CHANGES OF SERUM TSH LEVEL DURING ORAL GLUCOSE TOLERANCE TEST: COMPARISON OF MORNING AND EVENING TEST WITH PLAIN CIRCADIAN TSH RHYTHM

P. LANGER, 1E. MARTINO, L. KSNANTOVÁ, 1L. GLASSO, M. VIGAS

Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia; 1Istituto di Endocrinologia, Universita di Pisa, Tirrenia-Pisa, Italia
E-mail: uenlang@savba.savba.sk

Objective. To compare the changes of TSH level in serum during oral glucose tolerance test (OGTT) with those resulting from a plain circadian rhythm and, in addition, to compare such changes between the morning and evening hours.

Methods. Oral glucose tolerance tests were performed in groups of 8-20 adults after the oral administration of glucose (75 g in 400 ml tap water) at 8.00, 10.00 and 20.00 h. Blood samples for the estimation of TSH (supersensitive IRMA method) were taken in 30 min intervals for following 3 hours. In the same groups of subjects the blood samples were obtained between 8.00 and 13.00 h or between 20.00 and 23.00 h one week later for the assessment of plain circadian rhythm of TSH levels.

Results. The level of TSH in a group subjected to OGTT at 8.00 h was significantly decreased (P<0.05) between 8.30 and 10.30 h, i.e. 30-150 min after glucose administration which was parallel to the circadian decrease found in the same subjects. However, this was followed by an increase of TSH up to the original level reached at 11.00 h which was contrasting to a circadian decrease. Similar pattern was found also when OGTT was started at 10.00 h. In a group subjected to the evening OGTT at 20.00 h similar decrease of TSH level was found at 21.00 h which was contrasting to the circadian increase. However, this was followed by a remarkable increase of TSH level between 21.00 and 23.00 h which was parallel to the circadian trend, but much more abrupt than that found without the previous administration of glucose.

Conclusions. In both the morning and evening OGTT a decrease of TSH level was found between 30 and 90 min after glucose administration which was followed by an increase between 90 and 180 min after that. The decrease during the morning test was parallel to the circadian trend, while the increase was opposite to that. However, an inverse figure was found in the evening test, the decrease of TSH being opposite and following increase being parallel to the circadian trend.

Key words: Glucose tolerance test – Serum TSH – Circadian rhythm

There are several reports on the effect of changes in carbohydrate metabolism such as insulin induced hypoglycemia (Smythe et al. 1985; Lambert et al. 1986; Ježova et al. 1987; Watanabe et al. 1987), intracellular glucopenia after 2-deoxy-glucose (Martino et al. 1984; Painson et al. 1985) or oral glucose (Berelowitz et al. 1982; Grill et al. 1984) as well as exercise-induced glucose decrease (Scheen et al. 1998) either on the release of anterior or pituitary hormones or their level in blood which may be mediated by hypothalamic releasing or inhibiting factors.
So far, the interrelations between carbohydrate metabolism and thyrotropin were studied with the aid of TRH test (ROEJDMARK and NYGREN 1983; ROEJDMARK et al. 1989), insulin hypoglycemia (LAMBERTON et al. 1986; VIGAS et al., 1988) in man or with 2-deoxy-glucose in rats (MARTINO et al. 1984). Recently, KAMAT et al. (1995) reported a significant decline in serum TSH after normocaloric, but not after hypocaloric isobulk meal and suggested that this may result from food-induced elevation of somatostatin and consequent suppression of TSH secretion. Simultaneously YANG et al. (1995) found the suppression of TRH-stimulated TSH secretion by glucose-induced hypothalamic somatostatin release.

Since the the normocaloric mixed meal as used by KAMAT et al. (1995) does not appear to be precisely defined, we focused our attention to the administration of purely defined stimulus such as glucose. Moreover, we intended to test whether the administration of glucose could influence not only TRH-stimulated (YANG et al. 1995), but also basal TSH release and to compare the effect of glucose on TSH level in morning and evening hours as related to the circadian rhythm of TSH level.

Subjects and Methods

Healthy human volunteers who participated in this study gave their written informed consent.

Observation 1: 12 staff members of this Institute (9 males and 3 females, age range 27 to 43 years) and 8 medical students (all males, 21 years of age) were subjected to oral glucose (75 g glucose in 400 ml tap water) tolerance test (OGTT) between 8.00 and 11.00 h after overnight fast. One week later the blood samples were taken from 10 of those subjects between 8.00 and 13.00 h to obtain control values in respect to circadian rhythm of TSH level.

Observation 2: 20 medical students (all males, 21 years of age) were subjected to OGTT (see above) between 10.00 and 12.00 h after overnight fast.

Observation 3: The same group of 8 medical students (all males, age 21 years) who participated in the observation 1 was subjected to following 2 tests in one week intervals: 1. evening control test: blood sampling after 12 h fast every 30 min between 20.00 and 23.00 h; 2. evening OGTT between 20.00 and 23.00 h after 12 h fast (for time intervals see Line A, Fig 1).

Laboratory methods. The blood samples were obtained through indwelling catheter inserted into a cubital vein. The serum was obtained by centrifugation and kept frozen until assayed. The level of sensitive TSH was estimated with the aid of IRMA using the kits purchased from Travenol (Belgium). The samples from each observation were estimated in duplicate and in the same assay.

Statistical evaluation. The data on TSH level were evaluated with one way repeated measures ANOVA followed by Friedman’s repeated measures analysis of variance on ranks.

Results

As expected, in each observation the levels of glucose and insulin (not shown) increased significantly after the ingestion of glucose showing a maximum at 30 min and then declining up to about the initial level at 120 to 150 min. No deviations were found from normal pattern of OGTT.
**Morning tests.** As shown in Fig. 1, the level of TSH in a group subjected to OGTT at 8.00 h (Line A, Fig. 1) was significantly decreased (P<0.05) at 8.30, 9.00, 9.30, 10.00 and 10.30 h, i.e. 30-150 min after glucose administration. At all intervals mentioned the level of TSH was also significantly lower than that at 11.00 h. This means that at 11.00 h the TSH level increased to the original value.

The above described decrease of TSH within first 90 min (i.e. between 8.00 and 9.30 h) of OGTT (P<0.05) appears to be parallel to the physiological decrease of TSH level in such a period of the day due to the circadian rhythm. However, the increase of that level during the second period of OGTT (i.e. between 9.30 and 11.00 h) certainly is opposite to the continuing physiological decline. It should be mentioned that the control examination (Line B, Fig. 1) showed a continuous decrease for as long as 5 h, the TSH levels at 9.00, 10.00, 11.00, 12.00 and 13.00 h being significantly lower (P<0.05) than the initial level (i.e at 8.00 h).

In the group subjected to OGTT at 10.00 h (Line C, Fig. 1), significant decrease of TSH level between 10.00 and 11.30 h was also parallel to the circadian trend, but the increase of that at 12.00 h (P<0.05) was again opposite to the physiological trend of TSH levels and was similar to that found between 9.30 and 11.00 h during the OGTT performed at 8.00 h (Line A, Fig. 1).

**Evening test.** Similar, but somewhat different changes of TSH level were found in the evening OGTT (Line A, Fig. 2). Thus, even in this case a decrease of that (P<0.05) was found at 21.00 h as compared to the initial level at 20.00 h. However, it should be stressed that in this case such decrease was opposite to the increasing trend of circadian rhythm of TSH level (Line B, Fig. 2). In addition, a highly significant increase of TSH level was found between 21.00 and 23.00 h (i.e. 60-180 min after glucose administration). This was remarkably more expressed than that due to the circadian rhythm and was also apparently much more abrupt than that during the second half of OGTT in morning hours.

**Discussion**

From these results a decrease of TSH level during the first 90 min of OGTT followed by an increase during the next 90 min appears to be repeatedly established which was mostly convincing in the evening OGTT. Such phenomenon could be explained either by the changes in TSH secretion rate or by the changes in peripheral disposal rate of this hormone or by a combination of both.

Perhaps the most plausible would be a possibility of decreased TSH secretion after the administration of glucose which might be due to the increase of either peripheral or hypothalamic somatostatin release. The former possibility may be supported by repeated findings on a decrease of TSH level after the infusion of exogenous somatostatin (reviewed by BRAINT et al., 1989). In addition, in dogs and normal volunteers the increase of somatostatin occurring 90-120 min after ingestion of test meals have been reported which presumably reflect the release of somatostatin from the gut (SHIBASAKI et al. 1989; GREENBERG et al. 1993; MARTINEZ et al. 1993; HILDEBRAND et al. 1994).

The next question probably should be whether the glucose or insulin themselves may influence
the release of somatostatin from the hypothalamus. In this respect the observation by Grill et al. (1984) should be presented who found an increase of plasma somatostatin level at 30-90 min after oral glucose, but they did not answer the question whether such somatostatin comes from the brain or from the periphery. Several authors reported an increase of somatostatin release from the hypothalamus after 2-deoxy-glucose in rats in vivo (Lengyel et al. 1984; Paison et al. 1985) or in vitro (Berelowitz et al. 1982) and similar type of effect was described by Smythe et al. (1982) which was due to a decrease of brain glucose utilization after insulin injection in rats. Although Chihara et al. (1979) did not find any changes of somatostatin release from median eminence after injection of microquantities of glucose into cerebral ventricles, such finding must not be relevant to the present observation.

There are only few observations related to the effect of glucose or insulin directly on the TRH-TSH axis. Thus, in rats Martino et al. (1984) found a decrease of hypothalamic TRH and increase of blood TSH after intracellular glucopenia resulting from the administration of 2-deoxy-glucose. After insulin injection an increase of plasma TRH level in rats was reported by Matsuma et al. (1984). Roedmark et al. (1988) did not observe any difference of TSH level in TRH test between human volunteers subjected to marked hyperglycemia with the use of hyperglycemic clamp technic. In diabetic patients with hyperglycemia a blunted nocturnal peak of TSH level was found which was normalized after the correction of hyperglycemia (Bartalena et al., 1993).

Finally, a direct neuromodulatory effect of insulin on TSH release from the pituitary may be taken into account which was discussed previously on the basis of the observed decrease of TSH level during insulin induced hypoglycemia (Vigas et al., 1989). The present data support the view on such possible effect of insulin, since a decrease of TSH level was coincident with the increased level of insulin. In contrast, our findings do not support the view by Lamberton et al. (1986) who explained a decrease of TSH level after insulin administration as a result of stress effect from acute hypoglycemia, since in these observations we found a decrease of TSH together with increased glycemia.

Since there undoubtedly exists a circadian rhythm of TSH level in serum with a zenit at about 22.00 h to 4.00 and a nadir at about 10.00 to 16.00 h (Sowers et al. 1982; Greenspan et al. 1986; Rossmannith et al. 1988; Brabant et al. 1989), the question arose to which extent this might interfere with the observed changes during OGTT. It should be pointed out, first, that in the morning tests the decrease of TSH level after glucose is difficult to be distinguished from the physiological decrease of that level occurring at the same time as shown namely in Fig. 1. Second, there was an increase in TSH level at 180 min after glucose in the morning tests and, in addition, its direction was opposite to the physiological decrease. In contrast, in the evening test a decrease of TSH level after glucose tended against the physiological increase, while the increase at 2-3 h after glucose was remarkably more expressed than the increase due to the circadian rhythm and also than that in morning test.

Our findings are in agreement with those by Kamat et al. (1995) who recently showed a decrease of TSH level after normocaloric meal and suggested that this may result from food-induced elevation of somatostatin. Simultaneously Yang et al. (1995) found the suppression of TRH-stimulated TSH secretion by glucose-induced hypothalamic somatostatin release.

However, it appears that the mechanism resulting in the increase of TSH level which follows the initial decrease after glucose administration still remains to be explained. Actually, Kamat et al. (1995) described only a decrease of TSH level after lunch or dinner, while Yang et al. (1995) observed the suppression of TRH-induced TSH secretion including that after glucose pretreatment after the inhibition of somatostatin release by pyridostigmine. In our study, remarkably increasing TSH level was found during the second half of OGTT which was synchronous with the decrease of blood glucose and insulin level, but opposite to the circadian decrease of TSH. In contrast, similar increase of TSH level during the evening OGTT was much more abrupt and parallel to the circadian increase of TSH, although again synchronous with the decrease of glucose and insulin level.
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**Corresponding author:** Pavel Langer, M.D., DrSc.
Institute of Experimental Endocrinology
Slovak Academy of Sciences
Vlarska 3
833 06 Bratislava
E-mail: uenlang@savba.savba.sk