

## **A study of growth hormone release in man after oral administration of amino acids**

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### **Summary**

*A study was carried out in 15 male volunteers to evaluate qualitatively the secretion of growth factors following stimulation by oral amino acids. The results showed that oral administration of a combination of two amino acids (1200 mg l-lysine plus 1200 mg l-arginine) provoked a release of pituitary somatotropin and insulin. This phenomenon was reproducible and the growth hormone secreted in response to this stimulation had biological activity (as demonstrated by a radioreceptor assay and somatomedin induction). The effect appeared to be specific to the combination of the two amino acids; neither of the amino acids demonstrated appreciable stimulating activity when administered alone, even at the same doses.*

*Key words: Arginine – lysine – drug combinations – growth hormone*

### **Introduction**

It has been known for some time that intravenous administration of amino acids strongly stimulates secretion of growth hormone by the hypophysis.<sup>1,4,13,15,16,18-20</sup> This phenomenon has, in fact, been accepted and utilized as a test of pituitary function in growth disorders.<sup>2,8,9,12,17</sup> However, the physiological relevance of this release of human growth hormone (HGH) has not yet been fully established.

We have investigated, therefore, the following aspects of this phenomenon: (i) the exact biological nature of the HGH secreted in response to amino acid stimulation, (ii) the possibility of induction of peripheral mediators of HGH, (iii) a possible release of other growth factors in addition to HGH, (iv) whether specific amino acids (l-lysine and l-arginine), either alone or in combination, produce varying HGH responses, (v) the time course of the HGH release, and (vi) whether oral administration of the amino acids is effective.

### **Subjects and methods**

Fifteen healthy male volunteers, aged 15 to 20 years, free of all endocrine or metabolic abnormalities and who were not receiving any kind of medical treatment, were kept under observation for 2 days. After initial blood samples had been obtained, each subject received a single oral dose of 2400 mg amino acids (1200 mg l-arginine-2-pyrrolidone-5-carboxylate plus 1200 mg l-lysine hydrochloride) on an empty stomach. Blood samples were again drawn at intervals of 30, 60, 90 and 120 minutes

after amino acid administration. The experiment was repeated after intervals of 10 and 20 days.

Blood samples were tested for growth hormone by: (a) a radioimmunoassay (RIA) method, and (b) a radioreceptor assay (RRA). This bioassay evaluates somatotropin on a substrate of lymphocytes in monolayer culture (blastic lymphocytes IM9 infected with Epstein-Barr virus) after extraction and purification by gel-chromatography on a Sephadex column. The biological activity is evaluated in units and extrapolated to ng/ml.

Somatomedin activity (Somatomedin A, Asm) was also assessed. To evaluate this HGH-dependent serum factor a biological method was used which determines the incorporation of  $^{35}\text{SO}_4$  (the sulphatating activity of the serum) and of  $^3\text{H}$ -thymidine on piglet rib cartilage. This method, which we modified,<sup>11</sup> is extremely precise (for levels below 0.3) and quite sensitive. Somatomedin activity is expressed in U/ml. One unit indicates the sulphatating activity of 1 ml of pooled serum from at least 3 healthy subjects, as no international standard has yet been established for Asm. Normal values using this method are  $1 \pm 0.2$  U/ml, with confidence limits of 0.8 and 1.2.

Insulin levels were determined by radioimmunoassay.

In a second experiment, 8 subjects randomly selected from the 15 included in the first experiment underwent growth hormone assays after administration of single doses of various amino acids: (i) 1200 mg l-arginine-2-pyrrolidone-5-carboxylate, (ii) 1200 mg l-lysine hydrochloride, (iii) 1200 mg of both (total 2400 mg), as in the first experiment, and (iv) 2400 mg l-arginine-2-pyrrolidone-5-carboxylate. Blood samples were drawn at the same times as in the first experiment and assayed only for growth hormone.

## Results

The results are reported in Table I and Figures 1, 2, 3 and 4 for the first experiment and in Table II and Figure 5 for the second experiment.

**Table I. Plasma levels of growth hormone (HGH), insulin and somatomedin (Asm) after oral administration of 1200 mg l-arginine-2-pyrrolidone-5-carboxylate and 1200 mg l-lysine hydrochloride: mean ( $\pm$ S.D.) values for 15 subjects**

Measurement	Growth hormone (ng/ml)		RRA:RIA	Insulin ( $\mu\text{U/ml}$ )	Asm (U/ml)
	RIA	RRA			
Baseline	15.4 $\pm$ 5.0	15.0 $\pm$ 3.0		7.3 $\pm$ 3.2	0.9 $\pm$ 0.2
30 mins	86.2 $\pm$ 8.7			21.0 $\pm$ 1.5	0.9 $\pm$ 0.2
60 mins	82.0 $\pm$ 14.8	60.26 $\pm$ 9.0	0.73	11.7 $\pm$ 2.0	1.0 $\pm$ 0.1
90 mins	108.0 $\pm$ 7.4	112.97 $\pm$ 6.2	1.04	7.9 $\pm$ 3.1	1.0 $\pm$ 0.1
120 mins	102.7 $\pm$ 10.0			9.85 $\pm$ 3.8	
8 hours	48.0 $\pm$ 5.5				3.0 $\pm$ 0.1
10 days later (90 mins)*	97.5 $\pm$ 5.6			18.3 $\pm$ 2.5	
20 days later (90 mins)*	95.0 $\pm$ 12.05			17.7 $\pm$ 8.4	

\*The test was repeated after 10 and 20 days. The figures relate to the plasma samples taken at the time of maximal peak (90 mins) in the previous experiment

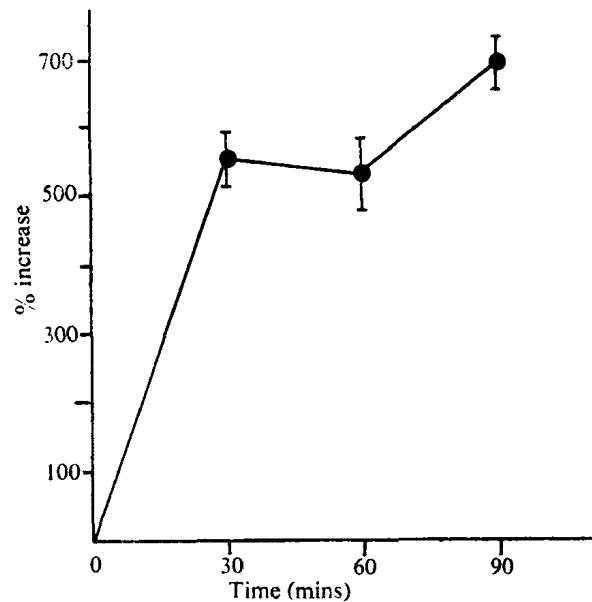
**Table II. Plasma levels (ng/ml) of growth hormone after single oral doses of l-arginine-2-pyrrolidone-5-carboxylate or l-lysine hydrochloride, alone or in combination: mean ( $\pm$ S.D.) values for 8 subjects**

Measurement	Arginine (1200 mg)	Arginine (2400 mg)	Lysine (1200 mg)	Arginine+lysine (1200 mg +1200 mg)
Baseline	7.3 $\pm$ 2.3	16.4 $\pm$ 4.1	4.8 $\pm$ 1.9	12.4 $\pm$ 2.5
30 mins	13.5 $\pm$ 7.5	3.4 $\pm$ 2.5	6.3 $\pm$ 3.8	29.0 $\pm$ 7.5
60 mins	10.0 $\pm$ 4.5	5.8 $\pm$ 4.0	7.8 $\pm$ 5.1	63.0 $\pm$ 7.8
90 mins	9.2 $\pm$ 4.7	5.9 $\pm$ 3.8	13.5 $\pm$ 5.2	98.5 $\pm$ 15.0
120 mins	9.4 $\pm$ 5.4	6.2 $\pm$ 4.1	15.8 $\pm$ 4.2	57.1 $\pm$ 10.0

From the results it would appear that the joint administration of the two amino acids (2400 mg total) brought about a marked biological response which was reproducible and did not reduce with time.

As will be seen from Figures 1 and 5, there was induction of a significant peak of immunoreactive HGH which reached a maximum at 90 minutes after administration and this was confirmed when the experiment was repeated.

**Figure 1. Percentage variations in plasma HGH (radioimmunoassay) with respect to baseline values after oral administration of 1200 mg l-arginine-2-pyrrolidone-5-carboxylate plus 1200 mg l-lysine hydrochloride: mean ( $\pm$ S.D.) values for 15 subjects**



The HGH released appeared to be biologically active both *in vitro* on RRA systems (Figure 2) and in the peripheral induction of mediators of somatomedin activity (Figure 3). The time lag between the HGH peak and the peak of somatomedin A corresponds to that previously reported.

Figure 2. Relationship between percentage variations from baseline values in plasma GHG, assessed by radioimmunoassay (RIA) and with a biological assay (RRA), after oral administration of the arginine plus lysine combination, as in Figure 1

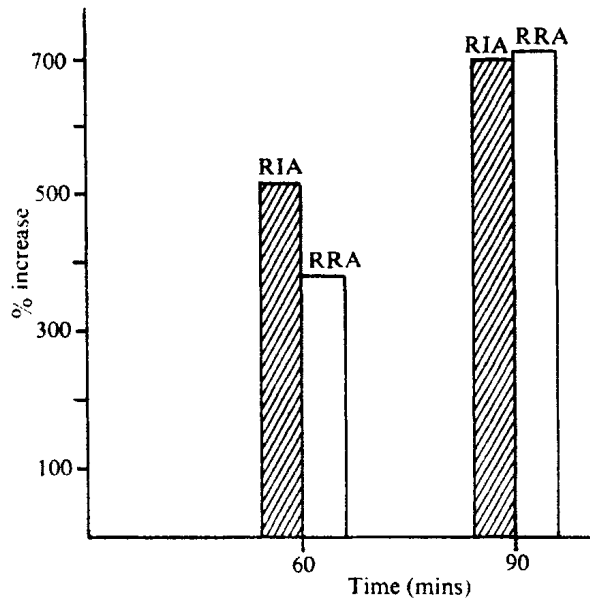
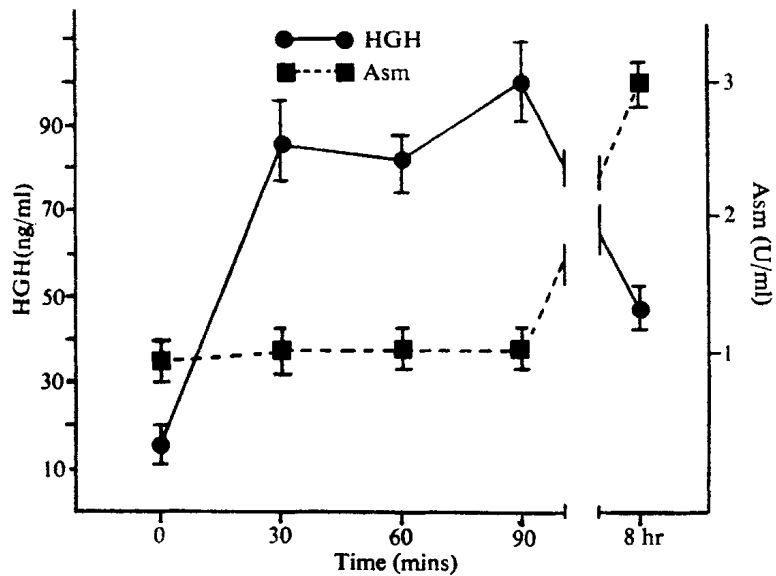
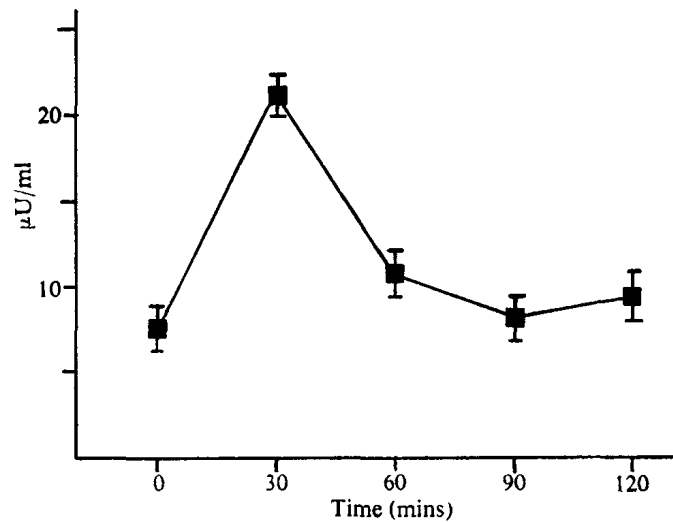


Figure 3. Plasma levels of growth hormone (GHG) and somatomedin A (Asm) after oral administration of the arginine plus lysine combination, as in Figure 1: mean ( $\pm$ S.D.) values for 15 subjects



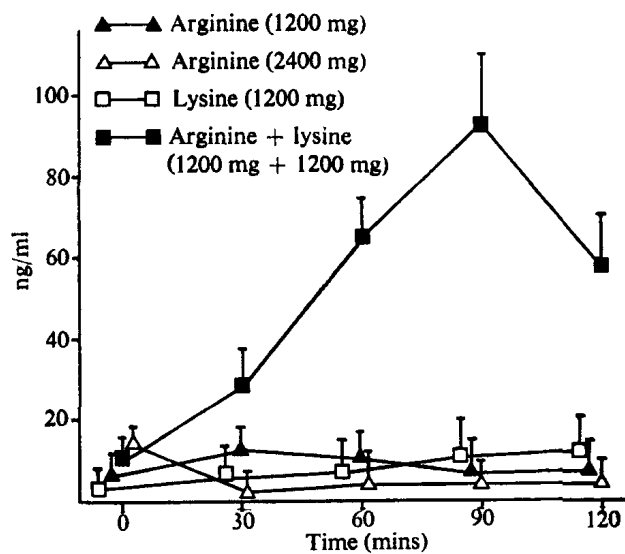
A significant secretion of insulin, another very important growth factor, was also induced (Figure 4).

**Figure 4. Plasma insulin levels (RIA) after oral administration of the arginine plus lysine combination, as in Figure 1: mean ( $\pm$ S.D.) values for 15 subjects**



The association of the two amino acids seemed to provoke a much greater GHG response than either of them demonstrated alone. Individually, their influence on GHG was practically non-existent (Figure 5), even when administered in doses equal to the total amino acid dosage of the combination.

**Figure 5. Plasma levels of GHG (RIA) after oral administration of arginine and lysine alone or in combination: mean ( $\pm$ S.D.) values for 8 subjects**



## Discussion

Our findings are clinically important in terms of the relevance of amino acids to growth disorders, particularly in relation to diagnostic tests in growth disorders.

The confirmation of biological activity of the HGH secreted in response to oral amino acid stimulation is very important. It is known that stimulation of the hypophysis by physiological processes<sup>6,7,10</sup> or pharmacological agents can result in the release of substances into the circulation, the majority of which possess immunoreactive properties enabling their identification by normal RIA systems, but the peripheral biological activity is often reduced or absent.<sup>21</sup> We have shown similar immuno-responses to the amino acid combination but, far more important, we could demonstrate that the association of the two amino acids does result in the release of biologically-active hormone able to affect peripheral cellular receptors and thus cell growth in general.

Insulin is, of course, an equally important factor in cell growth. Even if there are some uncertainties regarding the bioactivity of the substance assayed, there is no doubt that the amino acids stimulated this as well as HGH; hence, both of the physiologically important growth factors are affected. It should also be noted that the hypoglycaemia which follows the insulin peak is a further stimulus to HGH secretion.

Probably the most significant aspect of our findings is that these HGH responses have been demonstrated following oral administration of the amino acid complex. Previously, the only amino acid to have been tested orally is tryptophan,<sup>3,5,14</sup> so that our demonstration of oral activity of this arginine/lysine combination is clearly of considerable importance in clinical and diagnostic practice, where it offers a more practical and physiological approach.

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