Evaluation of endocrine tests. A: the TRH test in patients with hyperprolactinaemia

R. Le Moli[‡], E. Endert, E. Fliers[*], M.F. Prummel, W.M. Wiersinga

Department of Endocrinology and Metabolism, Academic Medical Centre of the University of Amsterdam, PO Box 22660, 1100 DD Amsterdam, the Netherlands, tel.: +31 (0)20-566 60 71, fax: +31 (0)20-691 76 82, e-mail: e.fliers@amc.uva.nl, [‡] present address: Department of Internal Medicine, Endocrinology and Metabolism, Garibaldi Hospital, University of Catania, Italy, [*] corresponding author

ABSTRACT

Background: In a previous study, we determined reference values for basal and thyrotropin-releasing hormone (TRH)-stimulated plasma concentrations of prolactin (PRL). The aim of the present study was to determine the clinical usefulness of the PRL response to TRH in the work-up of patients with hyperprolactinaemia.

Methods: We studied 92 consecutive patients referred for evaluation of hyperprolactinaemia. Patients with confirmed hyperprolactinaemia were divided into three groups: group A (pharmacological hyperprolactinaemia; n=2), group B (pathological hyperprolactinaemia; n=6) and group C (all other patients). Patients in group C underwent MRI of the pituitary and were subdivided into C1 (normal pituitary on MRI; n=6), C2 (slightly abnormal MRI; n=21), and C3 (evident microadenoma or macroadenoma on MRI; n=25 and 12, respectively). The MRI was technically insufficient in four patients. Basal PRL as determined by fluoroimmuno-metric assay and the PRL response to 400 µg TRH were determined in all patients.

Results: Hyperprolactinaemia was confirmed in 83% of the referred patients. Non-response, defined as a <2.5-fold PRL increase after TRH, occurred in one patient (50%) in group A, in 66% of patients in group B and in 99% of patients in group C. Within group C, basal PRL was not different between group C1 and C2, but higher (p=0.06) in group C3. The absolute PRL increase after TRH did not differ between the three subgroups. The relative PRL increase was smaller (p=0.03) in group C3 but overlapped considerably with groups C1 and C2. All patients except one in group C were so-called non-responders. Basal PRL

and absolute PRL increases after TRH correlated with the adenoma diameter on MRI (r=0.66, p=0.0002 and r=0.49, p=0.008, respectively).

Conclusion: In patients referred for elevated serum PRL, hyperprolactinaemia should be confirmed under standardised conditions. The absolute or relative PRL increase after 400 µg TRH does not help to differentiate between patients with prolactinoma or idiopathic hyperprolactinaemia. Therefore, the TRH stimulation test is not useful in the work-up of hyperprolactinaemia.

INTRODUCTION

Hyperprolactinaemia may be physiological (during pregnancy and lactation), pharmacological (for example by use of neuroleptics or oestrogens) or pathological. Among the pathological causes of hyperprolactinaemia are primary hypothyroidism, renal failure, hypothalamic or pituitary disease interfering with the secretion of dopamine to the pituitary, and prolactinomas. In a substantial number of patients with mild hyperprolactinaemia (between 25 and 100 µg/l) no cause can be found; this situation is usually referred to as idiopathic hyperprolactinaemia. The condition is reversible in a substantial percentage of patients and only occasionally develops further into a detectable pituitary adenoma.[1] In many patients, a detailed history and physical examination will reveal the cause of hyperprolactinaemia. In others, ancillary investigations may be necessary. In the older literature, a thyrotropin-releasing hormone (TRH)

stimulation test was advised since a diminished response of plasma prolactin (PRL) to intravenous TRH (<2.5-fold increase in plasma PRL after TRH) supported the presence of a prolactinoma, whereas a normal response was highly unusual.[2] However, a blunted response of PRL to TRH is not specific for prolactinoma and is also seen with other types of hyperprolactinaemia. Therefore, dynamic testing of PRL secretion may not add to basal PRL levels alone in the differential diagnosis of hyperprolactinaemia.[3] Furthermore, the advent of high-resolution imaging techniques such as magnetic resonance imaging (MRI) has made the TRH test obsolete in the work-up of hyper-prolactinaemia, according to many authors.[4] In a previous study, we established reference values for the plasma concentration of PRL and its response to TRH.[5] As part of an ongoing project aimed at standardising diagnostic procedures in our department, we proceeded and questioned the clinical usefulness of the TRH stimulation test in the work-up of patients with hyperprolactinaemia. Although indications for a TRH test are few[4] and some authors agree that TRH testing is not at all helpful, there is a paucity of studies clearly providing the evidence for this statement. To this end, we measured basal and TRH-stimulated plasma PRL under standardised conditions in 92 consecutive patients with hyperprolactinaemia, and analysed the results in relationship with the results of the pituitary MRI scan.

PATIENTS AND METHODS

Patients

We evaluated the clinical usefulness of the PRL response to TRH in the work-up of hyperprolactinaemia. Included were consecutive patients in whom clinical suspicion of hyperprolactinaemia was aroused by the existence of galactorrhoea, amenorrhoea, decreased libido or erectile dysfunction, or in whom hyperprolactinaemia had already been documented by the referring physician. Excluded were pregnant women (by assay of hCG in the urine) and breastfeeding women. Volunteers recruited by advertisements in a local newspaper served as controls.[5] Patients with confirmed hyperprolactinaemia according to the protocol and criteria described earlier[5] were divided into three groups. Group A had pharmacological hyperprolactinaemia. Group B had pathological hyperprolactinaemia caused by either renal insufficiency (plasma creatinine >200 μmol/L), severely impaired liver function, primary hypothyroidism or well-defined hypothalamic pituitary disorders clearly distinct from prolactinomas. Group C was composed of all the remaining patients and subdivided further based on pituitary MRI findings into group C1 (no abnormalities on MRI), group C2 (some abnormalities on MRI such as inhomogeneous pattern, pituitary asymmetry or partial

empty sella but no apparent mass lesion), and group C3 (evidence of pituitary microadenoma or macroadenoma).

TRH test

A TRH stimulation test was performed in all patients in the postabsorptive state and in recumbent position, starting between 8.30 and 9.30 am. Weight, height and blood pressure were recorded. An indwelling venous catheter was inserted (at t=-30 min) in an antecubital vein and a blood sample was taken at t=-15 min for measurement of PRL, creatinine, OT, PT and thyroid-stimulating hormone (TSH). At t=0 min, a second blood sample was taken for measurement of PRL. Additional blood samples were taken at t=20, t=60, t=120 and t=180 min after administration of 400 μg of TRH intravenously (TRH Relefact, Hoechst) at t=0 min. A subnormal PRL response was defined as an increase of less than 250% over the basal PRL concentration according to Assies *et al.*[2] Sera were stored at -20°C until assay.

Analytical and statistical methods

PRL was measured by a solid phase, two-site, time-resolved fluoroimmunometric assay (DELFIA Prolactin, Wallac Oy, Turku, Finland). The intra-assay coefficient of variation (CV) was 4 to 6% (5-24 μg/l); the interassay CV was 5.5 to 7.2% (4-50 μg/l). We calculated basal PRL as the mean of PRL at t=-15 min and t=0 min, the absolute PRL increase as peak PRL – basal PRL, and the relative PRL increase as [peak PRL – basal PRL]/basal PRL x 100%. The upper normal limit of basal PRL was taken as 25 μg/l for females and 19 μg/l for males as determined previously using precisely the same preanalytical and analytical methods.[5] Group differences were evaluated by non-parametric tests, i.e. the Kruskal-Wallis and Mann-Whitney U test. Correlations between basal PRL and PRL increases, and between PRL levels and prolactinoma size were evaluated by linear regression analysis. We used the SPSS 8.0 statistical package. In all tests, p values below 0.05 were considered statistically significant.

RESULTS

Sixteen of the 92 consecutively included patients had normal basal PRL values and were not analysed any further. From the remaining 76 patients (65 females and 11 males), two had pharmacological hyperprolactinaemia caused by penfluridole and ethinyl estradiol (group A), six had pathological hyperprolactinaemia caused by primary hypothyroidism (n=3), acromegaly (n=1), meningioma (n=1) and astrocytoma (n=1) (group B), thus leaving 68 patients for group C (*table 1*).
Basal PRL and the absolute PRL increase after TRH did not differ between groups A, B and C, although a tendency was

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Table 1

Basal PRL and TRH-stimulated PRL response in 50 healthy controls and in 76 hyperprolactinaemic patients (median values and range)

GROUPS[†]		CONTROLS	GROUP A	GROUP B	GROUP C	P VALUE
		n=50	n=2	n=6	n=68	group C vs group A+B
Sex (F, M)		25F, 25M	2F	6F	57F, 11M	
Age (years)		41 (22-66)	33 (32-35)	42 (27-58)	34 (19-79)	
BMI		24 (19-45)	26 (23-29)	21 (19-26)	25 (16-46)	
Basal	PRL (μg/l)	9 (4-25)	54 (42-66)	40 (26-225)	79 (23-13,000)	0.06
	PRL absolute (μg/l)	36 (2-120)	129 (84-173)	49 (10-103)	30 (-2-1250)	0.23
	PRL relative (%)	437 (18-1375)	273 (127-418)	74 (14-322)	29 (-8-345)	0.03
Non-responders[§]		24%	50%	66%	99%	

[†] *Controls derived from Le Moli et al.; group A = pharmacological hyperprolactinaemia, group B = pathological hyperprolactinaemia caused by primary hypothyroidism, acromegaly, meningioma, astrocytoma, group C = remaining patients including prolactinomas,* [§] *defined as relative PRL increase after TRH smaller than 250%.*

noted for higher basal PRL levels and lower absolute PRL increases in group C patients. The relative PRL increase after TRH was clearly lower in group C patients, giving rise to 99% of so-called TRH non-responders defined as a relative PRL increase after TRH smaller than 250%. In group C, MRI scans of four patients could not be assessed properly for technical reasons. Of the remaining 64 patients (53 females, 11 males), six had a normal pituitary MRI (group C1), 21 had slight MRI abnormalities (group C2) and 37 had clear evidence of pituitary adenomas (group C3, microadenomas n=25, macroadenomas n=12). Peak PRL levels after TRH were predominantly reached at t=20 min, but occurred at t=60 min in two patients from group C1, in three patients from group C2, and in five patients from group C3. Basal PRL was not different between group C1 and C2 but significantly higher in group C3 (*table 2* and *figure 1*). The absolute PRL increase did

not differ between the three groups, but the relative PRL increase after TRH was smaller in group C3.

Interestingly, all group C patients except one were so-called non-responders, a significant difference with the 24% TRH non-responders in the healthy controls ($p<0.001$). In the patients with definite microprolactinomas or macroprolactinomas (group C3), a significant relationship was observed between the adenoma diameter on MRI in millimetres and basal PRL ($r=0.66$, $p=0.0002$) and absolute PRL increase ($r=0.49$, $p=0.008$), but not with relative PRL increase ($r=0.06$, ns).
Body mass index (BMI) was not related to basal PRL or relative PRL increase in group C patients, but we did observe a negative relationship between BMI and the absolute PRL increase after TRH in patients of groups C1 and C2 (*figure 2*), which was absent in group C3 patients.

Table 2

Basal PRL and TRH-stimulated PRL response in 64 hyperprolactinaemic group C patients, subdivided according to pituitary MRI readings (median values and range)

GROUPS[†]		GROUP C1	GROUP C2	GROUP C3	P VALUE[*]	
		n=6	n=21	n=37	(C3vsC1)	(C3vsC2)
Sex (F, M)		4F, 2M	18F, 3M	31F, 6M		
Age (years)		38 (35-43)	32 (21-66)	33 (19-73)	ns	ns
BMI (kg/m²)		26 (23-41)	25 (16-36)	26 (19-46)	ns	ns
Basal	PRL (μg/l)	54 (34-71)	60 (24-170)	132 (23-13,000)	0.000	0.002
	PRL absolute (μg/l)	21 (6-93)	33 (3-252)	25 (-3-1250)	ns	ns
	PRL relative (%)	58 (12-130)	61 (9-345)	18 (-8-166)	0.09	0.001
Non-responders[§]		100%	95%	100%		

[†] *Group C1= normal pituitary MRI, group C2 = slight abnormalities on pituitary MRI but no mass lesion, group C3 = definite microadenoma or macroadenoma on pituitary MRI,* [§] *defined as relative PRL increase after TRH smaller than 250%,* [*] *no differences were observed between groups C1 and C2.*

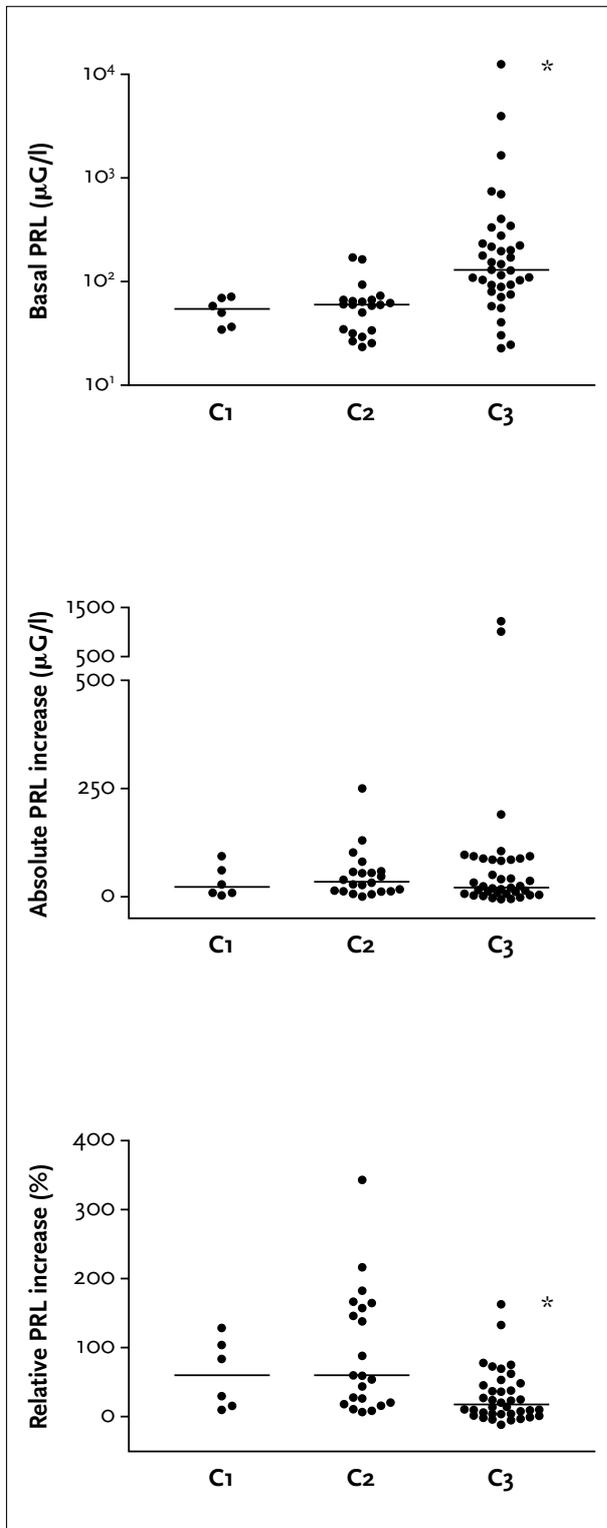Le Moli, et al. Evaluation of endocrine tests. A: the TRH test in patients with hyperprolactinaemia.

Figure 1

Basal PRL, absolute PRL increase after TRH, and relative PRL increase after TRH in 64 hyperprolactinaemic group C patients (for definition of groups see table 2)

Horizontal lines indicate median values. Basal PRL is not different between groups C1 and C2, but significantly higher in group C3. The absolute PRL does not show significant differences among the groups, but the relative PRL increase after TRH is significantly smaller in group C3. * Significantly different from groups C1 and C2.
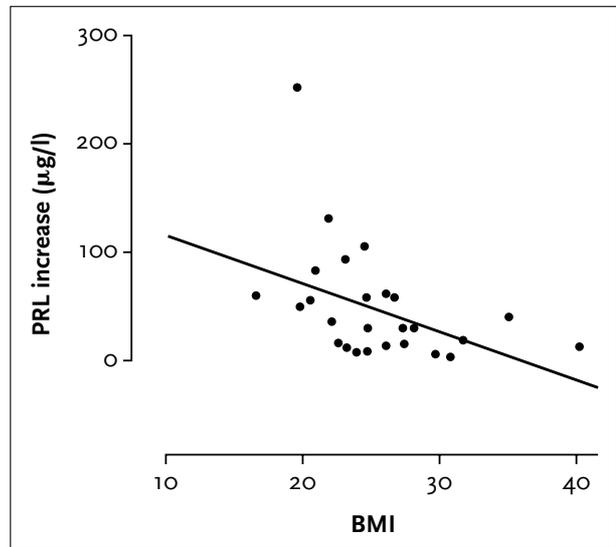

Figure 2

Relationship between BMI and the absolute PRL increase after TRH in hyperprolactinaemic group C patients with no or slight abnormalities on pituitary MRI (r=-0.43, p=0.02)

DISCUSSION

The present study was undertaken to determine the clinical usefulness of the TRH stimulation test in the setting of hyperprolactinaemia. Since many causes of hyperprolactinaemia are clear from the history, physical examination and routine laboratory tests (for example, pharmacological hyperprolactinaemia, renal failure), the question is whether the TRH stimulation test has any value in distinguishing idiopathic hyperprolactinaemia from prolactinoma. This differentiation has clinical relevance since idiopathic hyperprolactinaemia is a relatively benign and often self-limiting disease,[1] whereas patients with prolactinoma often require dopaminergical treatment as well as monitoring of tumour size in case of a macroprolactinoma.

In the present study, we used the protocol and reference values for basal plasma PRL described in our previous study.[5] Interestingly, hyperprolactinaemia was confirmed in only 83% of the referred patients. Since stress of any kind can cause a mild increase in serum PRL, our study reinforces the need to confirm hyperprolactinaemia under standardised conditions using an indwelling venous catheter before the patient is considered to have hyperprolactinaemia. Our present series of consecutive patients with confirmed hyperprolactinaemia contained only two patients with pharmacological hyperprolactinaemia and six patients with pathological hyperprolactinaemia. Five of these patients showed a <2.5-fold relative PRL increase after TRH, which is in accordance with other studies reporting a subnormal PRL response to TRH in more than 50% of patients with pharmacological and

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pathological hyperprolactinaemia (e.g.).[6] In the remaining patients (group C), we found a <2.5-fold PRL increase after TRH in 99% of patients irrespective of the presence of a pituitary tumour on the MRI. Apparently, a subnormal PRL response does not help to differentiate between idiopathic hyperprolactinaemia and prolactinoma, since group C1 consisted of six patients with a normal pituitary MRI. Responders were absent in group C1 even after lowering the threshold for a subnormal response to 150% as can be seen from *figure 1*. Shangold *et al*.[7] reported a subnormal PRL response to TRH (defined as a <2.0-fold PRL increase after 500 μg intravenous TRH) in 37 out of 49 patients with hyperprolactinaemia without signs of a prolactinoma as shown by polytomography or CT. Also Assies *et al*.[2] found subnormal PRL responses to TRH to occur as frequently in hyperprolactinaemic patients without signs of a pituitary adenoma as in patients with definite prolactinoma. Since the latter studies were performed before the availability of MRI, the possibility of undetected small microprolactinomas in these patients could not be excluded. The results of our present study favour the alternative explanation of subnormal PRL responses to TRH in the majority of patients with idiopathic hyper-prolactinaemia. In addition, we found 24% of subjects recruited from the general population to show a <2.5-fold PRL increase to TRH in our earlier study.[5] The majority of patients reached peak PRL levels after TRH at t=20 min, with only ten group C patients reaching peak PRL at t=60 min. Therefore, it is not necessary to extend the TRH stimulation test to t=120 or t=180 min before the maximal PRL response can be assessed. In accordance with earlier studies,[8] there was a positive and highly significant correlation of prolactinoma diameter with basal PRL and also with absolute PRL increase after TRH. In addition, hyperprolactinaemic patients without a clear adenoma on the MRI (group C1 and C2) showed a significant and negative correlation of absolute PRL increase after TRH and BMI. In obese women without hyperprolactinaemia, Donders *et al*.[9] showed a decreased PRL and increased TSH response to TRH as compared with normal weight women, possibly related to changes in serotonergic function. However, since a significant relationship between

BMI and PRL response to TRH was absent in our controls with similar BMI (*table 2*), this cannot be the only explanation. On the basis of the results of the present study, the TRH stimulation test can be omitted in the work-up of patients with hyperprolactinaemia. However, our study reinforces the need to confirm hyperprolactinaemia using standardised procedures for the assessment of basal PRL.

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