**Effect of Changes in Plasma Free Fatty Acids Level on Secretion of Human Growth Hormone**

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**Synopsis**

The relationship between the changes of plasma free fatty acids (FFA) and human growth hormone (HGH) levels was investigated in normal young male subjects.

The administration of nicotinic acid with its concomitant lowering of plasma FFA was followed by a rise of plasma HGH. The HGH rise could be inhibited by the simultaneous administration of heparin which prevented the fall of plasma FFA, or by glucose administration. The administration of heparin alone resulted in an increase of plasma HGH also in association with the fall of plasma FFA in the absence of any changes in plasma glucose levels. The rise of plasma HGH failed to occur when plasma FFA was kept elevated or when glucose was given.

From these results, it was suggested that the fall of plasma FFA has a stimulatory effect and in turn, the elevated plasma FFA has an inhibitory effect on HGH secretion.

By the development of radioimmunoassay, much information has been accumulated about the factors which influence the secretion of human growth hormone (HGH).

There is little doubt about the existence of a specific glucose sensitive growth hormone regulatory system. Secretion of HGH is stimulated by insulin induced hypoglycemia (Roth *et al.*, 1963a), falling blood glucose values without hypoglycemia (Roth *et al.*, 1963b) or the administration of 2-deoxy-D-glucose (Roth *et al.*, 1963b; Wegienka *et al.*, 1967). In addition, other stimuli such as exercise (Roth *et al.*, 1963b; Hunter and Greenwood, 1964) or prolonged starvation (Roth *et al.*, 1963b; Cahill *et al.*, 1966) have been shown to enhance HGH secretion.

From these observations it has been postulated that HGH secretion is stimulated by a shortage of carbohydrate substrate for energy metabolism. But many other factors independent of glucose utilization were reported to influence HGH secretion. Infusion of arginine or several other amino acids was demonstrated to increase HGH secretion (Knopf *et al.*, 1965).

Although growth hormone has profound effects on fat metabolism, little information has been obtained on the effects of changes in fat metabolism on HGH secretion. The oral ingestion of olive oil (Glick *et al.*, 1965) or 50 grams of butter (Irie, 1968) does not alter plasma HGH levels. Short chain fatty acids such as propionate or butylate (Hertelendy *et al.*, 1969) in sheep and ketone body infusion in monkey (Blackard and Heidingsfelder, 1969a) were reported not to stimulate HGH secretion.

When administered *in vivo* (Raben and Hollenberg, 1959) or added *in vitro* system (Fain *et al.*, 1965), growth hormone increases mobilization of FFA, which are normally the main source of the fat burned in the body. An attempt was made, therefore, to investigate a possibility that a negative feedback mechanism operates between plasma FFA levels and HGH secretion. A part of the present works was preliminarily reported elsewhere with a limited number of data (Irie *et al.*, 1967; Tsushima *et al.*, 1970).
Materials and Methods

All test subjects were normal young male volunteers, their ages ranging from 20 to 25 years old. They had no family history of diabetes mellitus or other metabolic disorders. Obese subjects were not involved. After an overnight fast (12–15 hr), they were kept at bed rest for at least 30 min before starting the tests, and avoided sleep during the tests, since sleep has been known to initiate secretion of HGH (Honda et al., 1969; Quabbe et al., 1966).

The study was divided into seven parts, according to the following purposes:
(1) Ten subjects were given 20 ml of saline intravenously at 0 time as controls.
(2) In fourteen subjects, 200 mg of nicotinic acid in 20 ml of saline, was administered intravenously in 2 divided doses at 0 and 20 min for the purpose of lowering plasma FFA levels.
(3) Eight subjects received 200 mg of nicotinic acid as mentioned above, combined with intravenous administration of 1000 units of heparin sodium at 10 min in order to inhibit the fall of plasma FFA by nicotinic acid.
(4) In five subjects, the injection of nicotinic acid as before was followed by oral administration of 25 g of glucose at 30, 60, 90 and 120 min each.
(5) Seven subjects received were intravenously with 1000 units of heparin at 0 time.
(6) In six subjects, 1000 units of heparin was injected intravenously at 0 time and further injection of heparin 500 units at 60 and 90 min was made to keep plasma FFA level elevated.
(7) Five subjects were given 50 g of glucose orally at 60 and 90 min, combined with intravenous injection of 1000 units of heparin at 0 time.

In all the subjects, an indwelling catheter was placed into antecubital vein and kept patent by a slow infusion of saline. All blood samplings and injections were made through this catheter. Blood specimens were drawn at 0 time and at 30 min intervals with 3 hr had passed with heparinized syringes and centrifuged immediately. Obtained plasma was stored at $-20^\circ\text{C}$ until plasma concentrations of HGH, immuno-reactive insulin (IRI), FFA, glucose and $\alpha$-amino nitrogen were determined. Plasma HGH was measured by a modification of the double antibody radioimmunoassay method of Schalch and Parker (1964). Wilhelm’s highly purified HGH (HS 1032 B) was used as a standard. Minimum detectable dose of plasma HGH in our laboratory was 0.1 ng/ml using 1:10 diluted plasma. Plasma concentrations of IRI, glucose, FFA and $\alpha$-amino nitrogen were determined by radioimmunoassay (Kanazawa et al., 1966), glucose oxidase method (Hugget and Nixon, 1957), Dole’s method (1956) and ninhydrin colorimetric method (Fisher et al., 1963) respectively.

Results

1 Effect of saline administration

In the control group, there were no significant changes in plasma IRI, glucose, FFA, $\alpha$-amino nitrogen and HGH values during 3 hr after saline administration (Fig. 1). Basal plasma HGH and FFA values in this group were $1.44\pm0.65$ ng/ml (mean±SEM), $586\pm55$ μeq/liter respectively.

2 Effect of nicotinic acid administration

In this group, there was an initial drop of plasma FFA to about 60% of the starting value ($545\pm58$ μeq/liter), then a return to the baseline at 90 min, followed by a sharp rebound (secondary rise of plasma FFA). Basal plasma HGH level was $1.58\pm0.40$ ng/ml, which was not different from those of the control group. Following nicotinic acid ad-

![Fig. 1. Plasma FFA (described as % of the baseline), $\alpha$-amino nitrogen, IRI, glucose and HGH values after administration of 20 ml of saline at 0 time. (mean±SEM, N: 10)](image-url)
administration, plasma HGH increased gradually, and the HGH values at 30 and 60 min were significantly higher than the baseline (p < 0.05). The peak value attained at 120 min was 14.27 ± 4.40 ng/ml, which is significantly higher than the value at 120 min in the control group. As shown in Figure 2, plasma IRI, glucose and α-amino nitrogen values showed no significant changes throughout the periods.

3 Effect of nicotinic acid administration with heparin

When nicotinic acid was administered combined with heparin, the initial drop of plasma FFA from the baseline (642 ± 84 μeq/liter) was inhibited and the secondary rise of plasma FFA was much reduced. Plasma FFA value at 180 min was 949 ± 70 μeq/liter, whereas it was 1169 ± 77 μeq/liter in the case of nicotinic acid administration without heparin. Basal HGH value was 2.37 ± 0.98 ng/ml and any appreciable changes in plasma HGH level could not be observed over 3 hr (Fig. 3). There were no significant changes also in plasma glucose and α-amino nitrogen concentrations.

4 Effect of nicotinic acid administration followed by glucose loading

When nicotinic acid injections were followed by glucose administration, plasma FFA was kept depressed below the baseline (586 ± 10 μeq/liter) throughout the periods. Basal HGH value was 1.78 ± 0.76 ng/ml and the elevation of plasma HGH failed to occur. The results were shown in Figure 4.

5 Effect of heparin administration

Intravenous administration of 1000 units of
heparin led to a sharp rise of plasma FFA (baseline, 557±62 μeq/liter), reaching a peak at 30 min (1366±135 μeq/liter) followed by a rapid fall. In association with the fall of plasma FFA from the peak value, plasma HGH (baseline, 2.51±0.77 ng/ml) showed a gradual increase as shown in Figure 5. The HGH values at 120, 150 and 180 min were significantly higher than the respective values of the control group (p <0.01). The peak HGH value at 150 min was 12.19±2.35 ng/ml. No appreciable changes were noted in plasma glucose, IRI and α-amino nitrogen levels.

6 Effect of repeated administration of heparin

In the experiment of repeated heparin injections, plasma FFA level was kept elevated above 250% of the starting value (490±56 μeq/liter) for the first 2 hours and then declined, but the value at 180 min remained high at 200%. Basal HGH value was 1.38±0.24 ng/ml and the rise of plasma HGH, which was observed in the experiment of heparin single injection, was abolished. The plasma concentrations of glucose and α-amino nitrogen remained unchanged for 3 hr as shown in Figure 6.

7 Effect of heparin administration followed by glucose loading

When heparin injection was followed by glucose administration, a rapid fall of plasma FFA was noted accompanied with hyperglycemia and there occurred no significant increase of plasma HGH. Basal plasma HGH and FFA were 1.09±0.49 ng/ml and 450±37
Discussion

On the basis of the evidences that stress (Glick et al., 1965; Meyer and Knobil, 1967) or exercise, not only stimulates HGH secretion, but increases FFA mobilization as well (Bogdonoff et al., 1959; Havel et al., 1963), test subjects were kept at bed rest during the test and an indwelling catheter was used in order to avoid frequent venous puncture. Under these circumstances, there were no significant changes of plasma FFA and HGH levels in the control experiment, which was in keeping with the finding of Frohman et al. (1967b).

In the present study, nicotinic acid was used to investigate the effect of depressed plasma FFA levels on HGH secretion. Nicotinic acid in vitro inhibits the stimulation of lipolysis in isolated adipose tissue by catecholamine (Carlson, 1963), ACTH (Fain et al., 1966) and

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**Fig. 6.** Plasma FFA (% of the initial), α-amino nitrogen, glucose and HGH values after intravenous administration of 1000 units of heparin at 0 time, and 500 units at 60 and 90 min. (mean±SEM, N: 6) μeq/liter respectively (Fig. 7).

**Fig. 7.** Plasma FFA (% of the initial), α-amino nitrogen, glucose and HGH values after intravenous administration of 1000 units of heparin at 0 time, combined with oral administration of 50 g of glucose at 60 and 90 min. (mean±SEM, N: 5).
growth hormone with glucocorticoids (Fain et al., 1966). Carlson and Oró (1962) demonstrated that acute administration of nicotinic acid to man caused the plasma FFA to decrease quickly followed by a secondary rise. It is in accordance with the present findings. The mechanism of the decrease in plasma FFA is not completely clear, but it has been proposed that nicotinic acid may exert its anti-lipolytic action by depressing the production of 3',5' Cyclic AMP in adipose tissue (Peterson et al., 1967).

Administration of nicotinic acid alone, with heparin or with glucose, resulted in the same degree of flush of the face and extremities. However, there occurred a marked elevation of plasma HGH only in the group treated with nicotinic acid alone. Thus, the increase of plasma HGH cannot be considered due to stress or the vasodilating effect of nicotinic acid. The rise of plasma HGH by nicotinic acid differs from the stimulation of HGH secretion by hypoglycemia or amino acids infusion, since no appreciable changes in plasma glucose, α-amino nitrogen and IRI levels were observed.

From these results, it is suggested that lowering of plasma FFA by nicotinic acid can stimulate HGH secretion and that the HGH rise is abolished when the decrease of plasma FFA is prevented by simultaneous administration of heparin. Though the HGH value at 60 min was significantly elevated from the baseline, the HGH response to nicotinic acid is rather delayed as compared to that in insulin induced hypoglycemia or arginine infusion. The reason for the delay remains to be determined.

Hartog et al. (1967) reported that during the exercise, the lowering of plasma FFA by the administration of nicotinic acid resulted in prolongation of high HGH levels, which is compatible with the present findings. On the other hand, in rhesus monkey nicotinic acid did not induce the increase of plasma GH (Blackard and Heidingsfelder, 1969b). This ineffectiveness may be due to anesthesia or elevated basal level of plasma GH, which is known to reduce GH responses to various stimuli (Frohman et al., 1967a).

The mechanism of the FFA rebound following nicotinic acid administration has not been established. The lack of FFA rebound in adrenalectomized-hypophysectomized rats indicates that both the pituitary and adrenal functions are required for the rebound (Pereira, 1967).

In hypopituitary subjects lacking HGH secretion, the rebound is significantly lower than in normal subjects (Irie et al., 1970b). It appears, therefore, that growth hormone is at least in part, responsible for the secondary rise of plasma FFA after nicotinic acid administration.

In the experiment of heparin, the rise of plasma HGH was observed also in association with the fall of plasma FFA, whereas it did not occur when plasma FFA was kept elevated above 200% of the basal level. However, the HGH rise following heparin administration is somewhat different from that by nicotinic acid. The secretion of HGH was initiated without the reduction of plasma FFA below the baseline. It is conceivable that the subjects who received heparin have adapted to the elevated FFA level and the fall of plasma FFA, even if not reduced below the baseline, can be a stimulus to HGH secretion. Schalch and Kipnis (1965) reported that plasma HGH remained unchanged after the fat meal-heparin regimen, but it was only 60 min observation after heparin injection and the plasma FFA value remained above 1300 μeq/liter during the periods. In our present experiment, there were also no changes in plasma HGH levels for the initial 60 min.

As in the case of nicotinic acid administration, heparin did not induce any significant changes in plasma glucose, IRI and α-amino nitrogen levels. The rise of plasma HGH, therefore, cannot be ascribed to these factors. Stress or direct effect of heparin can be ruled out, since neither repeated heparin injections nor heparin injection followed by glucose ad-
administration resulted in the elevation of plasma HGH.

These results suggest that the fall of plasma FFA, whether it is absolute or relative, can be one of the stimuli to HGH secretion and the continuous elevation of plasma FFA inhibits the response. Another line of the evidence for it, is our previous observation that administration of 3.3 g of acetyl salicylate in normal subjects resulted in an increase of plasma HGH in association with the fall of plasma FFA without any changes of plasma glucose levels (Irie, 1968). Furthermore, analysis of the relationship between the changes in plasma FFA and HGH levels during the 3 days fasting in five normal subjects, revealed that the fall of plasma FFA was associated with the rise of plasma HGH (Irie et al., 1970a).

It is not clear why the level of plasma FFA is related with the secretion of HGH. Recent observations demonstrated a stimulatory effect of α-adrenergic receptors and an inhibitory effect of β-receptors on HGH secretion (Imura et al., 1968; Blackard and Heidingsfelder, 1968). On the other hand, the mobilization of FFA by catecholamine in adipose tissue has been shown to be mediated through β-adrenergic receptors (Pinter and Pattee, 1967). A common regulatory mechanism might be postulated in FFA mobilization and HGH secretion. Though one possible mechanism is the adenyl cyclase-Cyclic AMP system to which adrenergic receptors are closely related (Robinson et al., 1967), further studies are needed to determine whether the action of Cyclic AMP can be regarded as specific to HGH secretion.

The secretion of HGH is generally accepted to be under the control of the hypothalamus and it has been believed that the brain is mainly dependent upon glucose as its energy source. Blanco et al. (1966) reported that an intrahypothalamic injection of minute amount of glucose prevented insulin induced growth hormone secretion in the presence of peripheral hypoglycemia, which indicates the existence of glucose sensitive growth hormone regulatory center in the hypothalamus.

However, other substrates have been shown to serve as an energy fuel to the brain. Beta-hydroxybutyrate and acetoacetate were reported to replace glucose as the predominant fuel for brain metabolism during the starvation (Owen et al., 1967). Though they could not find the A–V difference of FFA across the brain, several earlier studies demonstrated an ability for FFA oxidation (Vignais et al., 1958) and utilization of endogenous phosphatides (Geiger, 1958) by brain. There remains therefore a possibility that plasma FFA or its secondary metabolites have some effects upon brain metabolism and modify the secretion of HGH.

On the other hand, it is well recognized that the metabolism of FFA is closely linked with that of glucose. In the present studies, the administration of nicotinic acid or heparin resulted in the increase of plasma HGH without any changes of plasma glucose levels. But the concomitant administration of glucose with hyperglycemia abolished the HGH rise. Recently, Blackard et al. (1969) demonstrated that an infusion of 20% soybean oil emulsion resulting in 6 mM plasma FFA concentration completely inhibited insulin-induced growth hormone response in monkey. Furthermore, sodium octanoate infusion, which resulted in 3 mM plasma FFA concentrations also inhibited the response. These results may provide an additional possibility that FFA can abate the glucose requirement in the brain. Thus, it would be reasonable to state that growth hormone is secreted when energy substrate, whether glucose or FFA, is acutely deprived.

In evaluating the role of thus secreted growth hormone in the rise of plasma FFA, it must be taken into account that 2 or 3 hr elapse before the marked rise of plasma FFA following growth hormone administration. However, Rabinowitz et al. (1965) showed that HGH infused into brachial artery enhanced FFA uptake by muscle and reduced glucose uptake by forearm tissue promptly,
and that the release of FFA from adipose tissue occurred very soon at 40 min after the start of HGH infusion. This fact suggests that growth hormone secretion at times of acute carbohydrate or FFA need, could be responsible for supply of FFA as energy fuel and for sparing of glucose.

The fact that the mobilization of FFA is impaired (Merimee et al., 1967) and that recovery from hypoglycemia is delayed (Rimoin et al., 1966) in selective growth hormone deficient patients also supports the physiologically important role of growth hormone for energy metabolism.

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References