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Adding a protease inhibitor to sampling tubes increases the acylated ghrelin and decreases the desacylated ghrelin levels in girls

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INTRODUCTION

Ghrelin is a growth hormone-releasing peptide hormone stimulating the appetite, mainly produced in the stomach, and with an important role in pubertal development (1).

Two ghrelin forms have been described, acylated (AG) and desacylated (DAG), but it is debated whether DAG is an active hormone or only an artifact from degradation of AG in sampling tubes (2).

AIM

Our aim was to evaluate the effects of adding the protease inhibitor 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF) to sampling tubes and acidification of plasma on levels of AG and DAG in girls with suspected central precocious puberty (CPP).

METHOD

13 girls (6.6–10.1 years old) with suspected CPP were included when undergoing a gonadotropin-releasing hormone stimulation test during 2015-2017 at the Departments of Paediatrics at Örebro or Uppsala University Hospital.

For each girl, blood samples were collected at 0 min in precooled EDTA tubes both without and with AEBSF at a final concentration of 2 mg/ml. After cold centrifugation, hydrochloric acid (HCl, final concentration=50 µmol/L) was added to half of the plasma tubes containing AEBSF.

The AG and DAG concentrations were measured by specific, commercial ELISA kits.

Comparison was performed using one-way ANOVA for repeated measurements.

RESULTS

Mean (+/- SD) plasma AG levels were significantly higher after the addition of AEBSF (650.9 +/- 257.1 pg/ml) and addition of AEBSF + HCl (681.2 +/- 299.0 pg/ml) compared to the concentrations without additives (247.6 +/- 123.4 pg/ml, $p < 0.01$ for both comparisons).

There was no significant difference between AG levels after AEBSF and AEBSF + HCl addition (Figure 1).

Plasma levels of DAG were significantly lower after the addition of AEBSF + HCl (69.3 +/- 30.6 pg/ml) and even further lower after the addition of AEBSF only (56.3 +/- 30.9 pg/ml) compared to the levels in tubes without additives (149.9 +/- 73.7 pg/ml, $p < 0.01$ for both comparisons, Figure 2).

Figure 1.
The influence of different additives on the concentration of acylated ghrelin

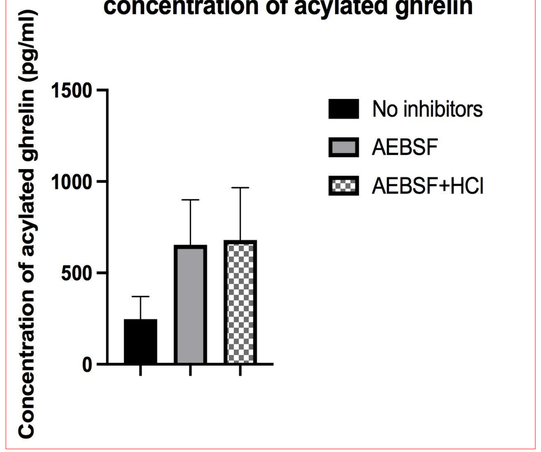
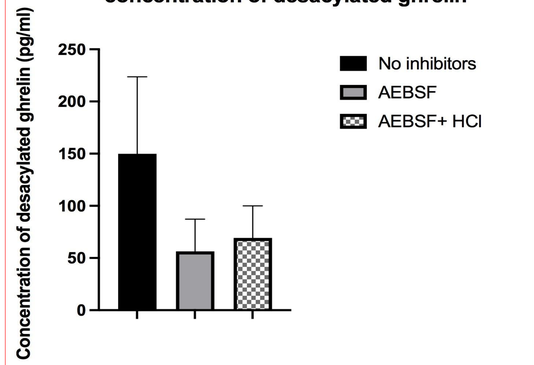


Figure 2.
The influence of different additives on the concentration of desacylated ghrelin



CONCLUSIONS

Due to the unstable nature of AG, special procedures are required for accurate measurement of its plasma levels in children, including the use of a protease inhibitor like AEBSF. However, DAG was still measurable after the addition of AEBSF, indicating that DAG may not only be an artifact from poor sampling procedures.

REFERENCES

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CONTACT INFORMATION

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