprotein (VLDL) apolipoproteins as described by Wardel et al. (1982) using ampholine pH 4-6. The concentration of free $T_4$ and TSH were determined by RIA using SPAC ET-F$T_4$ (Byk Sangtec, Dietzenbach, F.R.G.) and RIA-gnost human TSH (Hoechst Behring, Marburg, F.R.G.), respectively. The intra assay variation for free $T_4$ measurement was 2.5 %. All determinations for free $T_4$ were performed in one assay. IGF-I-c was measured by a radio-immunoassay in EDTA plasma, using methods described previously (Furlanetto et al.,1977). A commercial kit from Nichols Institute (San Juan Capistrano, California) was used. The intra-assay variation in the IGF-I measurement was 7.2 %. Measurements for IGF-I were performed in one assay. As IGF-I is known to be influenced by the period of fasting and its binding protein is known to have a 24-h cyclic variation (Busby et al., 1988), IGF-I samples were collected at a fixed time of the day after 10 h fasting.

**Statistical Analysis.**

Data are given as mean ± S.D. Statistical significance of differences was evaluated by unpaired or, if appropriate, paired Student's t-test. Correlation between different parameters was determined with linear regression analysis and multivariant regression analysis.

## RESULTS

### Hypothyroidism and plasma lipoproteins.

Hypercholesterolaemia in hypothyroidism was due to a significant increase in both LDL-cholesterol and IDL-cholesterol (Table 2.1). A negative linear correlation was found between LDL-cholesterol and free $T_4$ (r=- 0.62, $p<0.05$). There was no significant difference between concentrations in hypothyroid and euthyroid patients of total plasma triglycerides, HDL-cholesterol, VLDL-cholesterol and triglycerides in the various lipoprotein fractions. Plasma lipoproteins and IGF-I became normal in patients treated with L-$T_4$ until euthyroidism (Table 2.2). A correlation between the type of Apo E-polymorfism and the type of hyperlipoproteinaemia was not found (data not shown).

### Hypothyroidism and LPL activities

In overt hypothyroidism, HL was decreased to 38 % of the activity in euthyreotic state (Table 2.1). HL increased significantly during substitution with L-$T_4$ (Table 2.2), while LP- activity did not change.

### Hypothyroidism, concentrations of IGF-I and hypercholesterol-aemia.

In most patients with hypothyroidism, IGF-I was decreased and normalized after substitution treatment to euthyroidism (Table 2.2). A linear correlation between free $T_4$ and IGF-I was found ($r = 0.58$, $p<0.05$) (Figure. 2.1). Plasma cholesterol and IGF-I showed a negative linear correlation ($r = -0.68$, $p<0.05$), as did IGF-I and LDL-cholesterol ($r = -0.75$, $p<0.01$, Figure. 2.1). No correlation was seen between IGF-I and IDL-cholesterol, nor between IGF-I and HL. Multivariant regression analysis of the dependent variable LDL-cholesterol against IGF-I and free $T_4$ showed a regression coefficient for IGF-I of -5.25 ($p<0.05$), while the regression coefficient for free $T_4$ was only -0.20 (not significant). This indicates that the correlation of IGF-I to the concentration of LDL-cholesterol in hypothyroidism was independent from the concentration of $T_4$.

**Table 2.1: Concentrations of lipoproteins, lipases, thyroid hormone and IGF-I in 12 patients with primary hypothyroidism and in ten euthyroid controls.**

| | Hypothyroid | Euthyroid |
|---|---|---|
| IGF-I (mU/ml) | 0.44 ± 0.20 ** | 1.27 ± 0.47 |
| free $T_4$ (pM) | 3.17 ± 2.24 ** | 12.4 ± 1.90 |
| Total cholesterol (mM) | 7.27 ± 2.24 ** | 4.53 ± 0.72 |
| LDL cholesterol (mM) | 4.54 ± 1.69 ** | 2.37 ± 0.64 |
| IDL cholesterol (mM) | 0.37 ± 0.19 ** | 0.15 ± 0.09 |
| HDL cholesterol (mM) | 1.03 ± 0.33 | 1.40 ± 0.58 |
| Triglyceride (mM) | 1.38 ± 0.55 | 1.27 ± 0.47 |
| Lipoprotein lipase activity (mU/ml) | 80 ± 38 | 118 ± 33 |
| Liver lipase activity (mU/ml) | 108 ± 77 ** | 280 ± 11 |

Vales are means ± S.D. ** $P<0.005$ compared with controls (Student's t-test)

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**Table 2.2: Concentration of lipoproteins, lipases, thyroid hormone and IGF-I before and after substitution treatment with L-thyroxine of six hypothyroid patients.**

| | Before Treatment | After Treatment |
|---|---|---|
| IGF-I (mU/ml) | 0.40 ± 0.15 ** | 1.77 ± 0.24 |
| free $T_4$ (pM) | 2.47 ± 1.99 ** | 15.4 ± 4.01 |
| Total cholesterol (mM) | 7.79 ± 1.53 ** | 4.90 ± 0.70 |
| LDL cholesterol (mM) | 4.82 ± 1.03 ** | 2.84 ± 0.51 |
| IDL cholesterol (mM) | 0.37 ± 0.10 ** | 0.26 ± 0.11 |
| HDL cholesterol (mM) | 1.16 ± 0.28 | 1.02 ± 0.11 |
| Triglyceride (mM) | 1.25 ± 0.34 | 1.22 ± 0.25 |
| Lipoprotein lipase activity (mU/ml) | 88 ± 29 | 125 ± 26 |
| Liver lipase activity (mU/ml) | 100 ± 77 ** | 204 ± 14 |

Values are means ± S.D. ** $P<0.005$ compared with controls (Student's t-test)

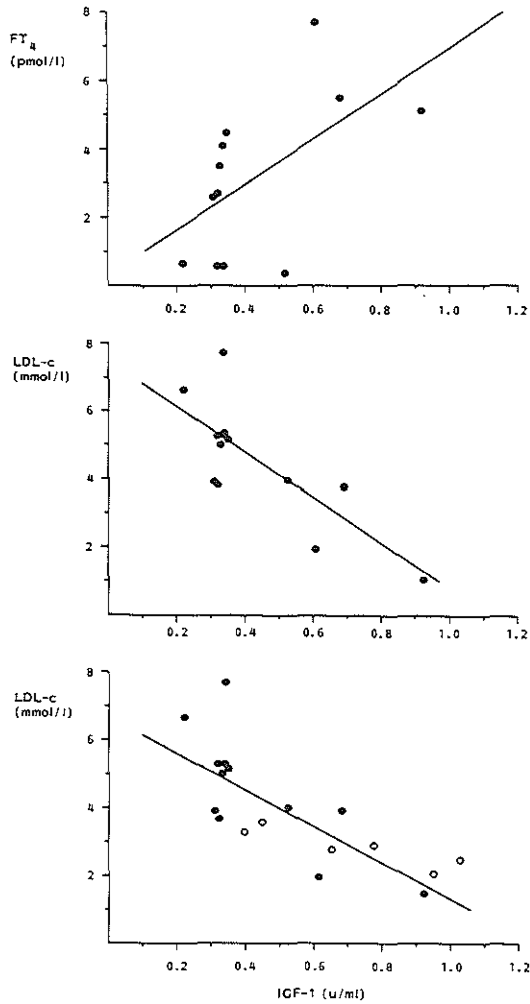


**Figure 2.1.:** Relationship in hypothyroidism between IGF-I and (a) free thyroxine ($T_4$) ($r = 0.58$, $p<0.05$), (b) low-density lipoprotein (LDL) cholesterol ($r = -0.75$, $p<0.01$). and (c) LDL cholesterol in 12 patients with primary hypothyroidism (•) and six patients after being treated with $T_4$ until euthyroid state was reached (o).

## DISCUSSION

It is not known exactly how hypothyroidism influences lipoprotein metabolism. Lipoprotein lipases are thought to play a role in the effect of hypothyroidism on lipoprotein changes (Valdemarsson, 1982). In our study HL was significantly reduced in hypothyroidism, but LPL was not affected. Evidence has been presented that liver lipase may play a role in the metabolism of HDL and IDL (Kraus et al., 1974; Jansen et al., 1980; Nozaki et al., 1986). We could not however, find a correlation between HDL-cholesterol or IDL-cholesterol and HL. It seems unlikely that the lowered HL-activity during hypothyroidism is a major determinant of the increase in these lipoprotein concentrations.Because we did not observe a correlation between lipases and plasma cholesterol or LDL-cholesterol, we were motivated to look for an other explanation of the effect of hypothyroidism on LDL metabolism.
Studies in animal models (Abrams et al., 1970) and children with GH-deficiency (Winter et al., 1979) suggest a relationship between IGF-I, a secondary plasma factor that mediates effects of GH (Daughaday et al., 1972), and lipoprotein metabolism. In hypothyroidism there is evidence for a lack of GH-activity (Chernausek et al., 1983) and thyroid hormones are regulators of IGF-I levels (Marek et al., 1981). In the present study we found a positive linear correlation between IGF-I and free $T_4$ concentrations and a negative correlation between IGF-I and plasma cholesterol, mainly LDL-cholesterol. In a multivariate regression analysis of LDL-cholesterol, as the dependent variable, against IGF-I and free $T_4$ we found a significant negative correlation between IGF-I and LDL-cholesterol concentrations but not between free $T_4$ and LDL-cholesterol. Our results suggest that the effect of IGF-I on cholesterol metabolism is directly on the LDL-cholesterol metabolism. It has recently been shown that IGF-I augments the binding capacity and uptake for LDL without altering the LDL-receptor affinity in porcine granulosa cells (Veldhuis et al., 1987). This finding supports the role of IGF-I in LDL metabolism. Thompson et al. (1981) demonstrated the existence of a defect of receptor-mediated LDL catabolism in hypothyroidism, while Chait et al. (1979) showed that tri-iodothyronine stimulates LDL degradation apparently by increasing the number of receptors in skin fibroblasts. The increase in LDL-cholesterol during hypothyroidism may, therefore, be due to a decrease in the LDL receptor-mediated uptake. We conclude that in hypothyroidism

IGF-I determines the concentration of LDL independent from free $T_4$. In addition hypothyroidism influences lipoprotein metabolism by lowering HL.

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## Chapter 3

# GROWTH HORMONE AND THYROXINE AFFECT LIPOPROTEIN METABOLISM IN HYPOTHYROID AND HYPOPHYSECTOMIZED RATS.

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## ABSTRACT

The aim of this study was to evaluate the effect of thyroxine ($T_4$) and GH replacement therapy on serum lipoproteins in rats with primary and secondary hypothyroidism and to see whether recovery of GH-activity was associated with normalization of serum lipoproteins. In both the primary and secondary hypothyroid rats, total cholesterol (TC) was higher than in normal controls, due to an increase in low-density lipoprotein cholesterol (LDL-cholesterol) and to a lesser extend also in high-density lipoprotein cholesterol (HDL-cholesterol). Treatment of hypophysectomized rats with GH induced a decrease in concentration of TC, LDL-cholesterol and HDL-cholesterol. The effect of GH on HDL-cholesterol was correlated with an increase in hepatic lipase (HL)-activity. $T_4$ replacement therapy of hypophysectomized rats normalized LDL-cholesterol, while HDL-cholesterol and HL were unaffected. During primary hypothyroidism, $T_4$ treatment induced GH-activity, increased HL activity and reduced the HDL-cholesterol concentration. GH treatment of primary hypothyroid rats had a similar influence on the lipid levels and HL as in hypophysectomized animals, although the lowering of HDL-cholesterol was less prominent.
These results demonstrate that GH determines serum lipoproteins during both primary and secondary hypothyroidism.

## INTRODUCTION

Hypothyroidism causes pronounced hypercholesterolaemia. In rats this is due to an increase in both low-density lipoprotein cholesterol (LDL-cholesterol) and high-density lipoprotein cholesterol (HDL-cholesterol) (Dory and Roheim, 1981; Gross et al., 1987). Studies in man revealed an increase in predominantly LDL-cholesterol, while the effect on HDL-cholesterol is still controversial (Hoogerbrugge - v.d. Linden et al., 1989; Chapter 2). The mechanisms responsible for these hypothyroidism-related changes in lipoprotein levels are not completely understood. During hypothyroidism, cholesterol synthesis, estimated by HMG-CoA reductase (the rate-limiting enzyme in cholesterol synthesis) activity in the liver, is decreased (Field et al., 1986). However, degradation of lipoproteins are retarded secondary to a

reduction of the number of LDL receptors, resulting in a rise in LDL-cholesterol (Chait et al., 1979; Thompson et al., 1981; Scarabottolo et al., 1986). The activity of heparin-releasable salt-resistant lipase from the liver (liver lipase activity, HL) is decreased during hypothyroidism (Hülsmann et al., 1977; Valdemarsson, 1982). A decrease in HL may be responsible for an increase in HDL-cholesterol, as HL has a key role in HDL-metabolism (Jansen et al., 1980; Jansen, 1988).
It is not clear whether these effects on lipoprotein concentrations are due to thyroid hormone itself. For instance Byers et al., (1970) suggested that the chief cause of hypercholesterolaemia during hypothyroidism is the latter's effect on the secretion of growth hormone (GH). They showed that the administration of not only thyroid extract, but also of bovine GH (in a supra-physiologically large dose) to thyroidectomized rats was effective in preventing the occurrence of hypercholesterolaemia. Recently we found that plasma LDL-cholesterol concentrations in hypothyroid women more strongly correlated with insulin like growth factor-I (IGF-I) than with $T_4$ (Hoogerbrugge-v.d. Linden et al, 1989; Chapter 2). These results suggest, that deficiency of GH is an important factor in the disturbances of plasma cholesterol levels in hypothyroid man.
Although both $T_4$ and GH can affect lipid metabolism, the specific effects of the individual hormones under physiological conditions, are not clear. The aim of this study was to investigate the separate effects of GH and $T_4$ on cholesterol and lipoprotein metabolism in rats with primary or secondary hypothyroidism.

## MATERIALS AND METHODS

### Animals

Male Wistar rats, 6-7 weeks of age and weighing 185 ± 10 (SD) g, were used in all experiments. They were housed at 21 °C on a 12 h cycle of light/darkness (lights on at 07.30 h). Vegetarian laboratory chow and drinking water were available ad libitum.

### Animal preparation

Hypophysectomy

Hypophysectomy was carried out by the parapharyngeal approach (Thongren et al., 1973). Rats were given a precautionary s.c. injection of 50.000 IU Penicillin after hypophysectomy. Hypophysectomy was judged to have been complete when, 2 weeks after hypophysectomy, rats showed <2 g weight gain, a serum $T_4$ concentration of less than 1 nmol/l and a decrease in testicular volume. At autopsy the pituitary fossa was inspected macroscopically for the absence of hypophysial tissue. The fossae were empty in all the rats used. A corticosterone pellet (C 2505, Sigma, St. Louis, Mo., U.S.A.) weighing 50 mg was implanted s.c., 2 days before administration of $T_4$ and GH.

Hypothyroidism

Hypothyroidism was induced by the addition of 0.05 % (w/v) 2-mercapto-1-methyl-imidazole (Janssen Chimica, 2340 Beerse, Belgium) to the drinking water and given during the entire study. The effect of treatment was checked by determination of $T_4$ concentration in the serum after 14 days of treatment. The concentration of $T_4$ had to be less than 1 nmol/l before substitution treatment was started.

**Animal treatment procedures**

Hormone administration was started 14 days after operation (hypophysectomized rats) or medication (hypothyroid rats). Hormones were given for 10 consecutive days by s.c. injection twice daily at 08.00 and 16.30 h. L-Thyroxine (Sigma) was dissolved in NaOH (0.1 mol/l) and diluted in 0.9 % (w/v) NaCL. A daily dose of 2.0 µg $T_4$/100 g body weight in 0.1 ml was used. Treatment with $T_4$ increased $T_4$ serum concentration (measured 4 h after injection) from less than 1 nmol/l to 118 ± 29 nmol/l, euthyroid controls had a $T_4$ concentration of 53 ± 12 nmol/l. Tri-iodothyronine ($T_3$) concentration in untreated rats was significantly decreased to 0.22 ± 0.37 nmol/l ($p < 0.01$) and normalized during $T_4$ treatment to 2.03 ± 0.37 nmol/l, compared with 1.89 ± 0.17 nmol/l in euthyroid controls. In hypothyroid rats, thyrotrophin (TSH) concentrations normalized during treatment with $T_4$ from 46.6 ± 11.2 mU/l to 0.6 ± 1.0 mU/l, compared with 1.2 ± 0.4 mU/l in euthyroid controls. In hypophysectomized animals TSH decreased below detection level.

For GH studies recombinant human GH (hGH, Norditropin, Novo-Nordisk, Denmark; 0.2 IU/100 g body weight) dissolved in 0.05 ml water was used

daily. This dose appeared to be physiological from dose-response studies by others (Jörgensen, 1987; Skottner et al., 1987). A combination of these hormones was given to a third group. Treatment with GH, alone or in combination with $T_4$, did not affect thyroid hormone concentration. Control groups of hypophysectomized and hypothyroid rats were given 0.1 ml 0.9 % NaCl sc twice daily. Blood was obtained from the orbital plexus the day before substitution started. On day 10 of substitution treatment, animals were killed by decapitation after 18 h of fasting. Trunk blood was collected. Blood sampling and decapitation was performed between 11.30 and 12.30 h. Body weights were determined every day at 8.30 h. Body weight gain was defined as the increase in body weight between days 2 and 9 of substitution.

**Analytical methods**

Total serum cholesterol and triglycerides were determined by enzymatic methods (Boehringer testkit combination, Boehringer, Mannheim, F.R.G.). Rat LDL (density (d) = 1.019 - 1.050 g/ml) and rat HDL (d = 1.050 - 1.121 g/ml) were isolated from pooled serum of two rats by density gradient ultracentrifugation (Redgrave et al., 1975). Lipoprotein fractions were separated by ultracentrifugation for 24 h, at 200.000 g using an SW 41 rotor in a Beckmann L5-50 ultracentrifuge and isolated by tube slicing. Additionally, HDL-cholesterol was determined in serum from individual rats, by separating HDL-cholesterol from other lipoproteins by ultracentrifugation at 15°C in a 42.2 Ti rotor at d 1.050 g/ml. for 3 h at 223.000 g. Cholesterol was measured in the infranatant. Purity of the fractionated HDL was checked by determination of Apolipoprotein $A_1$ by a commercially available kit (Boehringer). Apolipoprotein $A_1$ was detectable in the HDL and undetectable in the LDL fraction (data not shown).

The lipase activity in the livers was determined in liver homogenates as described by Jansen et al. (1980). Briefly, part of the liver was homogenized in an ice-cold solution of NaCl (154 mmol/l, pH 7.40), containing 5 U heparin/ml using a polytron tissue homogenizer. Homogenates were centrifuged for 5 min at 8000 g at 4°C and the supernatants, containing more than 80 % of all neutral lipase activity, were used. The activity was expressed as mU/mg liver; 1 mU represents the release of 1 nmol fatty acid from the substrate/min. Concentrations of $T_4$, $T_3$ and TSH were determined by

radioimmunoassay (Visser et al., 1975) in serum.

### Statistical Methods

Data are given as mean ± S.D. Paired and unpaired Student's t-tests were used for comparisons within and between groups.

## RESULTS

### Effects of primary and secondary hypothyroidism on serum TC.

The serum concentration of TC was increased ($p<0.01$) by 55 % in hypothyroid and by 67 % in hypophysectomized rats when compared with controls. This increase in TC was due mainly to an increase in LDL-cholesterol, but also in HDL-cholesterol concentration (Table 3.1 and 3.2). LDL-cholesterol was increased ($p<0.01$) by 251 % in hypothyroid and by 300 % in hypophysectomized rats; HDL-cholesterol was increased ($p<0.01$) by 31 % and 25 % respectively.

### Effects of hormonal treatment in hypophysectomized rats on cholesterol and lipoproteins.

Treatment with either $T_4$ or GH was effective in reducing TC in serum from hypophysectomized rats. The reduction in serum TC achieved by treatment with $T_4$ was significantly greater ($p< 0.05$) than with GH. Combined treatment with $T_4$ and GH normalized TC.

Treatment of hypophysectomized rats with $T_4$ induced a decrease of 75 % ($p<0.05$) in the LDL-cholesterol concentration to normal. $T_4$ did not influence HDL-cholesterol concentration nor HL (Table 3.1). Treatment with GH reduced LDL-cholesterol levels by 30 % ($p<0.05$), this reduction being less than that after treatment with $T_4$. In contrast to $T_4$, GH stimulated HL and caused a decrease in HDL-cholesterol. HDL-cholesterol measured in sera of individual rats, showed an HDL-cholesterol concentration of 2.04 ± 0.24 mmol/l in untreated and 1.67 ± 0.18 mmol/l in GH treated rats ($p<0.01$). Combined treatment with both $T_4$ and GH normalized lipid and lipoprotein concentrations in hypophysectomized animals.

**Table 3.1: Effect of substitution treatment with thyroxine ($T_4$), GH, or a combination of the two hormones in hypophysectomized (HPX) rats.**

| Treatment | (N) | TC (mM) | LDL-c (mM) | HDLc (mM) | HL (mU/mg liver) | BWG (g) |
|---|---|---|---|---|---|---|
| Control | (12) | 2.40 ± 0.28* | 0.35 ± 0.06* | 1.53 ± 0.16* | 1.18 ± 0.26* | 34.6 ± 6.7* |
| HPX | (13) | 4.01 ± 0.37 | 1.42 ± 0.36 | 1.92 ± 0.32 | 0.49 ± 0.12 | -5.6 ± 4.2 |
| HPX+$T_4$ | (13) | 2.67 ± 0.41* | 0.35 ± 0.26* | 1.87 ± 0.48 | 0.55 ± 0.12 | -6.2 ± 4.8 |
| HPX+GH | (16) | 3.02 ± 0.32* | 1.00 ± 0.16* | 1.64 ± 0.17 | 0.75 ± 0.14* | 16.8 ± 3.9* |
| HPX+$T_4$+GH | (16) | 2.49 ± 0.32* | 0.38 ± 0.07* | 1.55 ± 0.21* | 0.90 ± 0.13* | 16.4 ± 5.9* |

Data are means ± S.D. for the numbers of rats in parentheses; for determination of high-density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c) pooled sera of two rats were used.
* $P < 0.05$ compared to hypophysectomized rats (Student's t-test).

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**Table 3.2: Effect of substitution treatment with thyroxine ($T_4$), GH, or a combination of the two hormones in hypothyroid rats (HTH).**

| Treatment | (N) | TC (mM) | LDL-c (mM) | HDLc (mM) | HL (mU/mg liver) | BWG (g) |
|---|---|---|---|---|---|---|
| HTH | (12) | 3.71 ± 0.48 | 1.23 ± 0.28 | 2.00 ± 0.49 | 0.56 ± 0.12 | - 2.1 ± 4.4 |
| HTH+$T_4$ | (16) | 2.51 ± 0.30* | 0.36 ± 0.07* | 1.69 ± 0.32 | 0.87 ± 0.16* | 14.8 ± 7.4* |
| HTH+GH | (16) | 3.37 ± 0.27* | 1.02 ± 0.22 | 1.85 ± 0.24 | 0.81 ± 0.16* | 9.4 ± 3.2* |
| HTH+$T_4$+GH | 16) | 2.20 ± 0.19* | 0.15 ± 0.08* | 1.58 ± 0.09* | 1.00 ± 0.12* | 13.3 ± 5.4* |

Data are means ± S.D. for the numbers of rats in parentheses; for determination of high-density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c) pooled sera of two rats were used.
* $P < 0.05$ compared with values in hypothyroid rats (Student's t-test)

**Effects of hormonal treatment of hypothyroid rats on cholesterol and lipoproteins.**

Administration of GH to hypothyroid rats produced the same changes in lipid and lipoprotein concentrations as observed in hypophysectomized animals (Table 3.2). In contrast to its effects in hypophysectomized rats, $T_4$ treatment of hypothyroid rats induced a decrease in HDL-cholesterol and an increase in HL. This remarkable increase in HL was correlated to an increase in body weight ($r = 0.67$, $p<0.001$). In $T_4$ treated hypophysectomized animals, no body weight gain was seen (Table 3.1).

## DISCUSSION

The present study indicates that a physiological dose of GH can reduce hypercholesterolaemia in hypothyroid rats. A cholesterol-decreasing effect of GH was known from studies in GH-deficient children treated with GH (Blackett et al., 1982), and in patients with Acromegaly (Nikkilä and Pelkanen, 1975). This study shows that the cholesterol-decreasing effect of GH is mainly due to a decrease in LDL-cholesterol, although HDL-cholesterol is also lowered. Treatment of hypothyroid and hypophysectomized rats with GH had a stimulating effect on HL, thereby probably reducing HDL-cholesterol concentrations. In contrast to GH, treatment of hypophysectomized rats with $T_4$ alone did not affect HL, nor HDL-cholesterol, but produced an additional effect on HL when in combination with GH. Treatment of hypothyroid rats with $T_4$, on the other hand, stimulated HL and lowered HDL-cholesterol, which suggests induction of GH-activity by $T_4$. For estimation of GH-activity we measured body weight gain, a known bioassay (Groesbeck and Parlow, 1987). Body weight fell during primary and secondary hypothyroidism. In hypothyroid rats body weight increased during treatment with GH and more so with $T_4$, indicating an (at least partial) restoration of GH-activity during $T_4$ treatment in hypothyroid animals. In contrast, hypophysectomized animals did not show an increase in body weight after treatment with $T_4$.

The suggested decrease in GH-activity during primary hypothyroidism is shown by others too, e.g. the synthesis of GH in the pituitary gland is decreased in primary hypothyroidism (Evans et al., 1982; Casanova et al., 1985; Wood et al., 1987), resulting in decreased GH content of the pituitary

(Stachura, 1986) and decreased secretion of GH during stimulation tests (Root et al., 1985; Katakami et al., 1986). Furthermore, the normal pulsatile pattern of GH secretion is lost during hypothyroidism. Both production and secretion of GH can be restored by administering $T_4$ to hypothyroid rats (Martin et al., 1985; Katakami et al., 1986). When drawing conclusions from data on the effects of thyroid hormones on hypothyroid rats, it is important to take account of the fact that the secretion not only of thyroid hormone, but also of GH is low during primary hypothyroidism, while both hormones will be restored by the administration of $T_4$.
The main effect of $T_4$ on TC in hypothyroid and hypophysectomized is a decrease in the concentration of LDL-cholesterol. Although both $T_4$ and GH are able to lower the LDL-cholesterol concentration, the effect of $T_4$ is more potent, suggesting a role of thyroid hormone itself in LDL metabolism. A mediating role of GH in LDL-cholesterol metabolism in hypothyroid rats is suggested, as combined treatment had an additive effect in the decrease of LDL-cholesterol in these animals. The effects of thyroid hormone on LDL are known from in vitro experiments; $T_3$ was shown to promote the binding of LDL to its receptor in fibroblasts (Chait et al., 1979). More recently, it was shown that insulin like growth factor-I (IGF-I) has a positive effect on the number of LDL receptors in granulosa cells (Veldhuis et al., 1987), while we found that LDL-cholesterol levels are related more to the concentration of IGF-I, than to that of thyroid hormone in hypothyroid women (N. Hoogerbrugge-v.d.Linden et al., 1989; Chapter 2).
The present study in hypophysectomized rats shows that both $T_4$ and GH decrease LDL-cholesterol, while only GH and not $T_4$ decreases HDL-cholesterol by an increase in HL.

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## Chapter 4

# INTERACTION OF GROWTH HORMONE ACTIVITY AND THYROID HORMONE IN THE LIPOPROTEIN METABOLISM OF HYPOTHYROID RATS.

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## ABSTRACT

In hypothyroid rats hypercholesterolemia is present, due to an increase in both low density lipoprotein (LDL)-cholesterol and high density lipoprotein (HDL)-cholesterol. The activity of hepatic lipase (HL), an important enzyme in HDL metabolism, is decreased. LDL-cholesterol and HDL-cholesterol can be reduced and HL-activity stimulated by substitution with growth hormone (GH). In the present work the role of GH in the disturbed lipid metabolism during hypothyroidism was studied.

In a severely hypothyroid state in methimazole treated rats (TSH>46 mU/l), the IGF-I concentration was decreased by 51%. Treatment with a low daily dose of $T_4$ (0.25 μg/100g body weight (BW)), did not restore euthyroidism, but normalized IGF-I levels. During treatment with this low dose $T_4$, the LDL-cholesterol concentration was partially corrected, (-33%), but the HDL-cholesterol level and HL-activity did not change. When higher substitution doses of $T_4$ were given, LDL-cholesterol concentration decreased further and HL-activity increased dose dependently.

In hypophysectomized rats, substitution with $T_4$ (2 μg/100g BW per day) restored the euthyroid state, normalized the LDL-cholesterol concentration, but did not affect HL-activity.

Thus GH and/or IGF-I partly reduced LDL-cholesterol and HDL-cholesterol and increased HL-activity, while thyroid hormone stimulated HL-activity only in the presence of GH-activity. The LDL-cholesterol concentration is decreased by both hormones independently, with thyroid hormone having greatest effects.

## INTRODUCTION

Among the most prominent metabolic consequences of hypothyroidism is hypercholesterolemia (Epstein and Lande, 1922; Masom et al., Hunt and Hurxthal, 1930; Peters and Man, 1950; Valdemarsson, 1983; Heimberg et al., 1985). A number of reports have demonstrated that both the low density lipoprotein (LDL)-cholesterol and the high density lipoprotein (HDL)-cholesterol concentrations are increased in hypothyroid rats (Dory and Roheim, 1981; Gross et al., 1987), while hepatic lipase (HL)-activity, an

important enzyme in HDL metabolism, is decreased (Kuusi et al., 1988; Murase and Uchimura, 1980). The lipoprotein pattern reverts to normal after substitution with thyroxine ($T_4$) (Kuusi et al., 1988).
Thyroid hormone is thought to be responsible for these changes in lipid concentrations, but during hypothyroidism growth hormone (GH)-activity is also decreased (Chernausek and Turner, 1989; Katakami et al., 1986; Iwatsubes et al., 1967), indicating a possible role for GH-deficiency in the hypercholesterolemia as well. GH is known to decrease the serum cholesterol concentration in man and rats (Friedman et al., Elek, 1970; Friedman et al., 1974; Nikkilä and Pelkonen, 1975). In hypothyroid women we found that the LDL-cholesterol concentration was more strongly correlated to Insulin-like Growth Factor I (IGF-I) levels than to $T_4$ (Hoogerbrugge-vd Linden et al., 1989; Chapter 2). Hypothyroid rats develop hypercholesterolemia due to an increase in both LDL- and HDL-cholesterol, while in man hypercholesterolemia is predominantly a result of increased LDL-cholesterol. Hypophysectomized rats substituted with GH showed a decrease of both HDL- and LDL-cholesterol concentration (Hoogerbrugge-v.d.Linden et al., 1990; Chapter 3). Normalization of the levels of these lipoproteins was achieved when both GH and $T_4$ were substituted.
In the present study we have further investigated the relationship between GH, thyroid hormone and lipid metabolism of hypothyroid rats.

## MATERIALS AND METHODS

### Animals and treatments.

Animals

Male pubertal Wistar rats, 6-7 weeks of age and weighing 185 ± 10 (SD) g, were used in all experiments. They were housed at 21 °C on a 12 h cycle of light/darkness (lights on at 07.30 h). Chow and drinking water were available ad libitum. Vegetarian chow (containing a mixture of grains, herbs and seeds) was obtained from Puyk (Rabbit chow, Holland Diervoeders, Hilversum, The Netherlands).

Induction of hypothyroidism

Hypothyroidism was induced by the addition of 0.05 % (w/v) 2-mercapto-1-

methyl-imidazole (Janssen Chimica, 2340 Beerse, Belgium) to the drinking water and given during the entire study.

Hypophysectomy

Hypophysectomy was carried out by the parapharyngeal approach (Thongren et al., 1973). Rats were given a precautionary s.c. injection of 50.000 IU Penicillin after hypophysectomy. Hypophysectomy was judged to be complete when, 2 weeks after hypophysectomy, rats showed <2 g weight gain, a serum $T_4$ concentration of less than 1 nmol/l and a decrease in testicular volume. A corticosterone pellet (C 2505, Sigma, St. Louis, Mo., U.S.A.) weighing 50 mg was implanted s.c., 2 days before administration of hormones.

Administration of hormones

Hormone administration was started 14 days after medication (hypothyroid rats) or operation (hypophysectomized rats). Hormones were given twice daily for 10 consecutive days by s.c. injection. L-Thyroxine (Sigma) was dissolved in NaOH (0.1 mol/l) and diluted in 0.9 % (w/v) NaCL. The daily dose of $T_4$ used varied between 0.25 - 2 µg/100g BW. Recombinant human GH (hGH, Norditropin, Novo-Nordisk, Denmark) was given subcutaneously in a daily dose of 0.2 IU/100 g BW. After 10 days of substitution, animals were killed by decapitation after 18 h of fasting. Trunk blood was collected. Blood sampling and decapitation was performed between 11.30 and 12.30 h. Body weights were determined every day at 8.30 h. Body weight gain (BWG) was defined as the increase in body weight between days 2 and 9 of substitution.

**Analytical methods**

Total serum cholesterol and triglycerides were determined by enzymatic method (Boehringer testkit combination, Boehringer, Mannheim, F.R.G.). Rat LDL (density (d) = 1.019 - 1.050 g/ml) and rat HDL (d = 1.050 - 1.121 g/ml) were isolated by density gradient ultracentrifugation (Redgrave, et. al., 1975). The HL-activity was determined in liver homogenates as described by Jansen et al (1980). The activity was expressed as mU/mg liver: 1 mU represents the release of 1 nmol fatty acid from the substrate/min.

Concentrations of $T_4$ and $T_3$ were determined by radioimmunoassay (Visser et al., 1975), rat-TSH was determined by RIA using materials supplied by the NIDDK. TSH RP-2 was used as standard as described by Rondeel et al. (1988).

The rat IGF-I concentration was determined by radioimmunoassay, using a

test kit (Nichols institute diagnostics, San Juan Capistrano, CA 92675).

**Statistical Methods**

All data are expressed as mean ± S.D. Student's t-tests were used for comparisons between groups. The statistical significance of the differences within groups was determined using analysis of variance (ANOVA).

## RESULTS

**Effects of vegetarian food on lipids and lipoproteins in the rat.**

In all experiments the rats were fed a vegetarian diet. In a pilot study using normal lab chow, suspicion was raised for the presence of thyroid hormone in this chow. Methimazole treatment resulted in $T_4$ concentrations not lower than 12 ± 5 nM (Table 4.1). In rats fed a vegetarian lab chow, $T_4$ levels were significantly lower (Table 4.1).

Compared to the normally fed rats, the vegetarian fed rats showed an increase in serum cholesterol concentration of approximately 50 % ($p<0.01$, Table 4.1), due to an increase in both LDL-cholesterol and HDL-cholesterol. The LDL-cholesterol concentration in normally fed- and in vegetarian fed animals, was 0.25 ± 0.03 mM and 0.35 ± 0.05 mM ($p<0.01$), respectively, and HDL-cholesterol 0.91 ± 0.09 mM and 1.53 ± 0.16 mM ($p<0.01$), respectively. Total triglycerides (TG) and very-low density lipoprotein (VLDL)-TG were highly variable (1.20 ± 0.24 mM and 0.68 ± 0.14 mM respectively).

Besides differences in plasma lipids also the IGF-I concentration varied in rats fed the normal- or the vegetarian-chow. The IGF-I concentration in vegetarian fed hypothyroid rats was decreased with 50 % ($p<0.01$) as compared to euthyroid rats (Table 4.1).

**Severity of hypothyroidism and its effects on IGF-I and lipids.**

To induce differences in severity of hypothyroidism, groups of hypothyroid rats were treated with varying amounts of $T_4$ (0, 0.25, 0.5, 0.75, 1.0, 1.5 μg/100 g body weight per day). The severity of hypothyroidism was checked by the concentration of $T_3$, $T_4$ and TSH in serum (Table 4.2). With respect to the TSH concentration, normalization was achieved using a daily dose of 0.75-

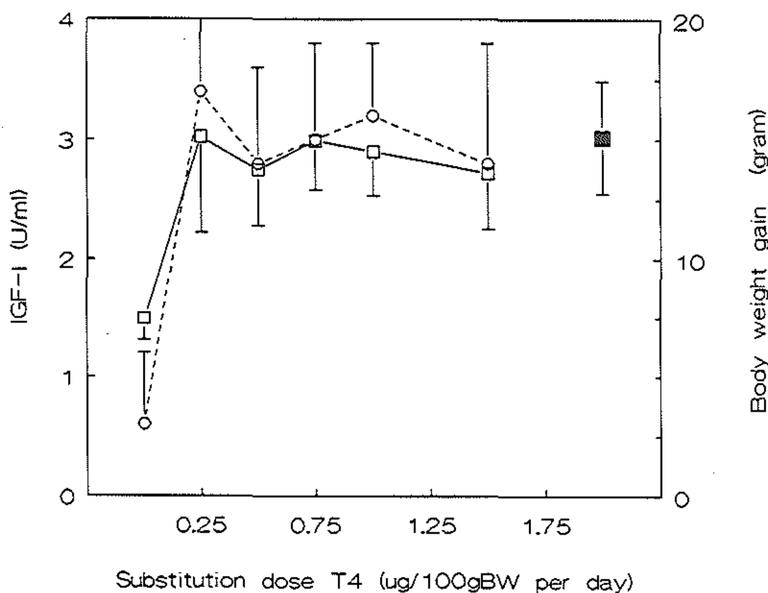


**Figure 4.1:** The effect of treatment of hypothyroid rats with different doses of thyroxine ($T_4$) on plasma insulin-like growth factor-I (IGF-I, squares, closed line) and body weight gain (BWG, circles, dotted line). IGF-I concentration of controls are indicated by a black square. Each group represents the data of 6 rats. Data are presented as mean ± SD.

1.0 µg $T_4$/100g. The lowest daily dose of $T_4$ (0.25 µg/100g BW) did not restore euthyroidism (Table 4.2), but normalized IGF-I concentration (Figure 4.1). A further increase in $T_4$ dose did not result in higher IGF-I levels. The increase in IGF-I concentration during treatment with $T_4$ was paralleled by an increased body weight gain (BWG, Figure 4.1), another parameter for GH-activity .The lowest dose of $T_4$ (0.25 µg/100g BW) led to a decrease in the LDL-cholesterol concentration from 1.06 ± 0.11 mM to 0.71 ± 0.06 mM ($p<0.01$). The LDL-cholesterol concentration in the group of animals treated with the highest dose $T_4$ (1.5 µg/100g BW) was decreased as compared to the group treated with the lowest dose of $T_4$ (0.25 µg/100g BW), to a concentration of 0.49 ± 0.04 mM ($p<0.05$, Figure 4.2). The HDL-cholesterol-concentration was affected by $T_4$ only in the group treated with 1.5 µg $T_4$/100

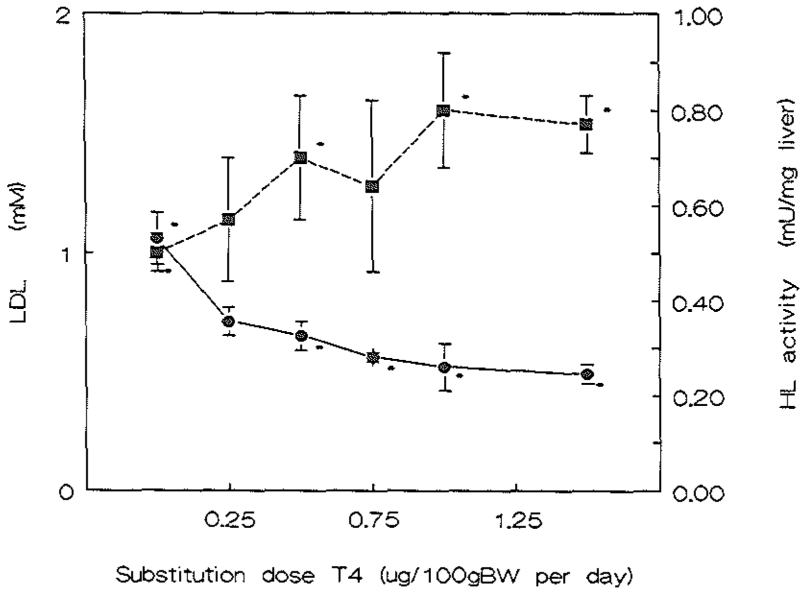


**Figure 4.2**: The effect of treatment of hypothyroid rats with different doses of thyroxine ($T_4$). on low density lipoprotein cholesterol (LDLc, closed line) and hepatic lipase (HL, dotted line). Values are means ± SD. Each group represents the data of 6 rats; for determination pooled sera of two rats were used. Data significantly different from the group treated with 0.25 µg/100g BW are marked with an asterisk ($p<0.05$).

g BW, in this group HDL-cholesterol levels normalized from 1.78 ± 0.01 to 1.51 ± 0.06 mM ($p<0.01$), respectively. The HL-activity was decreased during hypothyroidism, as compared to the euthyroid state (0.50 ± 0.04 mU/mg liver verses 1.36 ± 0.13 mU/mg liver ($p<0.01$), respectively). After treatment with 1.5 µg $T_4$/100 g BW, HL-activity increased to 0.77 ± 0.06 mU/mg liver ($p<0.05$).

## Effects of treatment with $T_4$ and GH on lipoproteins in hypophysectomized rats.

Treatment of hypophysectomized rats with $T_4$ in a daily dose of 2µg/100g BW, led to a decrease of 75 % ($p < 0.05$) in the LDL-cholesterol concentration

**Table 4.1: The effects of normal and vegetarian chow on cholesterol, thyroxine ($T_4$) and Insulin like Growth Factor I (IGF-I) in euthyroid and hypothyroid rats.**

| | Controls | Hypothyroids |
|---|---|---|
| **Normal Lab Chow** | | |
| n | (6) | (12) |
| cholesterol (mM) | 1.7 ± 0.08 | 2.5 ± 0.3 * |
| $T_4$ (nM) | 23 ± 5 | 12 ± 5 * |
| IGF-I (U/ml) | 5.28 ± 0.40 | 4.86 ± 0.55 |
| **Vegetarian Chow** | | |
| n | (6) | (6) |
| cholesterol (mM) | 2.5 ± 0.3 | 3.5 ± 0.3 * |
| $T_4$ (nM) | 40 ± 8 | 4 ± 5 * |
| IGF-I (U/ml) | 3.02 ± 0.47 | 1.49 ± 0.18 * |

* Significantly different compared to controls in a Student's T-test ($p<0.01$)

**Table 4.2: The effect of treatment with different doses of thyroxine ($T_4$) of hypothyroid rats for 10 days on serum $T_4$, triiodothyronine ($T_3$) and thyroid stimulating hormone (TSH).**

| Substitution Dose T4 µg/100g/day | $T_4$ nM | $T_3$ nM | TSH mU/l |
|---|---|---|---|
| **Controls** | | | |
| 0 | 40 ± 8 | 1.89 ± 0.17 | 1.2 ± 0.4 |
| **Hypothyroids** | | | |
| 0 | 4 ± 5* | 0.22 ± 0.14* | 46.6 ± 11.2* |
| 0.25 | 41 ± 8 | 1.03 ± 0.53* | 21.2 ± 9.9* |
| 0.5 | 82 ± 12* | 1.22 ± 0.21* | 5.7 ± 1.6* |
| 0.75 | 102 ± 11* | 1.27 ± 0.16* | 2.0 ± 0.9 |
| 1 | 128 ± 9* | 1.50 ± 0.21* | 0.7 ± 0.3 * |
| 1.5 | 140 ± 36* | 2.03 ± 0.37 | 0.6 ± 0.1* |

Each group represents the data of 6 rats. Data are presented as mean ± SD.
* Significantly different from controls in ANOVA ($p<0.01$).

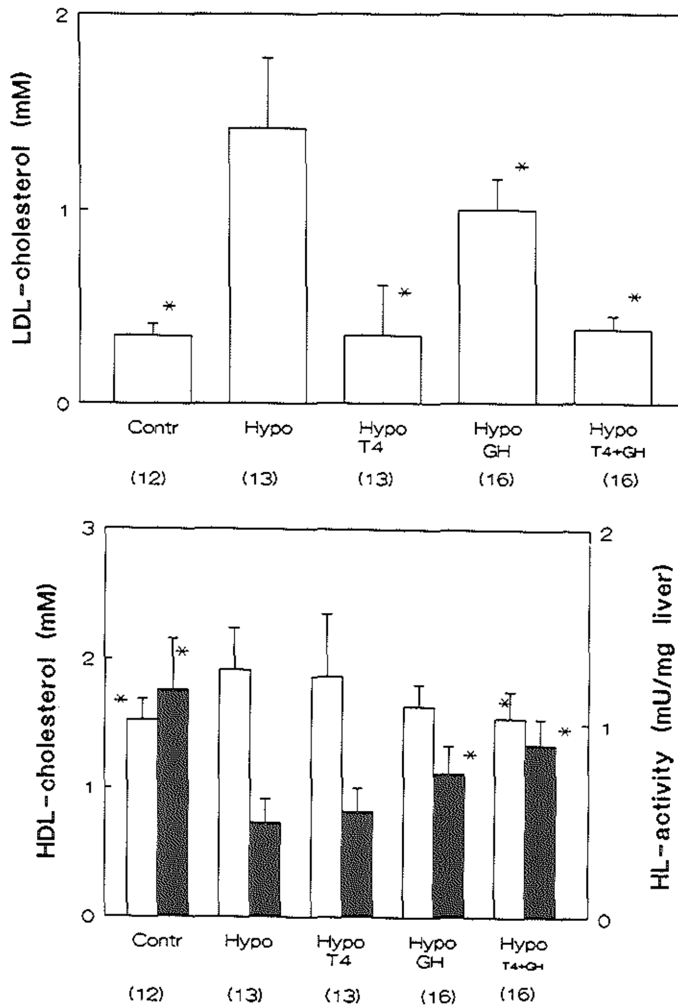


**Figure 4.3:** Effects of substitution treatment with thyroxine ($T_4$), growth hormone (GH), or a combination of the two hormones in hypophysectomized (hypox) rats on, **A**: low density lipoprotein (LDL) cholesterol, **B**: high density lipoprotein (HDL) cholesterol (open bars) and hepatic lipase (HL)-activity (closed bars). Values are means ± SD. for the numbers of rats in parentheses; for determination of HDL) cholesterol and LDL cholesterol pooled sera of two rats were used. Data significantly different from the hypophysectomized rats are marked with an asterisk ($p<0.05$).

(Figure 4.3), but did not influence the HDL-cholesterol concentration nor HL-activity (Figure 4.3). Treatment with GH reduced LDL-cholesterol levels by 30 % ($p < 0.05$). This reduction was less than with $T_4$ ($p<0.05$). In contrast to $T_4$, GH stimulated HL-activity ($p<0.05$) and caused a decrease in HDL-cholesterol (n.s.). Combined treatment with both $T_4$ and GH normalized HDL-cholesterol concentrations and induced a further increase in HL-activity with 20 % ($p<0.01$), compared to GH treated hypophysectomized rats (Figure 4.3). Administration of GH to hypothyroid rats produced the same changes in lipid and lipoprotein concentrations as observed in hypophysectomized animals (data not shown). The effects of hormonal substitution treatment of hypophysectomized rats on body weight are given in Figure 4.4.

## DISCUSSION

The effects of GH and thyroid hormone on LDL- and HDL-cholesterol concentrations were studied. We have previously found that LDL- and HDL-cholesterol in the rat are both affected by GH and thyroid hormone (Hoogerbrugge-v.d.Linden et al, 1990; Chapter 3). The thyroid hormone status influences the GH-activity, represented by the IGF-I concentration. As seen by others (Chernausek et al., 1983; Valcavi et al., 1987), we found that during hypothyroidism, IGF-I is greatly diminished and returned to control values after thyroid hormone substitution. The IGF-I levels we measured, are in accord with the findings of Skottner et al (1987). In the present study hypothyroid rats were treated with different amounts of $T_4$. The lowest dose of $T_4$ (0.25 µg/100g) increased $T_3$ and decreased TSH concentration considerably. Larger doses of $T_4$ led to a further response in these variables. GH-activity, as reflected in the IGF-I concentration and the body weight gain,was increased to control values at the lowest substitution dose of $T_4$ and did not increase further at higher doses.

The LDL-cholesterol concentration was decreased during the thyroid hormone substitution and showed the same biphasic response as $T_3$ and TSH. From these data it can not be concluded whether the first fast phase in the lowering of LDL-cholesterol is due to the increase in thyroid hormone or (and) IGF-I. However, since during the second slower phase IGF-I remains constant, it seems that the decrease in LDL-cholesterol during this phase is due to the

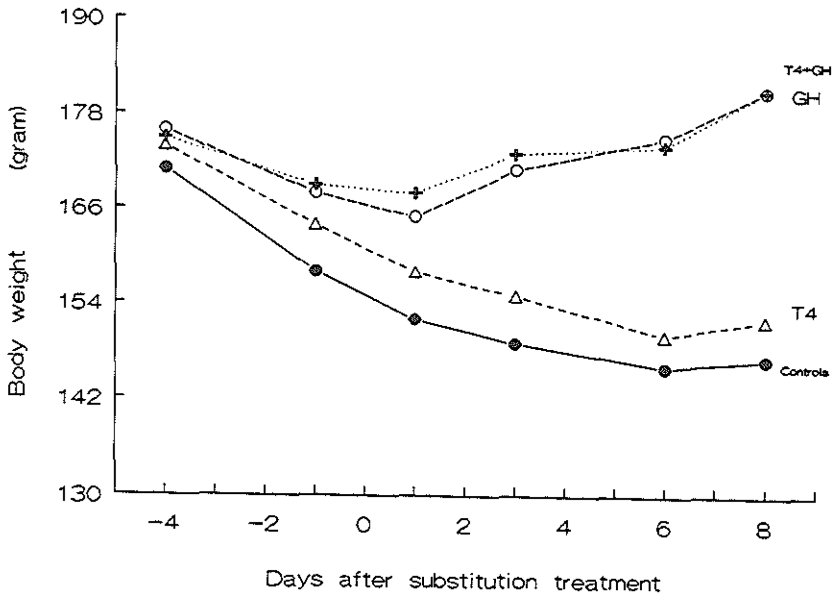


**Figure 4.4:** Effects of substitution treatment with thyroxine ($T_4$), growth hormone (GH), or a combination of the two hormones in hypophysectomized (hypox) rats on body weight. Each point represents the mean of 12 rats.

increase in thyroid hormone rather than to IGF-I. From these data it seems, that in the rat the LDL- cholesterol concentration is rather governed by thyroid hormone- than by the growth hormone-activity. To elaborate on this finding further, hypophysectomized rats were treated with $T_4$. Under thiscondition the thyroid hormone status is normalized, while IGF-I is only marginally affected (1.26 ± 0.30 U/ml versus 0.76 ± 0.14 U/ml). Body weight is not influenced during treatment with $T_4$ suggesting that GH activity is negligible. $T_4$ treatment led to a complete normalization of LDL-cholesterol, again indicating the dependency of LDL-cholesterol on thyroid hormone. However, GH substitution of the hypophysectomized rats lowered LDL-cholesterol also. This is in line with studies in humans, were we showed the plasma LDL-cholesterol during hypothyroidism is strongly related to IGF-I concentration (Hoogerbrugge-v.d.Linden et al., 1989; Chapter 2). Plasma

**Table 4.3: Effects of substitution treatment with thyroxine ($T_4$), growth hormone (GH), or a combination of the two hormones in hypophysectomized (hypox) rats.**

| | n | LDLc mM | HDLc mM | HL activity mU/mg liver |
|---|---|---|---|---|
| Control | (12) | 0.35 ± 0.06* | 1.53 ± 0.16* | 1.18 ± 0.26* |
| Hypox | (13) | 1.42 ± 0.36 | 1.92 ± 0.32 | 0.49 ± 0.12 |
| Hypox +$T_4$ | (13) | 0.35 ± 0.26* | 1.87 ± 0.48 | 0.55 ± 0.12 |
| Hypox +GH | (16) | 1.00 ± 0.16* | 1.64 ± 0.17 | 0.75 ± 0.14* |
| Hypox +$T_4$+GH | (16) | 0.38 ± 0.07* | 1.55 ± 0.21* | 0.90 ± 0.13* |

Values are means ± SD. for the numbers of rats in parentheses; for determination of high density lipoprotein cholesterol (HDLc) and low density lipoprotein cholesterol (LDLc) pooled sera of two rats were used. HL is hepatic lipase.
* Significantly different from hypophysectomized rats in a Student's t-test ($p<0.05$).

LDL-cholesterol concentration is determined mainly by its rate of removal, which is dependent on the LDL-receptor activity (Goldstein and Brown, 1986). Indeed in hypothyroidism LDL catabolism is decreased (Thompson et al., 1981). Recently we described that substitution with GH of hypothyroid rats stimulates LDL-receptor mRNA levels and activity of liver cells (Hoogerbrugge et al., Chapter 5)

HDL-cholesterol levels are largely dependent on HL activity, an enzyme which plays a key role in the metabolism of HDL (Jansen et al., 1980). Under all conditions studied in this experiment, both variables were strongly inversely correlated. HL-activity and the related HDL-cholesterol concentration showed a completely different picture in the response to thyroid hormone and GH. In hypophysectomized rats substitution with $T_4$ had no effect on either variable, indicating that the hypothyroidism as such was not responsible for the lowered HL activity in hypophysectomized rats. Treatment with GH increased the lipase activity significantly. But in the presence of both GH and thyroid hormone the activity was further increased to control values. Therefore, GH and/or IGF-I seems to be a permissive factor in the influence of thyroid hormone on HL. In hypothyroid rats thyroid hormone substitution led to an

increase in HL activity which was not directly related to either thyroid hormone or IGF-I concentrations. Probably the initial increase in IGF-I triggers the increase in HL which is then further enhanced by the increase in thyroid hormone. HDL-cholesterol followed inversely the HL-activity suggesting a causal relationship, which is feasible in the light of the proposed function of HL in the catabolism of HDL-cholesterol.
To obtain a truly hypothyroid state necessary for a deprivation of GH-activity, methimazol treated rats were deprived from exogenous thyroid hormone. As contamination of laboratory chow with thyroid hormone was suspected, rats were fed a vegetarian diet. Compared to the levels in control rats fed the usual laboratory chow, the vegetarian fed control rats showed an increase in the concentration of both LDL- and HDL-cholesterol (data not shown). Hypothyroidism increased these lipoproteins both in the vegetarian and in the control-fed animals.
In these studies we showed that GH and thyroid hormone are both involved in the regulation of two important lipoproteins in the rat, LDL and HDL. While thyroid hormone appeared to be of primary importance in the regulation of LDL-cholesterol, GH has a distinct additional LDL-cholesterol decreasing effect. In the regulation of HDL cholesterol, HL-activity appears to be of major importance. For the activity of HL, GH was shown to be a permissive factor, in the presence of which thyroid hormone can stimulate the activity.

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## Chapter 5

# GROWTH HORMONE ENHANCES mRNA AND ACTIVITY OF THE LOW DENSITY LIPOPROTEIN-RECEPTOR IN THE LIVER OF HYPOTHYROID RATS.

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Submitted

## ABSTRACT

In hypothyroid rats hypercholesterolemia, partially due to a decreased low-density lipoprotein-receptor (LDL-R) activity, is present. This hypercholesterolemia can be corrected to some extent by substitution of growth hormone (GH). In the present study the mechanism of this GH mediated effect was investigated.
Liver LDL-R mRNA levels and LDL-R concentrations in hypothyroid rats were diminished by 69% and 33% respectively. Substitution treatment with GH, to activity levels seen in euthyroid controls (as determined by plasma IGF-1 concentrations and restoration of growth), led to a normalization of both LDL-R mRNA and LDL-R activity.
In hypothyroids, HMG-CoA reductase activity was decreased by 64%. This activity rose after substitution of GH, excluding an indirect effect of GH on LDL-R via a decreased HMG-CoA reductase activity.
These data show that GH modulates hepatic LDL-R activity, by regulating LDL-R gene expression. These observations demonstrate the importance of GH in cholesterol homeostasis.

## INTRODUCTION

The regulation of cholesterol metabolism is multifactorial, diet and hormones playing major roles. Growth hormone (GH) has a profound but not yet completely understood effect on cholesterol metabolism: In hypercholesterolemic adults, supraphysiological doses of GH lower serum cholesterol (Friedman et al., 1974) and GH over production, as found in acromegaly, is accompanied by low serum cholesterol levels (Nikkilä and Pelkonen, 1975). Also in primary hypothyroidism, which is accompanied by GH deficiency (Katakami et al., 1986; Wood et al., 1987), hypercholesterolemia is present (Walton et al., 1965; Dory and Roheim, 1981; Sykes et al., 1981; Gross et al., 1987). We recently reported that in hypothyroid patients the elevated low-density lipoprotein (LDL) cholesterol concentrations are more strongly related to the IGF-I than to free thyroxine concentration (Hoogerbrugge-v.d.Linden et al., 1989; Chapter 2), indicating

the relevance of GH for cholesterol metabolism. In addition, it was found that the serum cholesterol concentrations in hypothyroid rats decreased upon substitution with GH (Byers et al., 1970; Hoogerbrugge-v.d.Linden et al., 1990; Chapter 3). The hypercholesterolemia in hypothyroidism is partially caused by an increase in LDL cholesterol, which is probably due to a reduced catabolic rate of this lipoprotein via the LDL receptor (LDL-R) (Thompson et al., 1981; Scarabottola et al., 1986). It has been demonstrated that the low LDL-R expression in the hypothyroid state can be stimulated by thyroid hormone (TH) (Chait et al., 1979). This effect of TH on LDL-R expression may either be direct or indirect via a TH-induced increase in GH synthesis and secretion (Martin et al., 1985; Katakami et al., 1986). The effect of treatment of hypothyroidism with TH on LDL-R can also be secondary to a lowering of the intracellular cholesterol concentration, as the number of LDL-R is subject to a negative regulation by this concentration: when this concentration falls, increased numbers of LDL-R are produced. Therefore we determined the effect of GH on the activity of hydroxy-methylglutaryl-Coenzyme-A (HMG-CoA) reductase, the rate limiting enzyme in cholesterol synthesis.
The present study was designed to examine the role of GH in the regulation of cholesterol metabolism during hypothyroidism in rats.

## MATERIALS AND METHODS.

### Animals and treatments.

Animals

Pubertal male Wistar rats weighing 185 ± 10 (SD) gram were used in all experiments. They were housed at 21 °C on a 12 hour light cycle. Vegetarian laboratory chow (free from thyroid hormone) and drinking water were available ad libitum.

Induction of hypothyroidism

Hypothyroidism was induced by the addition of 0.05 % (w/v) 2-mercapto-1-methyl-imidazole (Janssen Chimica, 2340 Beerse Belgium) to the drinking water during the entire study period of 24 days. Serum $T_4$ concentrations were determined after 14 days of treatment. If $T_4$ was less than 1 nmol/l, administration of GH was started.

Administration of GH

hGH (Norditropin) was a generous gift from Novo-Nordisk A/S Gentofte, Denmark. Dissolved in 0.1 ml 0.9% NaCl, it was given twice daily for 10 consecutive days by s.c. injection (total daily dose 0.2 IU hGH/100 g body weight). This dose appeared to be physiological from dose-response studies by others (Jörgensen et al, 1987; Skottner et al., 1987).

Animal preparation

At the end of the treatment period animals were fasted for 18 hr and killed by exsanguination under pentobarbital anaesthesia. Aortal blood was collected, allowed to clot at 0°C and the serum was stored at -20°C. The liver was removed immediately and rinsed with 0.9% NaCl. Part of the liver was frozen in liquid nitrogen. The remainder was used for isolation of microsomes by differential centrifugation (Havinga et al., 1987), and microsomes were stored at -80°C.

**LDL isolation and labeling**

Human LDL was purified by ultracentrifugation (d 1.019-1.063 g/ml) from fresh plasma of healthy donors (Zechner et al., 1986). LDL was labeled with $^{125}I$ as previously described (specific activity of 55 cpm/ng LDL protein)(McFarlane, 1956; Billheimer et al., 1972).

**Analytical methods**

Determination of LDL receptor activity.

LDL-R was quantified by a modified dot-blot assay (Maggi and Catapano, 1987): Microsomes were suspended in 50 mM Tris-maleate pH 6.5, containing 2 mM $CaCl_2$, 1.5 mM PMSF, 0.1 mM leupeptine and 40 mM ß-octyl-glucoside (buffer A)(leupeptine and ß-octyl glucoside were obtained from Boehringer Mannheim, Germany). The suspension was sonicated twice for 5 sec and the undissolved material was pelleted by centrifugation for 4 min in an Eppendorf centrifuge at 10.000 x g. The ß-octyl-glucoside soluble material was diluted to a final protein concentration of 0.2 mg/ml and 50 µl of the membrane suspension was applied to nitrocellulose membranes using a manifold apparatus (Bio-Rad). Individual spots were washed with 150 µl of a buffer pH 7.5, containing 10 mM Tris-HCl, 50 mM NaCl and 2 mM $CaCl_2$ (buffer B). After blocking the unbound places for 1 h in buffer B containing 50 mg/ml BSA, the nitrocellulose filter was incubated for 16 h at 21°C with 16 µl

labeled LDL (0.27 μg/ml) in buffer B, washed at least 5 times with 150 μl buffer B and dried for autoradiography (X-ray films were from Kodak, Badhoevedorp The Netherlands). Exposure was for 38 hours. Quantitative analysis was performed using a scanning densitometer (Videodensitometer model 620, Biorad). All measurement were done in triplicate. Specificity of binding was evaluated in the presence of a 20-fold excess of unlabeled LDL. The effects of disodium EDTA (30 mM) and heparin (4 mg/ml) added to the incubation medium were evaluated.

LDL receptor mRNA determination.

RNA was prepared by the guanidine isothiocyanate/cesium chloride procedure (Chirgin et al., 1979) from the liver of individual animals. Dotblot hybridizations of total cellular RNA were performed as described previously (Staels et al., 1989). A 0.3-kb BamHI restriction fragment of the human LDL receptor clone pLDLR-3, corresponding to nucleotide position 1079-1450, was used for LDL-R mRNA determination (Staels et al., 1990). A chicken 3 actin probe was used as a control (Cleveland et al., 1980), to normalize for events of differences in mRNA blotting. Filters were hybridized to 1.5 x $10^6$ cpm/ml of each probe as previously described (Staels et al., 1989). They were washed in 500 ml 0.5 x SSC (20 x SSC = 3.0 M NaCl, 0.3 M $Na_3$citrate) and 0.1 % SDS for 10 min at room temperature and twice for 30 min at 65°C and subsequently exposed to X-ray film (Hyperfilm-ßmax, Amersham, Arlington Heights, IL). Quantitative analysis was performed on autoradiograms of dot blot filters by scanning densitometry in the linear range of film sensitivity. Curves were plotted on a log/log scale relating the densitometric measurement to the amounts of mRNA in the corresponding dots. The relative amounts of mRNA were calculated in parallel parts of these curves. Part of this mRNA work was supported by a grant from Merck Sharp and Dohme to J. Auwerx.

HMG-CoA reductase activity

The activity of HMG-CoA reductase was determined in liver microsomes after dephosphorylation, as described by Balasubramanian et al. (1976). HMG-CoA reductase activity is expressed as pmol of [$^{14}$C] mevalonate formed per mg microsomal protein/min.

**Statistical Methods**

Data are given as mean ± standard deviation. For comparison between groups the Student's t-test and ANOVA were used.

## RESULTS

### Effects of hypothyroidism and GH substitution on IGF-I levels and body weight gain.

In hypothyroid animals plasma IGF-I concentrations were decreased compared to controls, 1.99 ± 0.24 U/ml vs. 3.36 ± 0.52 U/ml ($n=6$, $p<0.05$) respectively. After treatment of hypothyroid rats with GH for 10 days the concentration of IGF-I became comparable to values in control rats: 3.74 ± 0.48 U/ml vs 3.36 ± 0.52 U/ml ($n=6$).

Body weights of the hypothyroid rats did not change during the last 10 days of the experiment (data not shown). However, hypothyroid rats substituted with GH gained 9 ± 3 g during the 10 days of GH-substitution ($p<0.05$ vs untreated hypothyroid rats), indicating that treatment with human GH induced a physiological growth response in rats.

### Effects of hypothyroidism and GH substitution on liver LDL-R expression and LDL-R mRNA.

LDL-R was quantified by a ligand ($^{125}$I-LDL) dotblot assay. The binding of $^{125}$I-LDL to the blots exhibited the characteristics of binding of LDL to the LDL receptor. EDTA prevented, unlabeled LDL competed, and heparin completely reversed $^{125}$I-LDL binding to its receptor (Table 5.1).

The effects of hypothyroidism on LDL-R was determined by comparing membrane preparations of rats treated or not with methimazole. In hypothyroid rats the expression of the LDL-R decreased significantly to 67 ± 6 % of intact controls (Table 5.2). During GH treatment of hypothyroid rats the LDL-R expression was normalized. LDL-R mRNA fell in hypothyroid rats to 31 ± 6 % of the controls, while treatment with GH normalized LDL-R mRNA levels to those observed in intact controls (Figure 5. 1).

### Effects of hypothyroidism and GH substitution on hepatic microsomal HMG-CoA reductase activity.

In hypothyroid rats the microsomal HMG-CoA reductase activity was greatly diminished to 36%, as compared to euthyroid rats (Table 5.2). Treatment with GH for 10 days increased the HMG-CoA reductase activity significantly.

**Table 5.1:** **Effect of heparin, EDTA and excess unlabelled LDL on binding of $^{125}$I-LDL. LDL-R were incubated with labeled $^{125}$I-LDL for 1 hr in the presence of heparin (4mg/ml), EDTA (30mM) or excess LDL (20 fold). Data are means of triplicate determinations.**

| Experiment | Heparin | EDTA | excess LDL |
|---|---|---|---|
| 1 | < 10 % | 21 % | 25 % |
| 2 | < 10 % | 25 % | 26 % |

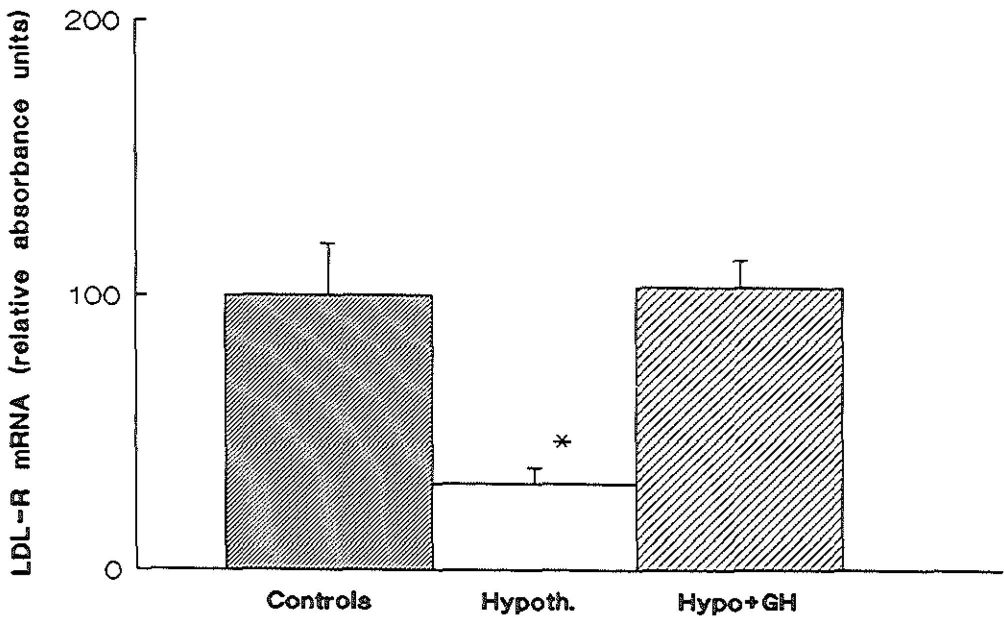


**Figure 5.1:** Influence of GH on liver LDL receptor mRNA in hypothyroid rats. Liver mRNA levels for LDL receptor were measured by dot blot hybridization. Significant difference from control values and GH-treated hypothyroid animals is indicated by an asterisk ($p<0.05$).

**Table 5.2: Effect of hypothyroidism with and without growth hormone (GH) substitution on low density lipoprotein receptor (LDL-R) expression and HMG-CoA reductase activity in liver microsomes. Data are presented as the mean ± SD for the number of rats in parentheses. Significant differences from control values and from the values of the GH treated animals, are indicated by an asterisk (p<0.05).**

| | n | LDL-R (relative absorbance units) | HMG-CoA reductase (pmol/mg protein/min) |
|---|---|---|---|
| **Controls** | 6 | 100 ± 23 | 126 ± 78 |
| **Hypothyroids** | 6 | 67 ± 6* | 45 ± 23* |
| **Hypothyroids+GH** | 6 | 109 ± 20 | 76 ± 29 |

## DISCUSSION

The depression of the rate of LDL-catabolism during hypothyroidism has been ascribed to the deficit of thyroid hormone (Valdemarsson, 1982; Gross et al., 1987; Staels et al., 1990). However, in this study we showed, that in the absence of thyroid hormone, physiological levels of GH stimulate hepatic LDL-R activity and LDL-R mRNA levels to normal values. This indicates that in the hypothyroid situation the deficit of GH influences the disturbances in lipid metabolism. The observed stimulation of LDL-R activity by GH (and/or IGF-I), can either be direct or secondary to another effect on cholesterol homeostasis. In favor of a direct effect of GH on LDL-R, Veldhuis et al. showed, that IGF-I has a direct stimulating effect on the number of LDL receptors in swine granulosa cells (Veldhuis et al., 1987). However, the number of LDL receptors is inversely related to intracellular cholesterol concentration (Brown and Goldstein, 1986). To exclude an indirect stimulatory effect of GH on the LDL-R via a reduction of the intracellular cholesterol concentration, because of a decreased HMG-CoA reductase

activity, the microsomal activity of this enzyme was determined. In this study an increased HMG-CoA reductase activity was observed after treatment of hypothyroid rats with GH, thereby excluding a secondary effect of GH on LDL-R via a lowered cholesterol synthesis.
The stimulation of both LDL-R and HMG-CoA reductase activity however, may be secondary to an increased use of cholesterol by the cells. For instance, in rapidly growing tissues, LDL-R are required to take up sufficient cholesterol to support new membrane synthesis (Goldstein et al., 1983). In our experiments the body weight of the pubertal rats increased considerably during substitution with GH indicating that augmented use of cholesterol is likely. Alternatively, an augmented use of cholesterol can result from a stimulated bile acid synthesis. Stimulation of bile acid synthesis by GH has been shown in rats (Day et al., 1989) and in GH deficient children substituted with GH (Heube et al., 1983). This leaves open the possibility of a secondary response of LDL-R to GH as a result of an augmented use.
The physiological importance of the influence of GH on cholesterol metabolism is not yet clear. Recently a protective role of GH with regard to cardiovascular mortality was reported (Rosen and Bengtsson, 1990). In a retrospective study in patients with pituitary insufficiency substituted for all hormonal deficiencies but GH an increased cardiovascular mortality was shown as compared to controls, suggesting that, in addition to the well known effects of a lack of thyroid hormone (Becker, 1985) and estrogens (Gordon et al., 1978), a deficiency of GH may also increase the incidence of premature atherosclerosis.
In summary, a stimulating effect of GH on hepatic cholesterol metabolism in hypothyroid rats is shown in this study. The decrease in LDL-cholesterol during GH treatment (Hoogerbrugge-v.d.Linden et al, 1990; Chapter 3), may be caused by an activation of the LDL receptor by GH and/or IGF-I.

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## Chapter 6.

# INSULIN-LIKE GROWTH FACTOR-I STIMULATES THE LDL-RECEPTOR ACTIVITY IN HEPG2 CELLS.

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## ABSTRACT

In man as well as in the rat hypothyroidism is accompanied by an increased LDL-cholesterol level, probably due to a decrease in LDL-receptor activity. Besides thyroid hormone also growth hormone (GH) is deficient in this state. The possible role of $T_3$, $T_4$, GH and insulin-like growth factor I (IGF-I) in the receptor mediated catabolism of LDL was studied in HepG2 cells.
HepG2 cells were incubated with fetal calf serum from hypo- and euthyroid animals. Both binding and internalization of $^{125}$I-LDL by HepG2 cells were approximately 50% ($p<0.05$) lower after incubation with hypothyroid FCS, as compared to using euthyroid FCS.
In serum free conditions "physiologic" concentrations $T_3$, $T_4$ and GH, were without effect on either binding or internalization of $^{125}$I-LDL by HepG2 cells. Insulin-like growth factor I (IGF-I) stimulated both binding and internalization of $^{125}$I-LDL by HepG2 cells cultured in serum free condition by 50% ($p<0.01$).
We conclude that IGF-I may play a regulatory role in cellular cholesterol metabolism by direct effect on LDL uptake by the liver.

## INTRODUCTION

Elevation of serum cholesterol concentration is characteristically seen in untreated hypothyroidism in man (Peters and Man, 1950; Mishkel and Crowther, 1977; Abrams and Grundy, 1981; Lithell et al., 1981; Muls et al., 1984). The catabolic rate of low-density lipoprotein (LDL), the major transport form of serum cholesterol, is slowed in hypothyroidism (Walton et al., 1965). This is mainly the result of a decreased expression of LDL receptors on the liver membranes (Thompson et al., 1981; Scarabottolo et al., 1986).
Generally, the effects of hypothyroidism on LDL-cholesterolholesterol catabolism are considered to be due to lowered thyroid hormone levels. However, during hypothyroidism both thyroid hormone and growth hormone (GH) are deficient (Iwatsubes et al., 1967; Katakami et al., 1986; Chernausek and Turner, 1989). In hypothyroid rats we showed that treatment with GH or

thyroxine ($T_4$) both enhance LDL metabolism (Hoogerbrugge-v.d.Linden et al., 1990; Chapter 3). In patients with untreated and treated hypothyroidism, we found that LDL-cholesterol was related to IGF-I levels rather than to those of free $T_4$ (Hoogerbrugge-v.d.Linden et al., 1989; Chapter 2).
We report here the effects of triiodothyronine ($T_3$), $T_4$, GH and IGF-I on LDL binding to its receptor and on LDL internalization in a human hepatoma cell line HepG2.

## MATERIALS AND METHODS

### Preparation of LDL.

Human plasma was prepared from pooled fresh blood obtained from healthy donors. LDL was isolated from plasma by sequential ultracentrifugation using the method of Zechner et al. (1986). Briefly, the lipoproteins of d 1.020-1.063 g/ml were prepared by ultracentrifugation in a fixed angle rotor, washed and concentrated by density gradient ultracentrifugation. This LDL preparation was immediately used for iodination. Radioiodination was performed according to the method of McFarlane (1956), as modified by Billheimer et al. (1972). After iodination LDL was dialyzed for 24 hr against Williams E supplemented with 20 mM hepes buffer (pH 7.4), penicillin and streptomycin, stabilized by the addition of 1% human serum albumin (HSA) and stored at 4° C. For binding experiments, $^{125}$I-LDL was diluted with unlabeled LDL to give a specific radioactivity of 35 - 40 cpm/ng LDL protein. The iodinated LDL was used within 2 weeks. The free iodine fraction of the $^{125}$I-labelled LDL samples was less than 1 %.
When not labelled with $^{125}$Iodine, LDL was immediately stabilized by the addition of 1% HSA and extensively dialyzed against Williams E supplemented with hepes (pH 7.4), penicillin and streptomycin.

### Culturing of HepG2 cells

The HepG2 cell line was maintained in Williams E (Flows laboratories, Great Britain), supplemented with 10 % heat inactivated fetal calf serum (FCS), penicillin (100 U/ml) and streptomycin (1 µg/ml) at 37 °C in a humidified 95 % air - 5% $CO_2$ atmosphere. The medium was renewed twice a week. For binding experiments the cells were seeded in 6-well plates (Costar,

Cambridge, MA). All experiments were initiated 6 days later after cells had been incubated for 48 hr in the presence of medium containing either 10% FCS, 10% hypothyroid FCS or 1% HSA. Hormones were added to the HSA containing media for the last 48 hr of incubation as indicated.

**LDL Binding experiments.**

The cells were washed shortly before the experiment, to remove serum, and incubated three times with Williams E supplemented with 1% HSA, during a period of 30 min at 37 °C. The experiment was started by replacing medium by 1 ml Williams E containing 20 mM Hepes pH 7.4, 1 % HSA and various amounts of $^{125}$I-labelled LDL. Non specific binding was determined by the addition of a 20 fold excess of cold LDL. Dishes without cells were used as controls. After 3 hr of incubation at 37 °C the medium was removed, each cell plate was cooled to 0°C and washed rapidly 3 times with 3 ml of ice cold solution containing 0.15 M NaCl, 50 mM Tris-chloride (pH 7.4) and 2 mg/ml of bovine albumin. The monolayers were then incubated twice for 10 min at 4°C with 3 ml of the above NaCl-Tris-albumin solution, followed by one rapid wash with 3 ml of solution containing 0.15 M NaCl and 50 mM Tris-Chloride (pH 7.4). After washing, the cells were incubated with heparin (10 mg/ml) containing NaCl-Tris buffer for 1 hr at 0 °C (Goldstein et al., 1976). The radioactivity released into the heparin buffer reflects the amount of $^{125}$I-LDL bound to the plasma membrane. Cells were dissolved in 1 M NaOH. The radioactivity in the cell lysate represents the total amount of $^{125}$I-labelled LDL that became internalized. A portion of the cell lysate was used for protein determination by the method of Lowry with BSA as standard. All experiments were done in duplicate.

**Materials.**

3,3',5-Triiodo-L-thyronine (sodium salt) was a product from Sigma Chemical Company (London, Great Britain). hGH (Norditropin) was a generous gift from Novo-Nordisk. Recombinant hIGF-I analog was obtained from Amersham (Buckinghamshire, Great Britain).

**Statistical methods**

All incubations were performed in triplicate and results are reported as mean

± SD. Differences were considered to be statistically significant when $p<0.05$ using Student's t-test.

## RESULTS

### Effects of hypothyroid FCS on LDL receptor expression of HepG2 cells.

In Figure 6.1, binding and internalization of $^{125}$I-labelled LDL by cultures of HepG2 cells incubated for 48 hr with medium supplemented with 10 % FCS or 10 % hypothyroid FCS are shown. The expression of the LDL-receptor, measured by binding and internalization of $^{125}$I-LDL is approximately 50 % ($p < 0.01$) lower after incubation with hypothyroid FCS as compared to FCS of euthyroid animals.

### Effect of $T_3$, $T_4$, GH and IGF-I on LDL receptor expression by HepG2 cells.

HepG2 cells were incubated in 1 % HSA with either $T_3$ (0.1, 1, 10 and 100 nM), T4 (0.1, 1, 10 and 100 nM), GH (0.1, 1, 10 and 100 µg/ml) or IGF-I (0.1, 1, 5 and 10 nM). The binding and internalization of $^{125}$I-LDL (10 and 50 µg/ml) were not affected by incubation with different doses of $T_3$, $T_4$ or GH (Data not shown). On the other hand IGF-I in a concentration of 10 nM increased both binding and internalization of $^{125}$I-LDL (10 and 50 µg/ml) by approximately 50 %, as is shown in Table 6.1 and Figure 6.2. Lower levels of IGF-I (5 nM or less) did not affect the expression of the LDL-receptor. Table 6.1 shows the data of binding and internalization of $^{125}$LDL, for the maximal hormone concentration used; that is 100 nM $T_3$, 100 µg/ml (=0.29 U/ml) GH and 10 nM IGF-I.

## DISCUSSION

We have presented evidence that the LDL-receptor of HepG2 cells is sensitive to stimulation by IGF-I at high concentrations. However the more "physiological" concentration, 1 nM did not stimulate LDL-receptor expression. For some years it is known that HepG2 cells have receptors for

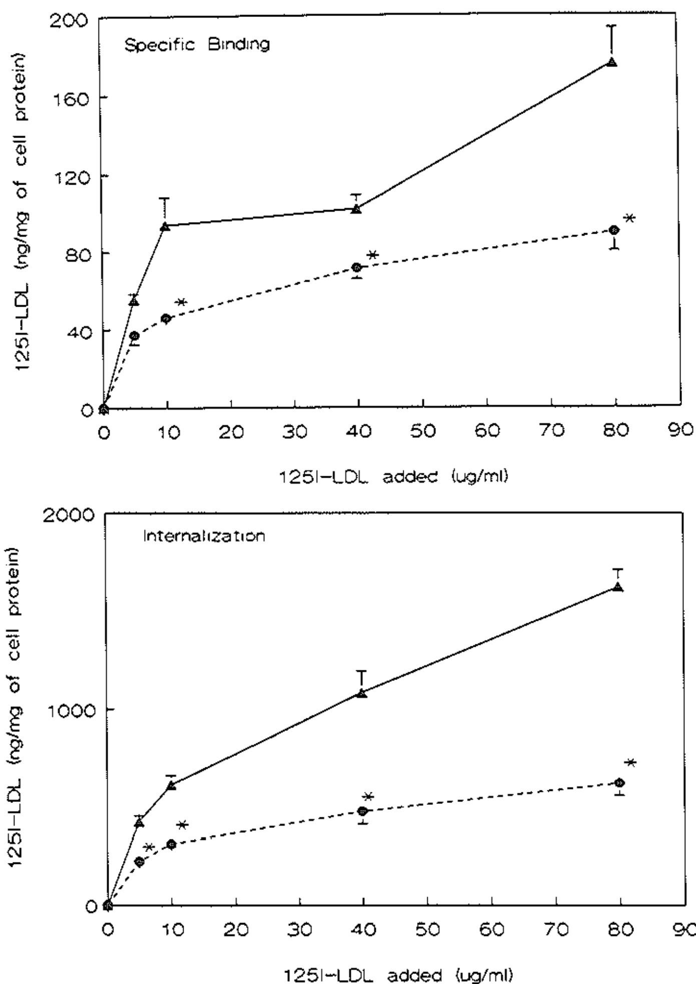


**Figure 6.1:** Effects of incubations of HepG2 cells for 48 hr with 10 % fetal calf serum (FCS) (triangles, drawn line) or 10 % hypothyroid FCS (dots, broken line) on the receptor-mediated binding and uptake of 5 - 80 μg/ml $^{125}$I-LDL. The inhibition by hypothyroid FCS was statistically significant for the LDL concentrations indicated by an asterisk ($p<0.05$). Symbols represent means ± SD of three wells for each condition.

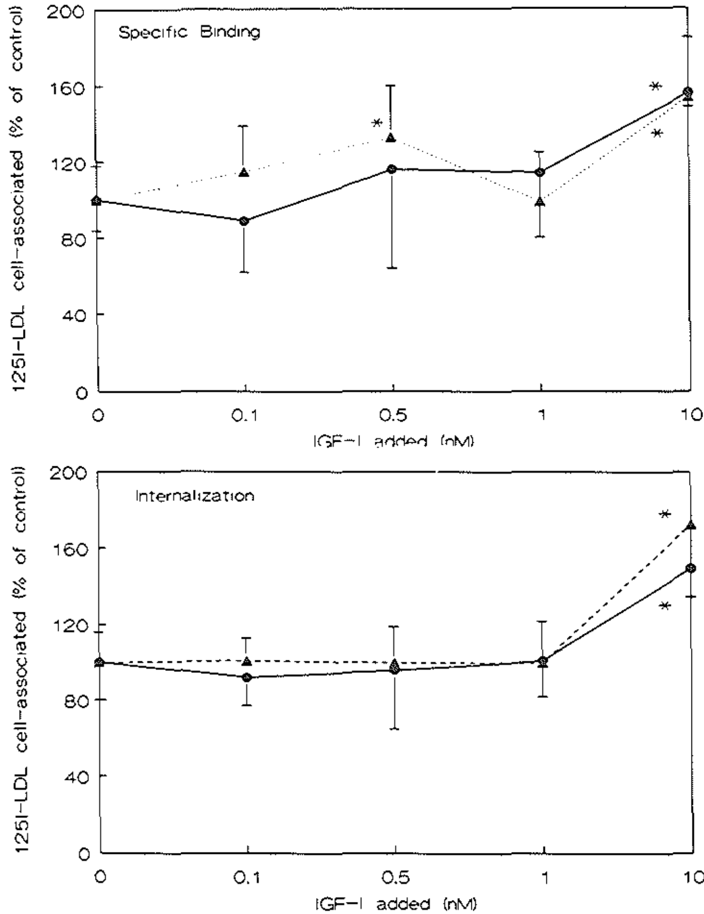


**Figure 6.2:** Effects of incubations of HepG2 cells for 48 hr in serum free medium supplemented with 1% HSA and insulin like growth factor-I (IGF-I) on the receptor mediated binding and uptake of 10 (triangles, dottewd line) and 50 µg/ml (dots, drawn line) $^{125}$I-LDL. The stimulation by IGF-I was statistically significant for the IGF-I concentrations indicated by an asterisk ($p<0.05$). Symbols represent means ± SD of two separate experiments.

**Table 6.1: Effects of incubation of HepG2 cells triiodothyronine ($T_3$) or growth Hormone (GH) on the receptor-mediated binding and uptake of 50 µg/ml $^{125}$I-LDL.**

| Addition | Relative binding to LDL-receptor % | Relative internalization by LDL-receptor % |
|---|---|---|
| **none** | 100 | 100 |
| **$T_3$** (100 nM) | 105 ± 15 | 90 ± 4 |
| **GH** (100 µg/ml) | 110 ± 13 | 105 ± 23 |
| **IGF-I** (10 nM) | 156 ± 7 * | 150 ± 15 * |

Cells were cultured for 48 hr in the presence of 1% HSA with the indicated concentrations of hormones. The specific binding and internalization was measured at 37 °C for 3 hr and expressed per mg cell protein. Results are mean ± SD for 2 independent experiments and are expressed relative to the binding or internalization in the absence of added hormones. The hormonal treatment did not affect the recovery of cell protein.
* Significantly increased as compared to cells without hormonal addition $p<0.01$.

IGF-I (Verspohl et al., 1988). Veldhuis (Veldhuis et al., 1987) showed that IGF-I enhanced the LDL-receptor activity in swine granulosa cells. So far it was not known whether IGF-I also affects LDL metabolism in parenchymal liver cells, the most important cell in LDL metabolism. A stimulatory effect of IGF-I on the LDL-receptor of liver like cells, as shown in this study, supports the hypothesis that GH-activity, has a function in the physiologic regulation of LDL metabolism and that a deficiency of GH activity, either primarily or for instance during hypothyroidism, will add to the impairment of LDL catabolism (Hoogerbrugge et al, 1990; Chapter 3).
IGF-I is thought to partly mediate the growth stimulating effects of GH (Daughaday et al., 1972). It is produced in many tissues, notably the liver (Phillips and Vassilopoulou-Sellin, 1980). In liver cells GH can directly induce

IGF-I mRNA (Norstedt and Möller, 1987), while in fibroblasts it was shown that GH stimulates IGF-I production in a dose dependent manner (Clemmons et al., 1980). In our study GH, in contrast to IGF-I, did not enhance the LDL-receptor activity. It was shown that in serum free medium the HepG2 cell line does not secrete significant quantities of IGF-I (Moses et al., 1983). Therefore, the amount of GH given to the HepG2 cells during incubation probably did not induce sufficient amounts of IGF-I to activate the LDL-receptor.

$T_3$ used in a concentration up to 100 nM, and also $T_4$ in a dose up to 100 nM, were not able to stimulate LDL-receptor binding and internalization. In fibroblasts a concentration of 10 nM $T_3$ stimulated both binding and internalization (Chait et al., 1979). Salter et al. (1988) showed that a dose of 10 nM $T_3$ induced a 30 % increase in LDL-receptor binding, in rat hepatocytes. The concentration $T_3$ that was used, was based on the physiological situation. Possibly higher concentrations of thyroid hormone could show an effect on the LDL-receptor in HepG2 cells. A stimulatory effect of 5000 nM Thyroxine ($T_4$) on the testosterone-estradiol-binding globulin production of HepG2 cells has been shown (Rosner et al., 1984). As the $T_3$ and $T_4$ concentration in FCS are 0.71 and 16 nM respectively, the use of FCS for cell culturing can not be the explanation for the difference in LDL-receptor expression between cells incubated with hypothyroid or normal FCS. Hypothyroid FCS differs from FCS in more respects than just the concentration of thyroid hormones e.g. lipoprotein levels, creatine phosphokinase activity and IGF-I concentration. Which factors are responsible for the decreased LDL-receptor expression after incubation with hypothyroid FCS we do not know, but a distinct role for IGF-I in LDL metabolism has been demonstrated.

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**Chapter 7**

# GROWTH HORMONE NORMALIZES HEPATIC LIPASE mRNA LEVELS AND ACTIVITY IN HYPOTHYROID RAT LIVER.

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Submitted

## ABSTRACT

The effect of growth hormone (GH) administration on hepatic lipase (HL) activity and mRNA levels were studied in pair-fed hypothyroid rats. In the hypothyroid state liver HL mRNA levels as well as liver- and postheparin plasma-HL activities were decreased. In hypothyroid rats GH normalized HL mRNA levels and HL-activity in postheparin plasma. GH also increased the activity of HL in the liver, which remained lower than in controls. These data indicate that at least part of the lowering of HL during hypothyroidism is due to the concomitant GH deficiency and that GH, rather than thyroid hormone, may regulate HL mRNA levels.

## INTRODUCTION

Hepatic lipase (HL) exerts a regulatory influence on the concentration and characteristics of lipoproteins in plasma (1,2). The activity of HL is affected by several hormones; e.g. estrogens, corticosteroids and insulin (3,4). During hypothyroidism one of the most consistent changes in lipid variables is a decreased HL-activity (5,6). In hypothyroids not only thyroid hormone is deficient, but also the synthesis, secretion and activity of growth hormone (GH) are decreased (7,8). Recently we reported that in hypothyroid rats treatment with GH increased HL-activity (6). Therefore, we studied the effects of GH replacement on mRNA levels in liver tissue and on HL-activity in plasma of hypothyroid rats. Since hypothyroids have a decreased basal metabolism and eat less than controls, while a decreased food intake is known to lower HL-activity (4,9), the studies were carried out with pair-fed animals.

## MATERIALS AND METHODS

### Animals

Male Wistar rats aged 6-7 weeks, weighing 140 ± 6 g were used.

### Animal treatment

Rats were fed a vegetarian laboratory chow and had free access to drinking

water.
Paired feeding Food intake of all animals was restricted to the amount ingested by the animals with lowest food intake. The animals were housed together, but fed separately for 2 hours a day. Hypothyroid rats had lowest food intake. The amount eaten by a hypothyroid rat was weighted, and the same amount was given to a control and a GH treated rat.
Hypothyroidism was induced by the addition of 0.05 % 2-mercapto-1-methyl-imidazole (Janssen Chimica, 2340 Beerse Belgium) to the drinking water. When after 14 days $T_4$ was less than 1 nmol/l the administration of GH was started.
GH (Norditropin, Novo-Nordisk, Denmark) was administered s.c. for 10 days twice daily, in a daily dose of 0.2 IU/100 g dissolved in 0.1 ml 0.9 % NaCl. This dose appeared to be physiological from dose response studies by others (11), and of maximal duration (12). Hypothyroid and control animals were injected twice daily with 0.05 ml 0.9 % NaCl.

**Animal preparation**

After overnight fast animals were anaesthetized with pentobarbital. Post heparin plasma was obtained from the aorta 6 minutes after i.v. administration of 50 IU heparin (Tromboliquine, Organon Oss, The Netherlands). The plasma was kept on ice and immediately used for the determination of HL-activity. Other rats were killed by decapitation, the livers were removed. A part of this liver was kept on ice for determination of HL-activity. An other part of the liver was frozen in liquid nitrogen and stored at -80°C until mRNA analysis.

**Analytical methods**

The IGF-I concentration in plasma was determined by radioimmunoassay, using a testkit (Nichols institute diagnostics, San Juan Capistrano, USA).
Determination of HL
HL-activity was determined by a triglyceridase assay pH 8.5 in 0.6 M NaCl using a gum acacia-stabilized glycerol [9,10(n)-$^3$H]trioleyl emulsion as substrate (2). Determination of HL-activity in plasma and liver samples were each done in one assay. Both assays were carried out in duplicate. The coefficient of variation of the assay was 10 %. In plasma, HL-activity was measured as the anti-HL inhibitable salt-resistant lipase using a polyclonal goat anti- HL.
HL mRNA determination.

HL mRNA determination was done, exactly as described (9).

**Statistics**

Data are given as mean ± standard deviation. Multiway analysis of variance was used to evaluate the results.

## RESULTS

**GH activity.**

In hypothyroid rats GH activity, reflected in the circulating IGF-I concentration, was decreased as compared to controls 1.50 ± 0.12 U/ml (n=12) and 3.30 ± 0.18 U/ml (n=12, $p<0.01$), respectively. During treatment with GH of hypothyroid rats, the plasma IGF-I concentration was increased as compared to hypothyroid rats to 2.43 ± 0.24 U/ml (n=13, $p<0.05$).

In control rats pair-feeding led to a less pronounced body weight gain (BWG) over the last 7 days of the experiments as compared to ad libitum fed rats 9 ± 6 g (n=4) and 33 ± 6 g (n=4, $p<0.05$), respectively. Pair-feeding did not affect BWG of hypothyroid rats or GH treated hypothyroid rats; while body weight of hypothyroid rats did not change, GH treated hypothyroid rats gained 9 ± 3 g over the last 7 days.

**Hepatic lipase activity.**

Food restriction decreased HL-activity in liver homogenates of controls from 1385 ± 193 mU/g (n=4) to 810 ± 44 mU/g (n=4; $p<0.05$). Under pair-fed conditions the activity of HL in hypothyroid rats was decreased as compared to controls ($p<0.05$, Table 7.1), while GH replacement stimulated the HL-activity in the liver homogenates with 21 % ($p<0.05$, Table 7.1).

HL-activity in the post-heparin plasma was also decreased in the hypothyroid rats, but to a lesser degree than in the homogenates (-15%). Upon GH administration the activity of HL in post-heparin plasma returned to control values (Table 7.1).

**Hepatic lipase mRNA.**

To study whether the effects of GH and HL-activity could be in the synthesis of the enzyme, the level of mRNA for HL was determined in liver

homogenates of pair-fed animals. Livers from hypothyroid rats contained lessHL mRNA than euthyroid controls (-27%, $p<0.05$). Treatment with GH of hypothyroid rats increased HL mRNA to control values (Table 7.1).

**Table 7.1:** Effect of hypothyroidism with or without growth hormone (GH) replacement, on the level of hepatic lipase (HL) mRNA and activity in homogenized liver tissue and on HL-activity in plasma obtained 6 min after intravenous injection with heparin 50 IU. Results are means ± sd. for a group of four rats. Significant differences from control values are indicated by an asterisk ($p<0.05$). Values of mRNA were measured of individual animals by a dotblot hybridization technique, autoradiograms were scanned. Values are expressed in arbitrary units (U) taking the mean value of control groups as 100 U.

| | **HL-activity liver** (mU/g) | **mRNA liver** (U) | **HL-activity plasma** (mU/ml.) |
|---|---|---|---|
| **Control** | 810 ± 44 | 100 ± 8 | 558 ± 27 |
| **Hypothyroid** | 479 ± 59* | 73 ± 20* | 474 ± 23* |
| **Hypothyroid+GH** | 578 ± 54 | 104 ± 12 | 544 ± 14 |

## CONCLUSION

The present study confirms earlier studies showing that during hypothyroidism HL mRNA levels and activity in the liver are decreased. During hypothyroidism GH activity, measured by body weight gain and plasma IGF-I concentration are decreased. GH administration restores IGF-I levels but not thyroid hormone concentations. In this situation, hypothyroidism with increased IGF-I concentrations, HL mRNA was found to be normalized. This suggests that the well-known lowering of HL during hypothyroidism is at least partly due to the accompanying GH deficiency rather than to the low thyroidhormone concentration it self. Earlier we showed that in hypophysectomized (GH deficient) rats, thyroid hormone did not affect HL-activity. However, after GH substitution thyroid hormone

induced an increase in HL-activity (6). Secretion of HL by rat liver cells in vitro, in the absence of GH, was found not to be affected by $T_3$ (12). We conclude that GH, presumably via IGF-I, regulates HL mRNA levels. This effect could be exerted on transcription or on the stability of the mRNA. Thyroid hormone alone does not regulate HL expression at the mRNA level, but may act (post)transcriptionally or and, presumably, only in the presence of GH (IGF-I).

An apparent discrepancy was found between postheparin plasma HL-activity, which was normalized by GH, and HL-activity in the liver, which increased but remained lowered during GH administration. Under the present conditions the plasma HL-activity may not represent the hepatic HL content as we found that during hypothyroidism HL-activity after heparin injection disappears less rapidly from the plasma, suggesting a delayed clearance rate, leading to erroneous plasma values. The HL-activity in the liver during T3 and GH substitution seems to reflect the physiologically activity, as we found in a previous study that under these conditions, plasma HDL-cholesterol concentrations varied oppositely to the HL-activity.

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## Chapter 8

# GROWTH HORMONE DECREASES PLASMA CHOLESTEROL INDEPENDENT FROM THYROID HORMONE ACTIVITY.

N. Hoogerbrugge, A. Binnerts, H. Jansen, J.C. Birkenhäger and S.W.J. Lamberts.

## ABSTRACT

Growth hormone (GH) replacement leads to a decrease in serum cholesterol concentration and an increase in serum $T_3$ in GH-deficient adults. While both GH and thyroid hormone can decrease serum cholesterol concentration in man and rat, there is no consensus whether the cholesterol decreasing effect of GH is mediated by an increase in $T_3$. We examined the effect of GH replacement therapy on plasma cholesterol levels and thyroid hormone concentrations in 8 GH-deficient human adults and in GH-deficient rats, as well as control rats.
In humans GH treatment caused a decrease in total serum cholesterol from $5.3 \pm 0.9$ mM at baseline, to $4.6 \pm 0.6$ mM after one month of substitution ($p<0.05$). During this period serum $T_3$ increased from $1.44 \pm 0.36$ to $1.95 \pm 0.42$ nM ($p<0.05$) together with a decrease in serum $T_4$ concentrations.
In hypophysectomized rats (n=16) treated with GH plasma cholesterol was decreased when compared to their controls ($3.0 \pm 0.0$ mM and $4.0 \pm 0.4$ mM ($p<0.05$), respectively). In rats made hypothyroid by methimazole treatment similar changes were observed. $T_3$, nor $T_4$ concentrations changed after replacement treatment with GH, neither in hypophysectomized (n=8), nor in hypothyroid rats (n=8).
It is still uncertain whether the cholesterol decreasing effect of GH in humans is mediated by an increase in circulating $T_3$, by of a stimulated peripheral deiodination of $T_4$. Our data in rats are consistent with a GH-induced decrease in plasma cholesterol independent from circulating thyroid hormone concentration.

## INTRODUCTION

Growth hormone (GH) administered in high doses to hypercholesterolemic adults acutely lowers the plasma cholesterol concentration (Friedman et al., 1974). This cholesterol decreasing effect of GH is confirmed in several other studies. For instance, in patients with acromegaly a lowered plasma cholesterol concentration is found (Nikkilä and Pelkonen, 1975), and in adults with GH-deficiency increased cholesterol levels have been described (Merimee et al., 1972; Merimee, 1980). Recently in a long-term (6 month) placebo controlled

GH-substitution study of GH-deficient adults it was shown that GH lowers cholesterol levels (Salomon et al., 1989). Also in GH-deficient (hypophysectomized) rats the administration of GH lowers plasma cholesterol (Hoogerbrugge-v.d.Linden et al., 1990; Chapter 3). Although a cholesterol decreasing effect of GH is generally accepted, the mechanism of its effect(s) on cholesterol metabolism has not been extensively studied.
Thyroid hormone has a well known lowering effect on the plasma cholesterol concentration in humans and animals (Abrams and Grundy, 1981; Dory and Roheim, 1981; Muls et al., 1984). Lately it was shown that GH treatment of GH-deficient adults stimulates the peripheral conversion of $T_4$ to $T_3$ (Grunfeld et al., 1988; Jorgensen et al., 1989). In the present study the effect of GH substitution on plasma lipid and thyroid hormone concentrations in GH-deficient patients are reported. In addition to this, data are provided on the effects of GH substitution on these parameters in GH-deficient (hypophysectomized or hypothyroid) and control rats.

## PATIENTS, ANIMALS AND METHODS

### Patients

Eight adults (3 men and 5 women, 25 to 46 years of age) with GH deficiency for at least 10 years (congenital or after surgery for craniopharyngioma) were studied. If present, other hormonal insufficiencies had been adequately substituted for at least one year before the study. The presence of GH deficiency was confirmed by the combination of a plasma IGF-I level of 0.3 U/ml or less (normal 0.34-1.90 U/ml in men and 0.45-2.20 U/ml in women), and a peak GH level of less than 10 mU/l after 1µg/kg GHRH i.v. None of the patients suffered from renal or liver disease, diabetes mellitus or had a history of malignancy. Written informed consent was obtained from each patient before the start of the study, which was approved by the Medical Ethics Committee of the University Hospital Dijkzigt.

### Patient treatment procedures

All individuals were studied for a period of 9 months, during which other hormonal substitution was not changed. The patients were asked not to alter their diet and level of physical activity. They received GH during the first 6

months only. GH (Humatrope; Eli Lilly and Co, Indianapolis, USA) was injected s.c. by the patients themselves at bedtime, in a dose of 25 μg/kg/day, with a maximum of 1.48 mg (4 IU). At the start of the study and at 1,3 and 6 months after the start as well as 3 months after the end of GH administration blood samples were taken after an overnight fast. Lipids were determined as well as lipoprotein lipase (LPL) and hepatic lipase (HL) activity in plasma obtained 20 min after the injection of 50 IU heparin/kg BW.

**Animals**

Male pubertal Wistar rats, 6-7 weeks of age and weighing 185 ± 10 (SD) g, were used. They were housed at 21 °C on a 12 h cycle of light/darkness (lights on at 07.30 h). Vegetarian chow and drinking water were available ad libitum.

**Animal treatment procedures**

Hypothyroidism

Hypothyroidism was induced by the addition of 0.05 % (w/v) 2-mercapto-1-methyl-imidazole (Janssen Chimica, 2340 Beerse, Belgium) to the drinking water and given during the entire study.

Hypophysectomy

Hypophysectomy was carried out by the parapharyngeal approach (Thongren, Hansson, Menander-Selman and Stenström, 1973). Rats were given a precautionary s.c. injection of 50.000 IU Penicillin after hypophysectomy. Hypophysectomy was judged to have been complete when, 2 weeks after the operation, rats showed <2 g weight gain, a serum $T_4$ concentration of less than 1 nmol/l and a decrease in testicular volume. A corticosterone pellet (C 2505, Sigma, St. Louis, USA) weighing 50 mg was implanted s.c., 2 days before administration of hormones.

Hormone administration

Hormone administration was started 14 days after the initiation of the medication (hypothyroid rats) or operation (hypophysectomized rats). Hormones were given for 10 consecutive days by s.c. injection twice daily. L-Thyroxine (Sigma) was dissolved in NaOH (0.1 mol/l) and diluted in 0.9 % (w/v) NaCL. A dose of 20 μg/kg/day was given in 0.2 ml. Recombinant human GH (hGH, Norditropin, Novo-Nordisk, Denmark) in a dose of 2 IU/kg/day (0.68 mg/kg/day)was administered in 0.1 ml/day. After 10 days of

substitution, animals were killed by decapitation after 18 h of fasting. Trunk blood was collected.

### Analytical methods

Total serum cholesterol and triglycerides were determined directly. Human HDL cholesterol was measured, after precipitation of VLDL and LDL with dextran sulfate-magnesium chloride, by enzymatic method (Boehringer testkit combination, Boehringer, Mannheim, Germany). Human LDL concentrations were calculated according to the formula of Friedewald. The hepatic lipase activity in rat liver was determined as described by Jansen et al (1980). The activity was expressed as mU/mg liver : 1 mU represents the release of 1 nmol fatty acid from the substrate/min. Concentrations of $T_4$ and $T_3$ were determined by radioimmunoassay (Visser et al., 1975). The IGF-I concentration in plasma was determined by radioimmunoassay, using a test kit (Nichols institute diagnostics, San Juan Capistrano, USA).

### Statistical Methods

All data are expressed as mean ± S.D. The statistical significance of the differences between groups was determined using analysis of variance (ANOVA). When significant overall effects were obtained, multiple comparisons were made with the Newman-Keuls test.

## RESULTS

### Effects of GH substitution in humans

After one month of GH substitution cholesterol had decreased by an average of 12 % ($p<0.05$, Figure 8.1), due to a decrease in LDL cholesterol ($p<0.05$), while HDL cholesterol was not affected (Table 8.1). The decrease in LDL cholesterol was significant after one month of replacement therapy, but the LDL concentration approached basal values after the treatment period of 6 month. Three month after discontinuation of the treatment LDL cholesterol had returned to pretreatment values. LPL activity decreased during GH substitution and normalized after withdrawal of treatment, although these changes did not reach statistical significance (Table 8.1). HL was not affected by GH treatment. GH induced a clear increase in circulating IGF-I and thyroid

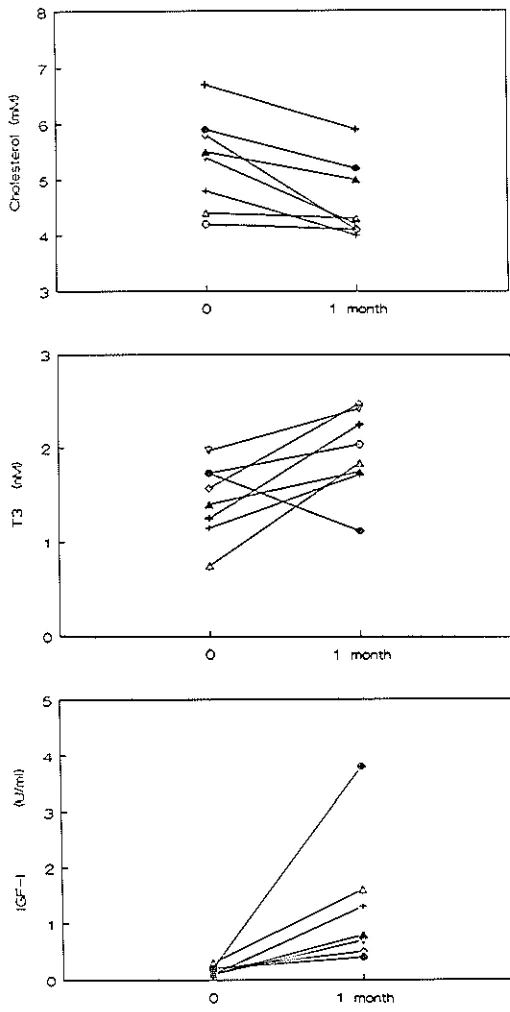


**Figure 8.1:** Effects of substitution with GH on individual values of plasma cholesterol, IGF-I and $T_3$ concentration, in 8 GH-deficient adults before and after 1 month of treatment (each symbol characterizes one patient).

**Table 8.1:** **Effects of treatment with GH of 8 GH-deficient adults on lipid values, thyroid hormones and IGF-I according to time. Values are means ± SD. Significant differences from baseline are indicated by an asterisk ($p<0.05$ by ANOVA).**

| | baseline | GH for 1 month | GH for 3 months | GH for 6 months | GH 3 months stopped |
|---|---|---|---|---|---|
| Chol(mM) | 5.3 ± 0.9 | 4.6 ± 0.6* | 4.9 ± 0.5* | 4.8 ± 0.7* | 4.9 ± 0.7* |
| LDL (mM) | 3.25 ± 1.06 | 2.69 ± 0.80* | 2.91 ± 0.54 | 3.05 ± 0.56 | 3.20 ± 1.00 |
| HDL (mM) | 0.96 ± 0.23 | 0.92 ± 0.17 | 1.08 ± 0.29 | 0.94 ± 0.21 | 0.96 ± 0.19 |
| TG (mM) | 2.3 ± 0.7 | 2.3 ± 1.0 | 1.9 ± 1.0 | 1.8 ± 0.9 | 1.6 ± 1.2 |
| LPL(mU/ml) | 96 ± 33 | 79 ± 22 | 78 ± 22 | 74 ± 20 | 94 ± 35 |
| HL (mU/ml) | 383 ± 91 | 400 ± 83 | 393 ± 86 | 372 ± 81 | 383 ± 108 |
| $T_3$ (nM) | 1.44 ± 0.36 | 1.95 ± 0.42* | 1.78 ± 0.36* | 1.66 ± 0.48 | 1.38 ± 0.41 |
| $T_4$ (nM) | 92 ± 16 | 74 ± 15* | 72 ± 28* | 81 ± 29 | 99 ± 25 |
| IGF-I(U/ml) | 0.2 ± 0.1 | 1.6 ± 1.3* | 1.6 ± 1.0* | 1.2 ± 0.7* | 0.2 ± 0.1 |

hormone in all but one patient. During the entire GH substitution period increased concentrations of $T_3$ were found ($p<0.05$), after cessation of treatment $T_3$ decreased to pretreatment levels. Simultaneously with the increase in $T_3$ a fall in $T_4$ concentration was observed.

### Effects of GH treatment in rats

Plasma cholesterol concentrations in hypophysectomized and hypothyroid rats, were increased as compared to euthyroid controls ($p<0.01$), 4.01 ± 0.37 mM, 3.71 ± 0.48 mM and 2.17 ± 0.21 mM respectively (Figure 8.2). Treatment with GH lowered cholesterol in the GH-deficient hypophysectomized and hypothyroid rats, but did not affect the plasma cholesterol concentration of euthyroid controls. Normalization of plasma cholesterol concentration was obtained by substitution treatment with a combination of GH and $T_4$. GH raised IGF-I concentration in hypophysectomized rats from 0.76 ± 0.14 U/ml to 1.62 ± 0.31 U/ml ($p<0.01$). In hypothyroid rats IGF-I concentration normalized during treatment with GH as well as with $T_4$ ($p<0.03$, Figure 8.3).

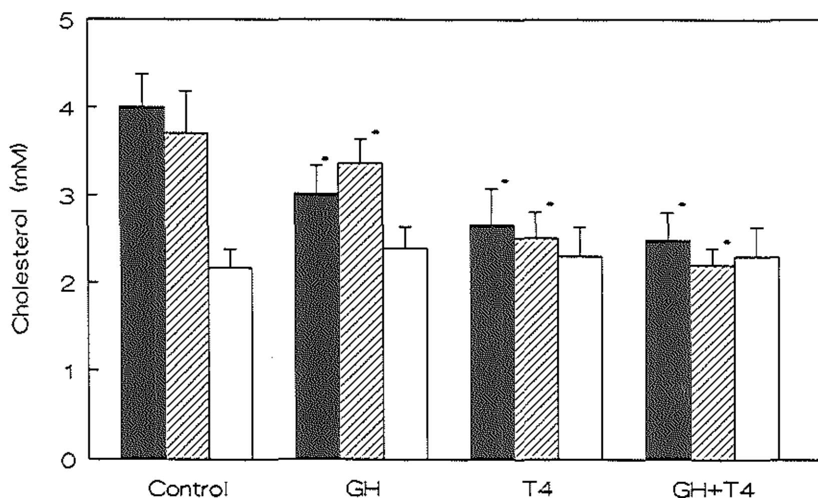


**Figure 8.2:** Effect of treatment with $T_4$, GH, or a combination of the two hormones on plasma cholesterol concentration in hypophysectomized, hypothyroid or euthyroid rats. Values are means ± SD for a group of 16 (hypophysectomized and hypothyroid rats, black and hatched bars, respectively) or 8 (euthyroid, open bars) rats. Data significantly different from control are marked with an asterisk ($p<0.05$).

The effects of treatment with GH, $T_4$ or a combination of these two hormones on thyroid hormone status are given in Table 8.2. GH treatment of hypothyroid or euthyroid rats did not affect the $T_3$ nor $T_4$ concentrations. The addition of GH to treatment with $T_4$ did not change $T_3$ nor $T_4$ concentrations as compared to treatment with $T_4$ alone in either of the three studied groups.

## DISCUSSION

Replacement therapy with GH caused a decrease in total serum cholesterol in GH-deficient humans as well as in rats. In GH-deficient humans the administration of GH lowered the cholesterol concentration only when

**Table 8.2:** Mean (± SD) values of serum thyroid parameters of 8 rats per group at the end of 10 days of treatment with Thyroxine ($T_4$), Growth hormone (GH) or a combination of the two hormones.

$T_4$ concentration (nM):

| | Hypophysectomized | Hypothyroid | Euthyroid |
|---|---|---|---|
| Control | <1 | 2 ± 4 | 56 ± 5 |
| GH | <1 | <1 | 49 ± 14 |
| $T_4$ | 112 ± 22 | 118 ± 29 | 123 ± 14 |
| $T_4$ + GH | 116 ± 40 | 120 ± 15 | 133 ± 13 |

$T_3$ concentration (nM):

| | Hypophysectomized | Hypothyroid | Euthyroid |
|---|---|---|---|
| Control | nd[0] | 0.46 ± 0.28 | 1.49 ± 0.11 |
| GH | 0.23 ± 0.03 | 0.23 ± 0.08 | 1.40 ± 0.12 |
| $T_4$ | 3.52 ± 1.03[#] | 2.63 ± 0.34 | 2.21 ± 0.46 |
| $T_4$ + GH | 2.60 ± 0.40 | 2.80 ± 0.36 | 2.34 ± 0.41 |

[0] nd means not determined. [#] determined in EDTA plasma instead of serum.

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baseline cholesterol was above 4.5 mM. This is in accordance with the changes in circulating cholesterol levels during GH administration, as observed by other investigators (Blacket et al., 1982; Asayama et al., 1984; Salomon et al., 1989). It supports the hypothesis that GH-activity influences cholesterol metabolism in man (Hoogerbrugge-vdLinden et al., 1989; Chapter 2). The mechanism of the cholesterol lowering effect of GH is still unsolved: it appears to consist of a decrease in LDL cholesterol, but it is not known whether this is the result of a direct effect of the activity of GH (or IGF-I) or whether this effect is mediated via an increase in thyroid hormone activity. An increase in $T_3$ and a decrease in $T_4$ levels were observed in patients treated with GH, suggesting activation of the peripheral de-iodination of $T_4$. Thyroid hormone is generally accepted to stimulate LDL catabolism and might contribute to or even totally mediate the observed changes in LDL-cholesterol concentrations during GH treatment in man.

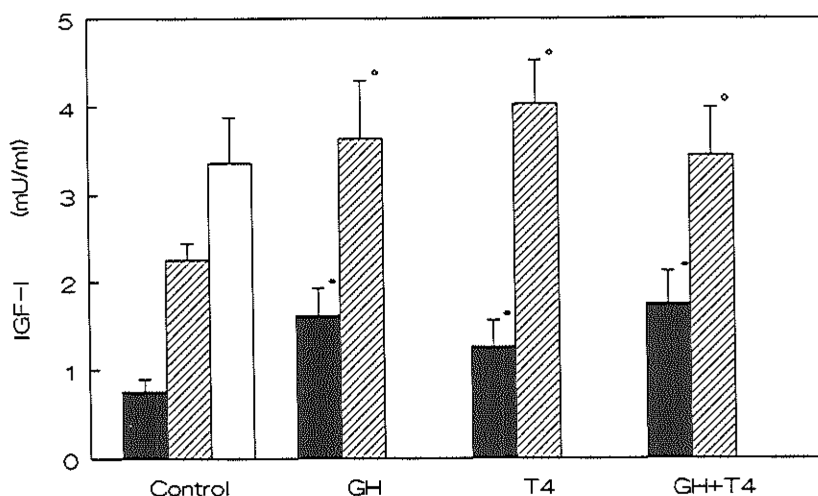


**Figure 8.3:** Effect of treatment with $T_4$, GH, or a combination of the two hormones on plasma IGF-I concentration, in hypophysectomized, hypothyroid or euthyroid rats. Values are means ± SD for groups of 8 rats. See also legend to Figure 8.2. Data significant different are marked ($p<0.05$); 0=different from hypophysectomized controls, *=different from hypothyroid controls).

During hypothyroidism the synthesis, secretion and activity of GH were shown to be deficient (Chernausek and Turner, 1989; Katakami et al., 1986). Thyroid hormone appeared to be necessary for GH gene transcription (Yaffe and Samuels, 1984). This means that not only hypophysectomized, but also hypothyroid rats are GH-deficient (Hoogerbrugge-v.d.Linden et al., 1990; Chapter 3). In these two groups of rats replacement therapy with GH led to a decrease in total cholesterol, due to a decrease in both LDL and HDL-cholesterol. However, in contrast to the human situation, substitution with GH in rats did not affect $T_3$ concentrations. The cholesterol decreasing effect of GH appears therefore at least in rats not to be mediated via changes in thyroid hormone activity.

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Chapter 9

# GENERAL DISCUSSION AND CONCLUSIONS

For almost 70 years it is known that deficiency of thyroid hormone induces a rise in serum cholesterol (1). The hypercholesterolemia of hypothyroidism can be reversed to normal by thyroid hormone replacement (2). In the huge amount of (controversial) data with respect to lipid metabolism in hypothyroidism, a decreased low-density lipoprotein (LDL) catabolism (3), and a decreased hepatic lipase-activity (4) are common findings. These effects on lipid metabolism are generally interpreted as a direct effect of a deficiency in thyroid hormone. In 1970 it was described that hypercholesterolemia in thyroidectomized rats can be prevented, not only by substitution with thyroid hormone, but also by growth hormone (GH) (5). Later it was shown that GH can lower the concentration of plasma cholesterol in humans, for example in GH-deficient adults (6), and in patients with primary hypercholesterolemia (7). Recently, it was revealed that thyroid hormone regulates GH gene transcription (8) and it was shown that in hypothyroid patients as well as in hypothyroid rats, GH secretion and plasma IGF-I concentration are reduced (9).

In this thesis, the effects of GH on lipid metabolism, especially during hypothyroidism were investigated. We studied whether the hypercholesterolemia present in hypothyroidism has to be attributed to a deficiency in thyroid hormone, a deficiency in GH or to both of them. Schematically this question is depicted in Figure 9.1.

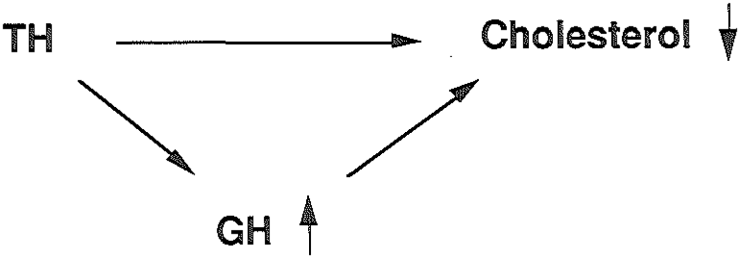


**Figure 9.1:** Schematic representation of the interaction that might occur between thyroid hormone (TH) and growth hormone (GH) as well as their effects on the concentration of plasma cholesterol.

The evaluation of the separate effects of either GH or thyroid hormone on lipoprotein metabolism, is complicated; substitution treatment of hypothyroid subjects with thyroid hormone will restore the pituitary secretion of GH and normalize the activity of both hormones. Next to this, replacement therapy of human adults with GH has been reported to stimulate thyroid hormone activity by increasing the peripheral de-iodination of $T_4$ (10). In humans as well as in rats, the effects of replacement therapy with thyroid hormone and GH on plasma lipoprotein concentrations and hepatic lipase activity were studied. We used hypophysectomized and methimazole- induced-hypothyroid rats, to investigate the individual effects of GH and thyroid hormone on lipoprotein metabolism in the hypothyroid state. Special attention was paid to the determinants (LDL-receptor) of plasma LDL-cholesterol and the activity of hepatic lipase, as these two parameters are generally reported to be affected in hypothyroidism.

## EFFECTS OF GH AND THYROID HORMONE ON THE CONCENTRATION OF CIRCULATING LDL CHOLESTEROL.

An indication that the concentration of plasma LDL cholesterol and the activity of GH are related was obtained in **hypothyroid women** (Chapter 2). In these women GH-activity was measured by determination of insulin-like growth factor-I (IGF-I), as most actions of GH are assumed to be mediated by IGF-I. Multivariate regression analysis of the dependent variable LDL cholesterol, against the concentration of plasma IGF-I and free $T_4$, showed that the concentration of LDL cholesterol is related to IGF-I and that this relation is independent from the concentration of free $T_4$. This suggests that IGF-I rather than thyroid hormone determines the concentration of plasma cholesterol in patients with hypothyroidism.
GH replacement therapy of hypophysectomized and **hypothyroid rats** reduces the plasma concentration of LDL cholesterol (Chapter 3). The LDL cholesterol decreasing effect of replacement therapy with GH was less pronounced than that with $T_4$ (Chapter 3 and 4). In hypothyroid rtas combined therapy with the two hormones appeared to have an additive effect on the decrease in circulating LDL cholesterol (Chapter 4). The decrease of the concentration of plasma LDL cholesterol, following GH replacement, was accompanied by an increase in the concentration of LDL receptor mRNA in

the liver. In addition the expression of the LDL receptor in liver was enhanced (Chapter 5). This suggests that GH stimulates LDL catabolism by an increase in the number of active LDL receptors in the liver. GH may exert its effect either directly or indirectly via a lowering in the intracellular concentration of cholesterol. An indirect effect resulting from consumption of cholesterol e.g. for cell membrane synthesis, is likely to occur; the pubertal hypothyroid rats treated with GH showed a significant weight increase, while untreated hypothyroid rats did not.
Although GH replacement increased the levels of mRNA for the LDL receptor and the expression of the LDL receptor to control values, it did not normalize the concentration of plasma LDL cholesterol. The addition of thyroxin to GH therapy was necessary to normalize the concentration of circulating LDL cholesterol in hypophysectomized rats. For some other hepatic mRNA's it has been shown that thyroid hormone enhances the expression of GH-stimulated mRNA's at a pretranslation level (e.g. 5 alpha-reductase and 16 alpha-hydroxylase, 11). Our data suggest a similar mechanism to occur for hepatic mRNA of the LDL receptor, in which GH stimulates the transcription and thyroid hormone might enhance posttranscription. Another explanation for these results might be, that once mRNA for LDL receptor is formed, it will be translated but the translation product remains in the endoplasmatic reticulum instead of reaching the plasma membrane. The cellular membrane preparations we used to study the LDL receptor expression consisted of a mixture of membranes from intracellular compartments and plasma membranes. This method is unable to distinguish an erroneously located nonfunctional LDL receptor protein from one at the physiological site of action. Pulse chase studies are needed to disentangle this mechanism.

GH replacement in rats led to an increase in the concentration of plasma IGF-I. The results obtained in hypothyroid women suggested that IGF-I mediates the effects of GH on the concentration of plasma LDL cholesterol. This was consistent with the effects found in ***in vitro*** experiments; in a human hepatocyte cell-line it was shown that LDL binding and internalization was stimulated following incubation with IGF-I, but not with GH. These results support the hypothesis that the effects of GH on the LDL receptor are mediated by IGF-I (Chapter 6). A replacement experiment with IGF-I in

hypothyroids *in vivo* has yet to be done, in order to prove the hypothesis that the deficiency of IGF-I that occurs in hypothyroidism is important for the hypercholesterolemia during this condition. Recently it was reported that the administration of IGF-I to patients with Laron type dwarfism caused a decrease in plasma cholesterol concentration (12). As these patients are characterized by high plasma levels of GH, but an inability to generate endogenous IGF-I, due to a GH receptor defect, these results support the previous hypothesis.

Once the diagnosis hypothyroidism is made, treatment of this patient with thyroid hormone can not be postponed to study the effects of GH in this condition. In order to investigate the effects of GH (or IGF-I) on the concentration of plasma cholesterol, we studied GH-deficient adults before, during and after long-term GH replacement therapy. GH replacement in these patients led to a decrease in plasma (LDL) cholesterol and a rise of the level of circulating IGF-I, but also in the concentration of serum $T_3$ (Chapter 8). This means that in humans next to a "direct" effect of GH / IGF-I on the concentration of plasma cholesterol, GH might also affect cholesterol

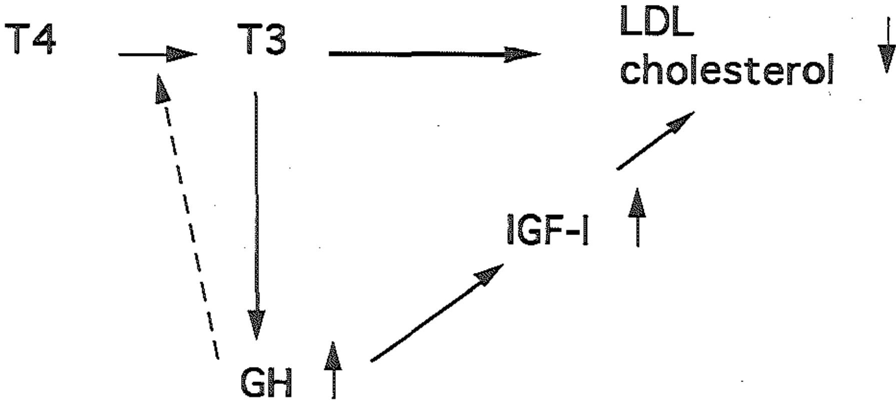


**Figure 9.2**: Diagram of the effects of $T_3$ on the activity of growth hormone (GH) and plasma LDL cholesterol concentration GH enhances the de-iodination of $T_4$ and decreases the concentration of circulating LDL cholesterol, the latter effect is mediated by IGF-I. The decrease in plasma LDL concentration induced by replacement therapy with $T_4$ is more potent than the decrease obtained by GH replacement therapy.

metabolism via $T_3$. In rats we did not observe an increase in $T_3$ concentration during replacement therapy with GH. In the human situation GH and thyroid hormone stimulate each others activity; an outline of the situation assumed to exist in humans is shown in figure 9.2.

## EFFECTS OF GH AND THYROID HORMONE ON HEPATIC LIPASE

**In rats** the effect of replacement treatment with GH on the activity of hepatic lipase in the liver, appears to differ from the effect of replacement with thyroid hormone: GH replacement treatment of hypophysectomized rats, enhanced the activity of hepatic lipase, while substitution with thyroid hormone did not affect the activity of this enzyme. However, replacement with a combination of thyroid hormone and GH, stimulated the activity of hepatic lipase more than GH alone. Thus only in the presence of GH, thyroid hormone seems to stimulate hepatic lipase activity. In other words, GH appears to be a permissive factor for the stimulatory effect of thyroid hormone on hepatic lipase activity in hypothyroidism (Chapter 3 and 4). A diagram of this situation is given in figure 9.3.

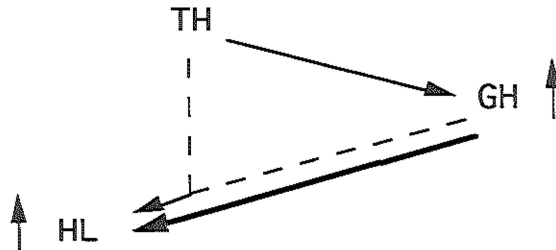


**Figure 9.3**: Diagram of the effects of GH and thyroid hormone (TH) on the activity of hepatic lipase (HL) **in rats.** GH is a more potent stimulator of HL-activity than TH, and serves as a permissive factor for the effects of TH on HL.

In hypothyroid rats the concentration of mRNA for hepatic lipase in liver cells was found to be decreased. It returned to control values after treatment with GH, suggesting a stimulatory effect of GH on the synthesis of this enzyme (Chapter 7). Hepatic lipase exerts its function bound to the sinusoidal cells, from which it can be released by the administration of heparin. Treatment of hypothyroid rats with GH normalized the activity of hepatic lipase in post heparin plasma, indicating that GH stimulates HL-activity at its site of action. Our results in rats suggest that GH stimulates HL synthesis. However, freshly isolated liver parenchymal cells from hypothyroid rats, substituted for 10 days with GH, secreted the same amount and activity of HL as cells from hypothyroid rats, i.e. approximately 60 % of the amount produced by cells from control rats (unpublished data). A GH induced increase in HL synthesis has yet to be shown, e.g. by incubation with GH of liver parenchymal cells in maintenance culture. So far, the increased activity of HL bound to the vascular endothelium might as well be caused by a GH induced increase in HL binding places. However, modulation of the mass of specific mRNA species is a major mechanism of growth hormone action (13). It is unlikely that a significant change in the concentration of hepatic lipase mRNA occurs without an increase in the protein encoded by that mRNA. In parallel to the effects of GH on LDL receptor mRNA and function, we suggest that HL protein synthesis is enhanced but that the protein might accumulate during the complicated process that is necessary to form an active enzyme. Pulse chase experiments are needed the investigate this process. Thyroid hormone is known to regulate hepatic enzymes by multiple mechanisms, involving both transcriptional and posttranscriptional steps (14). The additive stimulatory effects of thyroid hormone in the presence of GH, suggests that GH stimulates the transcription while thyroid hormone is necessary for posttranscriptional processes (such a regulatory mechanism was shown to occur for apolipoprotein B mRNA) (15).

**In humans** the effects of GH and thyroid hormone on the activity of HL are not clear. Comparable to the situation in rats the activity of HL was decreased in hypothyroid patients and normalized after substitution with thyroid hormone (Chapter 2). However, the activity of HL in GH deficient euthyroid individuals was normal and replacement with GH did not affect its activity (Chapter 8). Perhaps euthyroid GH deficient patients are not deprived of all growth hormone activity, as thyroid hormone can induce some IGF-I

production. In contrast, in patients with acromegaly, having a high concentration of circulating GH, HL is increased. We speculate that growth hormone does have a stimulating effect on the activity of hepatic lipase in humans too, but that this can only be shown either during absolute deprivation of growth hormone as exists in profound hypothyroidism, or in the presence of excessive amounts (e.g. acromegaly).

## GENERAL CONCLUSIONS

From the studies reported in this thesis we conclude that the dyslipidemia of hypothyroidism has to be attributed both to thyroid hormone and GH. Schematically this is depicted in figure 9.4.

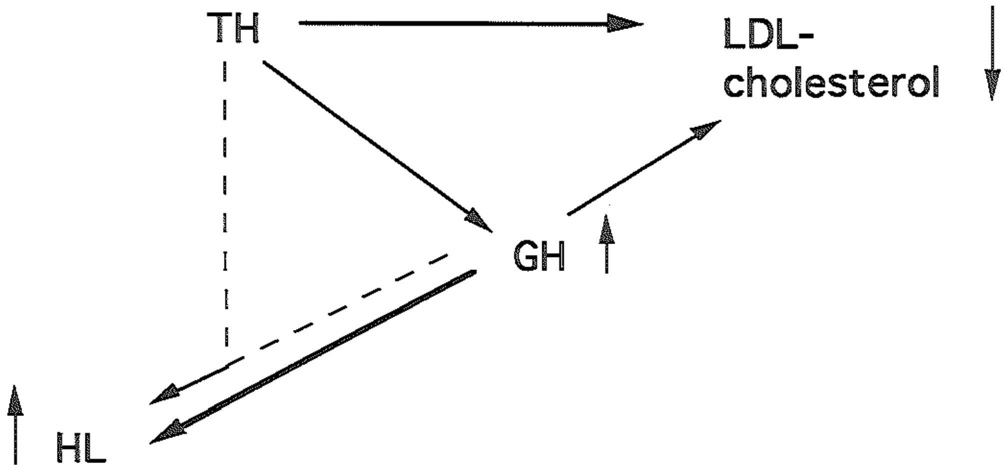


**Figure 9.4:** Schematic representation of the supposed interaction between thyroid hormone (TH) and growth hormone (GH), as well as their effects on the concentration of circulating LDL-cholesterol and the activity of hepatic lipase (HL) in the rat.

Thyroid hormone and GH stimulate each others activity; GH enhances the deiodination of T4 in humans, while $T_4$ stimulates GH synthesis and secretion in the pituitary and the number of GH receptors in rat liver (16). Although our

experiments in rats clearly demonstrate that thyroid hormone and GH can act independently, it is likely that in the intact animal these hormones will also interact to regulate LDL receptor and hepatic lipase activity. The precise mechanism by which these hormones stimulate cholesterol metabolism is (still) not known. Thyroid hormone might act as a positive regulator of GH-stimulated hepatic mRNA levels, active in cholesterol metabolism.

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# Chapter 10

## SUMMARY

An increased concentration of circulating low-density lipoprotein (LDL) cholesterol, is causally related to an increased risk of coronary heart disease. There is substantial evidence that lowering LDL-cholesterol levels as well as increasing high-density lipoprotein (HDL)-cholesterol concentration, will reduce the incidence of coronary heart disease. Hormones are important regulators of the concentration of these lipoproteins. However, the effects of most hormones on lipid metabolism are complex and poorly understood. Abnormalities in hormone secretion may participate in the pathophysiology of lipoprotein aberrations and contribute to the atherogenic risk accompanying common diseases such as hypothyroidism. Understanding of the factors that control cholesterol metabolism may provide new openings in the treatment of dyslipidemia. In this thesis the disturbance of lipid metabolism during hypothyroidism were studied.

Since early in this century it is known that hypothyroidism causes hypercholesterolemia. Nowadays we know that this hypercholesterolemia results from an increase in LDL-cholesterol. In man the influence of hypothyroidism on the concentration of total plasma HDL-cholesterol is rather equivocal, but the activity of hepatic lipase, a key enzyme in HDL metabolism, is uniformly reported to be decreased. These changes in lipoprotein metabolism during hypothyroidism have been ascribed to direct effects of the decreased circulating thyroid hormone (TH). However, as TH is necessary for the production and secretion of growth hormone (GH); hypothyroid individuals have a deficiency of GH-activity too. In other conditions GH itself is thought to affect lipoprotein metabolism. We studied the effects and involvement of TH, GH and IGF-I on lipid metabolism, especially in hypothyroidism.

In patients with hypothyroidism plasma lipids and the level of circulating IGF-I, a mediator for GH action, were measured. As compared to healthy controls, an increased concentration of plasma LDL-cholesterol and a decreased IGF-I concentration were found. The concentration of plasma LDL-cholesterol appeared to be highest in those patients with the lowest concentration of plasma IGF-I. In a multivariate regression analysis of LDL-cholesterol, as the dependent variable, against IGF-I and free $T_4$ a significant negative correla-

tion between IGF-I and LDL-cholesterol concentrations but not between free $T_4$ and LDL-cholesterol was shown. This suggests that the effect of IGF-I on cholesterol metabolism is directly on the LDL-cholesterol metabolism. Furthermore, a cholesterol decreasing effect of GH was seen in euthyroid, but GH-deficient adults. Substitution of these patients with GH resulted in a reduction of the concentration of plasma LDL-cholesterol. However in this group of patients GH increased the concentration of circulating $T_3$, while serum $T_4$ concentration fall. In these euthyroid patients it could not be concluded whether the decrease in plasma cholesterol concentration was the result of GH (or IGF-I) activity itself, or an indirect effect via enhancement of the conversion of $T_4$ into $T_3$.

Hypothyroidism is a serious disease; once it is diagnosed, substitution with TH has to be started. Postponement of this substitution, in order to study the effects of GH on lipid metabolism in hypothyroid patients is not permissible. For this reason we used hypophysectomized and hypothyroid rats as a model to study the effects of GH and TH on lipoprotein metabolism in vivo. Single GH treatment or GH treatment in addition to $T_4$ therapy did not affect the concentration of either $T_4$ or $T_3$ in hypophysectomized, hypothyroid and euthyroid rats. However treatment of hypophysectomized rats with GH, decreased the plasma LDL-cholesterol concentration. We concluded that at least in rats the cholesterol lowering effect of GH is not mediated by TH. Replacement with TH was more potent than replacement with GH in the regulation of LDL-cholesterol concentration.
In hypothyroid rats the LDL-cholesterol decreasing effect of GH was shown to be accompanied by a rise in the level of mRNA for the LDL-receptor in the liver. The LDL-receptor expression in the liver was stimulated too by GH. This indicates that GH stimulates the number of active LDL-receptors by an increase in LDL-receptor synthesis. A human liver tumor cell line (HepG2), was used to show that LDL-receptor activity, both LDL binding and internalization, can be stimulated by incubation with IGF-I. In contrast incubation with GH did not affect LDL-receptor function. This suggests that the effects of GH on the concentration of LDL-cholesterol in hypothyroids are mediated by IGF-I.

The activity of hepatic lipase is decreased both in hypothyroid humans as well

as in rats and increases during substitution with TH. In hypophysectomized and hypothyroid rats treatment with GH stimulated the activity of hepatic lipase in the liver. TH also stimulated hepatic lipase-activity, but only in the presence of GH. The administration of GH to hypothyroid rats increased both the concentration of mRNA for hepatic lipase and the activity of hepatic lipase in liver homogenates. Hepatic lipase exerts its function bound to the sinusoidal cells, from which it can be released by the administration of heparin. Treatment of hypothyroid rats with GH normalized the activity of hepatic lipase in post heparin plasma. This indicates that GH stimulates hepatic lipase synthesis (determined by mRNA), resulting in an increased activity at its site of action, the vascular endothelium of the liver.

In view of the experiments presented in this thesis, it is concluded that the dyslipidemia that occurs in hypothyroidism results from a deficiency in both TH and GH. In hypothyroid rats it was shown that the concentration of circulating LDL-cholesterol is mainly determined by TH, while GH has a minor LDL-cholesterol decreasing effect. On the other hand GH has a prominent role in the regulation of hepatic lipase-activity. GH exerts its effect on LDL-cholesterol and hepatic lipase-activity via an induction of hepatic mRNA encoding for the LDL-receptor and for hepatic lipase.

## Chapter 11

# SAMENVATTING

Een verhoogde concentratie van cholesterol circulerend in low-density lipoproteïnen (LDL), is mede verantwoordelijk voor een toegenomen risico op de voortijdige ontwikkeling van hart- en vaatziekten. Er zijn gegronde aanwijzingen dat verlaging van de plasma concentratie van LDL-cholesterol of verhoging van de plasma concentratie van cholesterol in high-density lipoproteïnen (HDL), het optreden van hart- en vaatziekten vermindert. Hormonen hebben een regulerende invloed op de concentratie van deze lipoproteïnen. De wijze waarop hormonen het vetmetabolisme beïnvloeden is complex en slechts gedeeltelijk bekend. Een afwijking in de normale hormoonproduktie kan leiden tot ziekelijke veranderingen in het metabolisme van lipoproteïnen en aanleiding geven tot een versnelde ontwikkeling van atherosclerose, zoals optreedt bij patiënten met hypothyreoïdie. Kennis van de factoren die in het cholesterolmetabolisme van belang zijn, kan leiden tot een meer specifieke behandeling van dyslipidemie. In het onderzoek beschreven in dit proefschrift is de regulatie van het lipidenmetabolisme tijdens hypothyreoïdie bestudeerd.

Sinds het begin van deze eeuw is bekend dat hypothyreoïdie een hypercholesterolemie veroorzaakt. De laatste jaren is bekend geworden dat deze hypercholesterolemie het gevolg is van een toegenomen plasma LDL-cholesterol concentratie. Bij de mens heeft hypothyreoïdie geen duidelijk effect op de totale plasma concentratie van HDL-cholesterol; wel is een verlaging van de activiteit van hepatisch lipase beschreven. Hepatisch lipase wordt verondersteld een rol te spelen in het HDL-metabolisme. De veranderingen in het lipidenmetabolisme tijdens hypothyreoïdie zijn toegeschreven aan een verminderde aanwezigheid van schildklierhormoon. Aangezien schildklierhormoon noodzakelijk is voor de produktie en secretie van groeihormoon (GH), is er tijdens hypothyreoïdie niet alleen een verlaagde schildklierhormoon-activiteit, maar ook een verminderde activiteit van GH aanwezig. Ook van GH is bekend dat dit het lipoproteïnemetabolisme beinvloedt. Wij bestudeerden daarom het afzonderlijk effect van GH en schildklierhormoon op het lipidenmetabolisme, met name gedurende hypothyreoïdie.

Iñ patiënten met hypothyreoïdie werden de plasma lipiden concentraties en de concentratie van circulerend IGF-I, een maat voor de activiteit van groei-

hormoon, gemeten. Vergeleken met gezonde controle personen werd in deze patiënten, zowel een verhoogde concentratie van LDL-cholesterol, als een verlaagde IGF-I concentratie, gevonden. De hoogste plasma LDL-cholesterol concentraties bleken aanwezig te zijn bij patiënten met de laagste plasma IGF-I concentraties. In een multivariate regressie-analyse van de concentraties van LDL-cholesterol, tegen IGF-I en vrij $T_4$, werd een negatieve correlatie tussen LDL-cholesterol en IGF-I gevonden, maar niet tussen LDL-cholesterol en de vrije $T_4$ concentratie. Dit suggereert dat de activiteit van GH, onafhankelijk van schildklierhormoon, de LDL-cholesterol concentratie beïnvloedt. Ook bij patiënten met een normale schildklierfunctie, maar een deficiëntie voor GH, werd aangetoond dat toediening van GH de plasma LDL-cholesterol concentratie verlaagt. Bij deze euthyreote patiënten bleek echter, dat tijdens behandeling met GH, de serum concentratie van $T_3$ werd verhoogd en de serum $T_4$ concentratie werd verlaagd. Uit deze gegevens bij patiënten met een normale schildklierfunctie kon daarom niet worden geconcludeerd of de verlaging van de plasma LDL-cholesterol concentratie tijdens behandeling met GH, een gevolg was van de GH activiteit zelf, of werd veroorzaakt door een toegenomen omzetting van $T_4$ in het biologisch actieve $T_3$.

Hypothyreoïdie is een ernstige ziekte waarbij zodra de diagnose is gesteld dient te worden begonnen met behandeling, die bestaat uit het toedienen van schildklierhormoon. Uitstel van behandeling, om het effect van GH te bestuderen, is derhalve niet gewenst. Om het afzonderlijke effect van schildklierhormoon en GH op het lipidenmetabolisme *in vivo* te kunnen bestuderen, hebben wij als model gehypofysectomeerde en hypothyreote ratten gebruikt. Toediening van GH aan gehypofysectomeerde, medicamenteus hypothyreoot gemaakte of euthyreote ratten, veroorzaakte geen verandering in de concentratie van circulerend $T_3$ en $T_4$. Behandeling van gehypofysectomeerde ratten met GH leidde wel tot een verlaging van de plasma LDL-cholesterol concentratie. Geconcludeerd werd dat, tenminste in de rat, het cholesterol verlagend effect van GH niet wordt gemedieerd door schildklierhormoon. Substitutie van schildklierhormoon bleek de LDL-cholesterol concentratie meer te verlagen dan substitutie van GH.
In met GH behandelde hypothyreote ratten werd naast een LDL-cholesterol verlagend effect, een toename in de concentratie van mRNA coderend voor de LDL-receptor in de lever waargenomen. De expressie van de LDL-receptor

bleek eveneens te worden gestimuleerd door behandeling met GH. Dit suggereert dat GH het aantal actieve LDL-receptoren doet toenemen via een verhoogde synthese van deze receptoren. In een humane tumorcellijn (HepG2), werd aangetoond dat zowel de binding als de internalizatie van LDL door de receptor, kon worden gestimuleerd door incubatie met IGF-I, terwijl incubatie met GH de LDL-receptoractiviteit niet veranderde. Dit maakt waarschijnlijk dat het effect van GH op de concentratie van LDL-cholesterol wordt gemediëerd door IGF-I.

De activiteit van hepatisch lipase was verlaagd, zowel in hypothyreote patiënten als in ratten, en nam toe tijdens substitutie met schildklierhormoon. Behandeling van gehypofysectomeerde en hypothyreote ratten met GH stimuleerde de activiteit van hepatisch lipase in gehomogeniseerd leverweefsel, terwijl behandeling met schildklierhormoon de activiteit van hepatisch lipase alleen kon verhogen in aanwezigheid van GH. Toediening van GH aan hypothyreote ratten leidde niet alleen tot een toename in activiteit van hepatisch lipase, maar ook tot toename van de mRNA concentratie voor hepatisch lipase in leverweefsel. Hepatisch lipase oefent haar functie uit gebonden aan de sinusoidalecellen van de lever, en kan van deze plaats worden losgemaakt door toediening van heparine. Behandeling van hypothyreote ratten met GH normaliseerde de activiteit van hepatisch lipase in het postheparine plasma. Hieruit werd geconcludeerd dat GH de synthese van hepatisch lipase stimuleert, met als gevolg een toename van de hoeveelheid aan endotheelcellen gebonden enzym.

Uit de in dit proefschrift beschreven experimenten wordt geconcludeerd dat de afwijkingen in het lipidenmetabolisme tijdens hypothyreoidie, worden veroorzaakt door een deficiëntie van zowel schildklierhormoon als GH. De concentratie van circulerend LDL-cholesterol bleek, althans in de rat, voornamelijk te worden bepaald door schildklierhormoon, terwijl GH hierin een ondergeschikte rol vervult. In de regulatie van de activiteit van hepatisch lipase bleek de rol van GH belangrijker. Het effect van GH op de concentratie van LDL-cholesterol en de activiteit van hepatisch lipase verloopt via inductie van mRNA coderend voor respectievelijk de LDL-receptor en hepatisch lipase.

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## CURRICULUM VITAE

De schrijfster van dit proefschrift is geboren op 26 juli 1958 te Delft. Na het behalen van het diploma Atheneum-B aan het Christelijk Lyceum te Delft, werd in 1976 aangevangen met de studie Geneeskunde aan de Erasmus Universiteit te Rotterdam. Het doctoraal examen werd in 1981 (Cum Laude) afgelegd en in 1983 het arts examen (Cum Laude).
In april 1983 begon zij de opleiding tot internist in het Diakonessenhuis Refaja te Dordrecht (opleider destijds: C.Verdoorn). In mei 1985 werd de opleiding tot internist voortgezet in het Academisch Ziekenhuis Dijkzigt op de afdeling Inwendige Geneeskunde III (opleider: Prof. Dr. J.C. Birkenhäger). Tijdens de opleiding werd aangevangen met het in dit proefschrift beschreven onderzoek. Op 1 mei 1988 volgde inschrijving in het specialisten register. Vanaf 1 September 1988 is zij als internist verbonden aan de afdeling Inwendige Geneeskunde III (afdelingshoofd: Prof. Dr. J.C. Birkenhäger), waar het in dit proefschrift beschreven onderzoek werd verricht.