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LIPOPROTEIN COMPOSITION IN HYPOTHYROIDISM

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Summary

Sixteen patients with hypothyroidism have had their lipid status assessed before and during replacement therapy. More than 60% had hypercholesterolaemia and more than 60% had hypertriglyceridaemia. Significant reductions in plasma cholesterol, but not in plasma triglyceride, were seen during replacement therapy. A high cholesterol : triglyceride ratio was observed in VLDL and this relationship tended back to normal during treatment. This raises the possibility that in hypothyroidism, as in Type III hyperlipoproteinaemia, an abnormality in VLDL conversion to LDL is present.

Introduction

There is evidence, some of it conflicting, that patients with hypothyroidism have more coronary artery disease than do euthyroid patients [1—5]. Inasmuch as elevated serum cholesterol [6] and triglyceride [7] concentrations are risk factors for ischaemic heart disease, these lipids are of interest in hypothyroidism. There is general agreement that hypercholesterolaemia is a feature of hypothyroidism [8,9] and there is some evidence that hypertrigylceridaemia is also a feature [10,11]. Thus, it would appear that there is a general disturbance of lipoprotein metabolism associated with the disease and the question arises as to where that disturbance might be. Data have accumulated implicating several mechanisms regulating lipoprotein metabolism. Examples would be lipolytic activity involved in the catabolism of VLDL [10—15], lipoprotein interconversions [16—18], and the removal of low density lipoprotein [19—21]. In this study, chemical analysis of lipoprotein fractions before and during treatment for hypothyroidism, was undertaken as part of an investigation of the lipoprotein metabolic abnormality.

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Patients

Sixteen patients with hypothyroidism were studied. They were consecutive patients who had been referred to the Department of Nuclear Medicine with suspected hypothyroidism. There were 12 females and 4 males and the patients' ages ranged from 15 to 84 years with a mean ±S.E.M. of 48 ± 5. The diagnosis of hypothyroidism was based on the finding of a free thyroxine index below the normal range, or, in 2 cases where the free thyroxine index was borderline, on an elevated TSH value. In 14 of the 16 patients, TSH measurements were made and each of these patients had an elevated TSH (upper limit of normal 5 ng/ml) confirming the diagnosis; the mean ±S.E.M. for these patients was 92 ± 12 ng/ml. One patient with Hashimoto's disease was on the contraceptive pill throughout the study. None had evidence of pituitary disease. The causes of hypothyroidism were: Hashimoto's disease, 5; post-thyroidectomy, 2; post-irradiation, 1; and primary, 6; in 2 cases the cause is unknown as Hashimoto's thyroiditis was not excluded. Patients with primary hypothyroidism did not have enlarged thyroid glands and had low anti-thyroglobulin and anti-microsomal antibodies. There was one male in each of the four diagnostic categories.

Blood was taken after an overnight fast for lipid determinations at the same time as blood was drawn for the thyroid function tests.

With the exception of one patient, a woman aged 52, whose diagnosis of hypothyroidism was made following the recognition of hypercholesterolaemia, no patient was on a diet at the time of diagnosis. No dietary advice was given during the study.

Identifiable factors which may have contributed to any hyperlipidaemia in the patients were the contraceptive pill in a 33 year old woman with Hashimoto's disease and an above average alcohol intake in a 53 year old man with primary hypothyroidism.

All patients received at least 200 μg of L-thyroxine a day as replacement therapy. Simultaneous re-assessment of thyroid function and fasting plasma lipids was made 86—250 days after commencing therapy, with a mean treatment period of 140 ± 12 days.

Methods

Thyroid function tests performed on each patient at diagnosis were T_3 resin uptake (T_3RU), expressed as a percentage, serum thyroxine (T_4) and free thyroxine index (F.T.I. = T_3 RU/100 × T_4) [22]. Some patients also had a serum TSH measurement by radioimmunoassay [23]. Two patients, in follow-up, had an effective thyroxine ratio (ETR) measurement [24] instead of the above tests.

Plasma cholesterol was measured using a colorimetric method based on the Lieberman-Burchard reaction. It was assayed in a Technicon Auto-Analyser II [25]. Triglycerides were determined simultaneously by the fluorimetric method of Kessler and Lederer [26], based on the Hantzsch reaction.

Lipoproteins were isolated by ultracentrifugation as described by Havel [27] and also by polyanion precipitation [28]. Heparinized plasma, at 4°C and den-

sity 1.006, containing 0.1—1 mM EDTA (dipotassium ethylene diamine tetra acetate) was centrifuged in cellulose acetate tubes in a Spinco L2-50 Preparative Ultracentrifuge at 39 000 rpm for 16 h using a 40.3 fixed angle rotor (105 000 × *g*). The supernatant containing VLDL was removed from the top by a tube slicing technique. LDL were precipitated from the infranatant by adding 1000 units of heparin sulphate and 0.25 ml of 1 mM manganous chloride to every 5 ml of infranatant. The precipitate was centrifuged at 4°C for 20 min at 2000 rpm in a refrigerated centrifuge and the supernatant containing HDL was decanted from the pellet (LDL). The pellet was suspended in physiological saline.

Lipoprotein lipids were extracted with Dole's solution [29] using a ratio of 10 ml of Dole's solution for 2 ml of lipoprotein solution. Lipids were then separated from the precipitated proteins using heptane (Dole's/heptane/water, 10 : 6 : 8, by vol.) Aliquots were then analyzed for cholesterol and triglyceride.

Recoveries averaged 78% for triglyceride and 77% for cholesterol. Determinations were corrected for recovery assuming a constant loss in each lipoprotein fraction.

Results

Thyroid function tests carried out on patients are shown in Table I. When evaluated during treatment, all but one patient had returned to within the normal range for free thyroxine index (F.T.I.) and all had returned to within the normal range of serum thyroxine (T_4) (Table I).

More than 60% of patients with hypothyroidism had either hypercholesterolaemia or hypertriglyceridaemia and about 40% had mixed hyperlipoproteinaemia (Table II). Fourteen out of the sixteen (88%) had one or the other lipid increased. Treatment achieved significant reductions in total serum cholesterol for the group (Tables II and III), but not in total serum triglyceride (Tables II and IV).

TABLE I

THYROID FUNCTION TESTS BEFORE AND DURING TREATMENT

Means ± S.E.M. are shown together with range in parentheses. Significance of effect of treatment is based on paired *t* test. Normal range of T_3 resin uptake (T_3RU) is 26.5—34.5%, for serum thyroxine (T_4) 4.5—10.5 μg/100 ml and for free thyroxine index (F.T.I.) 1.4—3.0. Number of patients (*n*) shown is 14 of the 16 assessed. This is because 2 patients had ETR's (effective thyroxine ratios) measured in follow-up rather than T_3RU, T_4 and F.T.I. The initial T_3RU, T_4 and F.T.I. in these patients were below the normal range and the initial serum TSH was elevated in both cases. Two patients had pre-treatment F.T.I.'s of 1.48, in the lower normal range, but elevated TSH values (55 and 160 ng/ml). Excluding these patients the range was 0.14—0.92. One patient had an F.T.I. during treatment of 1.14, below the lower limit of the normal range. This patient's pre-treatment F.T.I. was 0.92. Excluding this patient, the range of F.T.I.'s during treatment was 1.67—4.50.

	T_3 resin uptake	Serum thyroxine	Free thyroxine index
Before treatment	23 ± 1	3.2 ± 0.5	0.74 ± 0.13
(*n* = 14)	(10 — 29)	(0.4 — 8.5)	(0.14 — 1.48)
During treatment	32 ± 2	9.1 ± 1.0	2.88 ± 0.30
(*n* = 14)	(24 — 44)	(4.2 — 14.4)	(1.14 — 4.50)
P	<0.001	<0.001	<0.001

TABLE II

FREQUENCY OF HYPERLIPIDAEMIA IN PATIENTS WITH HYPOTHYROIDISM BEFORE AND DURING TREATMENT

Hypercholesterolaemia is taken as a value greater than 250 mg/100 ml and hypertriglyceridaemia as a value greater than 150 mg/100 ml.

	Hypercholesterolaemia	Hypertriglyceridaemia	Mixed hyperlipidaemia
Before treatment (n = 16)	11 (69%)	10 (63%)	6 (38%)
During treatment (n = 16)	8 (50%)	10 (63%)	7 (44%)

TABLE III

PLASMA CHOLESTEROL CONCENTRATIONS (mg/100 ml) IN PATIENTS WITH HYPOTHYROIDISM BEFORE AND DURING TREATMENT

Means ± S.E.M. are shown. Number of subjects is shown in parentheses. VLDL = very low density lipoprotein (<1.006); LDL = low density lipoprotein (1.006—1.063); HDL = high density lipoprotein (1.063—1.21). Significance of effect of treatment is based on a paired t test. n.s. = not significant.

	Total	VLDL	LDL	HDL
Before treatment	345 ± 33 (16)	69 ± 28 (12)	209 ± 24 (12)	42 ± 9 (12)
During treatment	261 ± 17 (16)	37 ± 10 (12)	171 ± 16 (12)	44 ± 6 (12)
P	<0.01	N.S.	N.S.	N.S.

TABLE IV

PLASMA TRIGLYCERIDE CONCENTRATIONS (mg/100 ml) IN PATIENTS WITH HYPOTHYROIDISM BEFORE AND DURING TREATMENT

For explanation of abbreviations see legend to Table III.

	Total	VLDL	LDL	HDL
Before treatment	296 ± 90 (16)	178 ± 96 (12)	104 ± 23 (12)	29 ± 7 (12)
During treatment	200 ± 34 (16)	106 ± 33 (12)	67 ± 9 (12)	24 ± 3 (12)
P	N.S.	N.S.	N.S.	N.S.

TABLE V

RATIO OF PLASMA CHOLESTEROL CONCENTRATION TO PLASMA TRIGLYCERIDE CONCENTRATION IN PATIENTS WITH HYPOTHYROIDISM BEFORE AND DURING TREATMENT

For explanation of abbreviations see legend to Table III.

	Total	VLDL	LDL	HDL
Before treatment	1.73 ± 0.22 (16)	0.51 ± 0.14 (12)	2.57 ± 0.33 (12)	1.80 ± 0.25 (12)
During treatment	1.61 ± 0.15 (16)	0.35 ± 0.04 (12)	2.87 ± 0.33 (12)	1.96 ± 0.21 (12)
P	N.S.	N.S.	N.S.	N.S.

Although there was a trend for VLDL and LDL cholesterol and triglyceride to fall with treatment, these changes were not significant (Tables III and IV). HDL cholesterol and triglyceride also did not change significantly (Tables III and IV).

The ratio of cholesterol to triglyceride in the VLDL fraction prior to treatment was high at 0.51 (Table V) by comparison with data on normolipidaemic subjects from this laboratory where the ratios range from 0.21 to 0.31 (unpublished observations) and with a mean ratio from another laboratory of 0.32 [30]. In general, in hyperlipoproteinaemia, this ratio is even lower than in normolipidaemia [31]. There was a trend for the ratio to fall with treatment, but this was not significant.

Discussion

This study confirms that hypercholesterolaemia and hypertriglyceridaemia are frequently observed in association with hypothyroidism [10,11]. Others have found that both respond to replacement therapy [11]. The variability in responsiveness of hypertriglyceridaemia may depend on other determinants of triglyceride concentration, especially diet as found by O'Hara and co-workers [32]. It is possible that with longer observation in each patient or additional thyroxine, reductions in total plasma triglyceride concentration might have been seen in this study. Another possibility is that hypothyroidism may induce a relatively resistant abnormality of lipoprotein metabolism in some patients.

It is of particular interest that hypothyroid patients appeared to have a relatively cholesterol enriched VLDL and that some correction of this may have been achieved with treatment. A similar finding has recently been reported by Rössner and Rosenqvist [33]. This is the type of abnormality observed in Type III hyperlipoproteinaemia [34,35] in which there is increasing evidence for the accumulation of a VLDL remnant or intermediate lipoprotein possibly involved in the conversion of VLDL to LDL. Thus, this study would appear to provide some support for the view that thyroid hormones act, at least in part, at the level of lipoprotein interconversion. By what mechanism this might be effected cannot be deduced from the present study.

There is evidence of concurrence of Type III hyperlipoproteinaemia and hypothyroidism in the literature [8]. Furthermore, an arginine-rich apoprotein appears to be associated with both hypothyroidism and Type III hyperlipoproteinaemia [36]. This may actually be a normal apoprotein [37] present in increased amounts. Further investigation of this apoprotein may provide insight into the mechanism whereby myxoedematous patients are more likely to become hyperlipidaemic.

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