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Originals

Increased Ketonaemia in Hyperthyroidism

Evidence for a β-Adrenergic Mechanism

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Summary. The metabolic status of 16 hyperthyroid patients and 22 control subjects was studied in the post-absorptive state after 3 days on a standard diet (35 kcal/kg body weight/day). In hyperthyroidism the ranges of blood ketone body (19-1159 vs 17-233 μmol/l, $p < 0.001$) and glycerol (59-285 vs 15-69 μmol/l, $p < 0.001$) concentrations were increased relative to controls despite slight hyperglycaemia (4.9 ± 0.6 vs 4.6 ± 0.4 mmol/l, mean ± SD $p < 0.05$). Plasma non-esterified fatty acids, immunoreactive insulin and glucagon levels were not significantly different. A positive correlation was found between thyroid hormone and ketone body ($p < 0.02$) and glycerol ($p < 0.05$) levels and between glycerol and ketone bodies ($p < 0.05$). There was no correlation of non-esterified fatty acids with either thyroid hormone or ketone body concentrations. In hyperthyroid patients propranolol administration for 4 days induced a decrease in triiodothyronine (349 ± 140 to 229 ± 107 ng/100 ml, $p < 0.01$) and a dramatic fall in ketone body (18-295 μmol/l, $p < 0.001$) and glycerol (44-130 μmol/l, $p < 0.001$) levels. Non esterified fatty acids were unchanged. There was no longer a correlation between thyroid hormones and ketone bodies or glycerol. Placebo administration to 6 other hyperthyroid patients had no significant effect. In euthyroid obese subjects on a 600 kcal/day diet, propranolol administration did not change ketone body or glycerol levels. These data provide evidence for an increase in both lipolysis and ketogenesis in hyperthyroidism which might, at least in part, be dependent upon a catecholamine β-receptor mediated mechanism.

Key words: Hyperthyroidism, blood ketone bodies, glycerol, catecholamines, starvation, β-blockade.

Hyperthyroidism is associated with many metabolic abnormalities. Some, such as weight loss, negative nitrogen balance, increased lipid mobilisation and gluconeogenesis [1] closely resemble those observed during starvation. Nevertheless, others, like heightened expenditure of calories and increased fuel consumption, are quite opposite. Recent human studies have described during starvation a faster increase of ketonaemia in hyperthyroid patients [2] and in obese subjects treated with triiodothyronine [3]. However Wahren et al. [4] found no difference in plasma ketone body levels in the post-absorptive state between hyperthyroid and euthyroid subjects. Thus, we reinvestigated, under a strictly controlled diet, the metabolic status of hyperthyroid patients with special attention to ketone bodies. Moreover, as catecholamines are ketogenic hormones in man [5] and as a β-receptor mediated effect of catecholamines has been implicated in some symptoms of hyperthyroidism, we studied the effect of propranolol administration upon the metabolic status of hyperthyroid patients.

Methods

Subjects

Three groups of subjects were studied.

1) The control group consisted of 22 healthy female volunteers from the medical staff (mean age 32 years, range 22–55). All were within 10% of their ideal body weight (mean 102%, range 93–107).

2) The hyperthyroid group consisted of 22 women (mean age 37 years, range 25–65; mean 86% of ideal body weight, range 73–105, $p < 0.01$ compared to the control group) hospitalized for hyperthyroidism and free of other disease.

3) The euthyroid obese group consisted of 8 female subjects admitted to the hospital for obesity. These patients were 42% (mean) over their ideal body weight (range 25–63%).

In all subjects thyroid status was assessed clinically and by determination of thyroid hormone levels. No subject had a per-

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Table 1. Blood metabolites, plasma non-esterified fatty acids and serum or plasma hormone values in control subjects and in hyperthyroid patients before and after propranolol administration. Venous blood was collected at 0800 h after a 12 h overnight fast. The hyperthyroid patients were studied before and after administration of propranolol (40 mg orally every 6 h for 4 days). Values are expressed as mean ± SD for normal distribution, or as *mean and range (in parentheses)*. Total ketone bodies means the sum of acetoacetate and 3-hydroxybutyrate

	Blood glucose	Blood alanine	Blood glycerol	Blood total ketone bodies	Plasma non-esterified fatty acids	Serum T3	FTI	Serum IRI	Plasma IRG
	mmol/l	μmol/l	μmol/l	μmol/l	μmol/l	ng/100 ml		mU/l	ng/l
Control subjects n = 22	4.6 ± 0.4	230 ± 60	41 [15–69]	62 [17–233]	172 ± 73	100 ± 30	2.2 ± 0.9	8.0 ± 3.0	110 ± 30
Hyperthyroid patients n = 16									
before propranolol	4.9 ± 0.6[a]	214 ± 64	134[c] [59–285]	304[c] [19–1160]	192 ± 45	349 ± 140[c]	6.3 ± 1.8[c]	7.3 ± 3.5	120 ± 38
with propranolol	5.0 ±0.4[a]	196 ± 43	71[b,e] [44–130]	102[e] [18–295]	152 ± 42	229 ± 167[b,d]	7.3 ± 2.7[c]	7.1 ± 3.6	120 ± 40

[a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$, means significantly different from the control group
[d] $p < 0.01$; [e] $p < 0.001$, means significantly different from the hyperthyroid group before propranolol

sonal or familial history of diabetes nor was propranolol contraindicated in any case. None took any drug before the starting of the study. All were informed of the purpose, nature and possible risks of the study and gave their informed consent.

Protocols

Except for the euthyroid obese subjects all studies were initiated after 3 days of a controlled diet (35 kcal/kg body weight/day, 40% fat, 40% carbohydrate, 20% protein) which was used throughout the study. Venous blood samples were collected at 0800 h in the post-absorptive state, after a 12 h overnight fast. All subjects lay down for 30 min before sampling.

In the first study, blood was collected from the control group and from 16 patients of the hyperthyroid group. The latter then took propranolol (40 mg orally every 6 h) for 4 days and blood was collected again. In 6 other hyperthyroid subjects blood was sampled before and after four days of treatment by a placebo. The subjects were allocated blindly to propranolol or placebo.

In the second study the metabolic response of the euthyroid obese group to a 600 kcal/day diet was studied over a seven day period. Blood was collected at 0800 h on days 1, 3, 5 and 7. Propranolol (40 mg every 6 h) or placebo were administered in a double-blind manner from day 3 to the end of the study. These subjects were instructed to follow their usual unrestricted food intake before the study was initiated.

Assays

Blood for metabolite determinations was deproteinized with ice-cold perchloric acid (6% v/v), the supernatant neutralized, and glucose [6], glycerol [7], alanine [8], acetoacetate (AcAc) [9] and 3-hydroxybutyrate (3-OHB) [10] were measured by standard enzymatic methods. Total ketone body refers to the sum of AcAc and 3 OHB. AcAc was assayed within 12 h and the other metabolites within a week. The precision of the AcAc and 3-OHB measurement was determined in neutralized samples of a 10 μmol/l standard solution in 6% perchloric acid.

The coefficients of variation for AcAc and β-OHB assay were 12% and 8% respectively. For both metabolites this coefficient was 3% for concentrations higher than 30 μmol/l. The recovery of added metabolites in 6% perchloric acid was between 90 and 103%. Non-esterified fatty acids (NEFA) [11], immunoreactive glucagon (IRG) [12], immunoreactive insulin (IRI) [13] and immunoreactive triiodothyronine (T3) [14] were determined in plasma or serum which were immediately frozen until the day of assay. Thyroxine (T4) and T3 resin uptake were determined using a kit from Abbott Laboratory. Free thyroxine index (FTI) was calculated as T4 × T3 resin uptake/100.

Statistics

Concentration of hormones and metabolites in the different groups are expressed as mean ± SD when the experimental values followed a normal distribution. In the other case a normal distribution was obtained by logarithmic transformation. Student's t test for paired or non-paired data were performed where appropriate.

Results

Metabolic Status of Hyperthyroidism

Metabolite and hormone values in the control and hyperthyroid groups are summarized in Table 1.

Hyperthyroidism was associated with a slight increase of blood glucose ($p < 0.05$) and a highly significant increase of both ketone bodies ($p < 0.001$) and glycerol ($p < 0.001$). Other metabolites, plasma NEFA, IRI and IRG were unchanged. Figure 1 shows the distribution of individual values of acetoacetate, 3-hydroxybutyrate and glycerol in both groups. All these metabolites were abnormally distributed. The distribution of other metabolites and of hormones was normal. There was a good positive linear correlation of triiodothyronine and free thyroxine index with total ketone bodies ($r = 0.61$, $p < 0.02$, $y = 1.26 x - 131$, and $r = 0.59$, $p < 0.02$, $y = 94 x - 285$ respectively) and with glycerol ($r = 0.54$, $p < 0.05$, $y = 0.28 x + 36$, and $r = 0.50$, $p < 0.05$, $y = 20 x + 10$ respectively) in the hyperthyroid group. These correlations were not significant in the control group. A positive correlation between glycerol and ketone bodies ($r = 0.52$, $p < 0.05$, $y = 2.06 x + 32$) also existed in the hyperthyroid group but was absent in the control subjects. No correlation could be found either between NEFA and thyroid hormones levels or between NEFA and ketone bodies or glycerol.

Effects of Propranolol in Hyperthyroidism. Hormone and metabolite values in hyperthyroidism after 4 days of propranolol treatment are summarized in Table 1. Propranolol administration induced a significant decrease in heart rate (92 ± 7 to 79 ± 5 beats/min, $p < 0.001$), systolic arterial blood pressure (133 ± 13 to 121 ± 10 mm Hg, $p < 0.02$), and

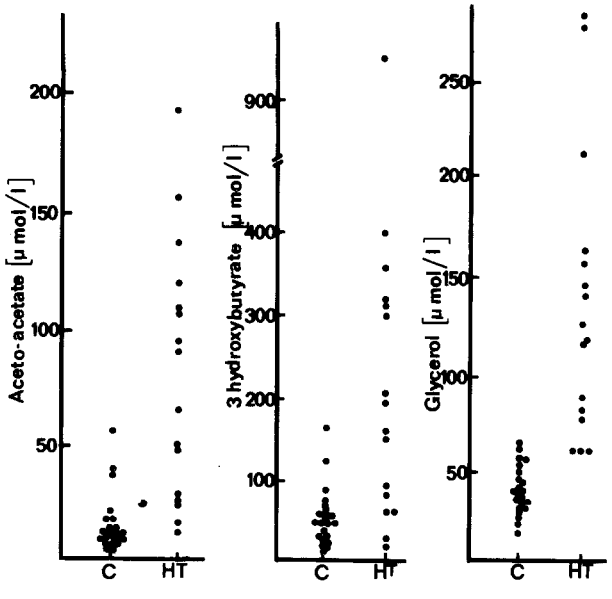

Fig. 1. See text

Table 2. Effect of propranolol on the metabolic response of 8 euthyroid obese subjects to a 600 kcal/day diet. Venous blood samples were collected at 0800 h after a 12 h overnight fast. Placebo or propranolol (40 mg orally every 6 h) were given from day 3 to day 7 of the hypocaloric diet in a double blind manner. n = 4 in each group. Values are expressed as mean ± SD, or mean and range (in parentheses) for obviously non-normal distributions. The response to the hypocaloric diet was the same in the two groups

	Placebo				Propranolol			
	Day 1	Day 3	Day 5	Day 7	Day 1	Day 3	Day 5	Day 7
Blood glucose mmol/l	4.5 ± 0.3	4.1 ± 0.2	4.0 ± 0.4	3.7 ± 0.4	4.7 ± 0.4	3.8 ± 0.4	4.0 ± 0.4	3.8 ± 0.5
Blood alanine µmol/l	268 ± 47	219 ± 41	200 ± 41	193 ± 53	257 ± 60	213 ± 60	209 ± 60	188 ± 80
Blood glycerol µmol/l	65 [49–80]	90 [55–145]	83 [40–150]	78 [45–130]	68 [52–83]	94 [65–126]	100 [75–115]	99 [80–120]
Blood total ketone bodies µmol/l	105 [50–230]	520 [250–1180]	858 [580–1520]	1130 [670–1900]	109 [70–190]	340 [155–640]	690 [600–870]	1050 [880–1255]
Plasma non-esterified fatty acids	146 ± 49	202 ± 42	230 ± 65	220 ± 100	124 ± 45	181 ± 83	201 ± 67	278 ± 132
Serum IRI mU/l	13.7 ± 8.5	8.5 ± 4.2	11.4 ± 5.2	11.8 ± 8.0	13.0 ± 6.5	9.3 ± 4.5	9.1 ± 4.9	9.2 ± 5.5

in triiodothyronine ($p < 0.01$). Thyroxine, free thyroxine index, IRI, IRG, glucose and NEFA remained unchanged. The most striking result was the dramatic fall of both ketone body ($p < 0.001$) and glycerol ($p < 0.001$). Blood total ketone bodies returned to normal values but glycerol was still higher than in the control group ($p < 0.01$). There was no longer a correlation between thyroid hormone levels and glycerol or ketone bodies. The correlation between glycerol and ketone bodies persisted ($r = 0.71$, $p < 0.01$, $y = 2.02 \text{ x } -41$). To exclude the possibility that the modification observed after β-blockade was due to spontaneous day to day variation and/or to rest linked to hospitalization the study was performed with placebo instead of propranolol in 6 other hyperthyroid patients: there was no significant change in triiodothyronine (453 ± 144 vs 500 ± 213 ng/100 ml) glycerol (98, range 65-147, vs 92, range 65-115 mmol/l) and ketone bodies (398, range 168-747, vs 347, range 170-480, mmol/l).

Effect of Propranolol on the Metabolic Response to Hypocaloric Diet. To test if the observed effect of propranolol on ketone bodies and glycerol was specific to hyperthyroidism we studied the effect of propranolol on blood ketone bodies, glycerol and NEFA in euthyroid obese subjects on a hypocaloric diet. Table 2 shows that on a 600 kcal/day diet the increase in glycerol, NEFA and ketone bodies and decrease in glucose, alanine and IRI were similar with either placebo or propranolol administration.

Discussion

Our data clearly show that blood levels of total ketone bodies and glycerol are increased in hyperthyroidism (Table 1, Fig. 1). This increase appears to be related to thyroid hormone excess. Our results agree with those of Bartels et al. [2] and Carter et al. [3], and also with those of Tibbling [15] showing an increase of glycerol levels in spite of increased glycerol turnover in hyperthyroidism. They disagree with those of Wahren et al. [4] who found no difference in plasma ketone body levels between hyperthyroid and euthyroid subjects. However their values [4] in euthyroid subjects are surprisingly high whereas ours agree with the values reported by Foster et al. [16] and Ruderman et al. [17].

The increase in ketone bodies may involve several factors. An absolute decrease of their oxidation seems unlikely since hyperthyroidism is associated with increased fuel consumption [1]. A relative decrease with imbalance between production and oxidation, as observed in fasted obese subjects [18], is possible. Nevertheless increased ketogenesis seems the most likely explanation of the observed increased ketonaemia. This increase could be in favour of an "accelerated starvation state" in hyperthyroidism and could be due merely to an inappropriately low caloric intake in the face of an increased need of calories. However, the metabolic status of hyperthyroid patients seems somewhat different from that of starved subjects. The increased ketonaemia was associated with an elevated blood glucose and normal alanine, IRI and IRG levels (Table 1) instead of the decreased glucose, alanine and IRI levels and increased IRG level usually observed in starvation [19]. The metabolic status of hyperthyroid patients could also suggest the existence of an insulin-resistant state. However the blood glucose of hyperthyroid patients responds normally to exogenous insulin [20]. Our results are not in favour of an insulin-deficient state. Data in the literature show considerable disagreement on the effect of hyperthyroidism on insulin secretion. Experimentally, in rats, thyroid hormones excess reduces glucose-induced insulin secretion [21–22], but in man increased [23] as well as normal [24] or decreased [25] responses have been reported. Johnston et al. [26] showed that somatostatin infusion results in a greater increase of ketone body levels in triiodothyronine treated subjects than in control subjects. Thus the increased ketogenesis of hyperthyroidism seems independent of modifications of insulin or glucagon secretion.

The increased ketogenesis could be due either entirely to enhanced lipolysis [15, 27], or also to a diversion of hepatic metabolism of fatty acids to ketogenesis. The correlation between glycerol and ketone bodies supports the first hypothesis. But NEFA, as in the study of Bartels et al. [2], were not significantly increased and no correlation between NEFA and ketone body levels could be found. This finding of increased glycerol but near normal NEFA levels suggests that the reesterification of NEFA in adipose tissue may be enhanced [28]. It is difficult therefore to establish whether the amount of NEFA delivered to the liver is increased or not. Whatever the role of lipolysis, a direct effect of thyroid status on ketogenesis has been recently demonstrated in the perfused rat liver [29].

Hyperthyroidism may influence lipolysis and ketogenesis by a catecholamine mediated mechanism. The dramatic fall of ketone body and glycerol (Table 1) upon propranolol administration would support this hypothesis. This fall contrasts with the lack of effect of β-blockade upon the metabolic response of euthyroid obese subjects to half-starvation (Table 2). This suggests that the mechanisms

responsible for lipolysis and ketogenesis in these two circumstances may be different. The effect of propranolol in hyperthyroidism could be related to the decrease of triiodothyronine (Table 1). However it should be emphasized that upon propranolol administration 1) the correlation between thyroid hormones and ketone bodies or glycerol disappeared, 2) levels of ketone bodies and glycerol were lower than those expected for the corresponding triiodothyronine levels, ketone bodies in particular returning towards control values despite high triiodothyronine levels. The effect of propranolol is probably not related to an action on insulin or glucagon secretion since, in our study, it did not modify basal levels of IRI and IRG. Thus propranolol may have acted by suppressing a β-receptor mediated effect of catecholamines. Since plasma catecholamines concentrations are subnormal in hyperthyroidism [30–31] the effect of propranolol suggests an increased sensitivity of tissues to catecholamines. Such an hypothesis is supported by in vivo [32] and in vitro [33] studies showing an increased responsiveness of adipose tissue to the β-receptor mediated effect of catecholamines in hyperthyroidism. The lack of effect of propranolol in euthyroid subjects agrees with the studies showing a decrease in the activity of the sympathetic nervous system during starvation [34–35].

Thus our data in men suggest that the increased lipolysis and ketonaemia observed in hyperthyroidism may be due, at least in part, to a β-receptor mediated effect of catecholamines. A direct effect of thyroid hormones themselves remains obviously possible.

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