STABILITY OF ACYLATED AND UNACYLATED GHRELIN LEVELS IN HUMAN BLOOD TREATED WITH AEBSF

CONTEXT

The acylated/unacylated ghrelin (AG/UAG) ratio has been reported to range from 0.02 to 0.3, suggesting biologically relevant independent regulation of each ghrelin isoform. However, AG is deacylated to UAG by esterases in blood samples, and esterase inhibition is critical for their accurate measurement.

Our hypothesis is that at least part of the variation in reported AG and UAG values is due to inconsistent sample preparation.

MATERIALS

- Vacutainers BD cat# 367899; 6 ml K2 EDTA
- AEBSF (4-(2-Aminoethyl) benzenesulphonyl fluoride hydrochloride) stock solution at 200 mg/ml
- Acylated Ghrelin (human) Easy Sampling ELISSA kit (cat# A05306)
- Unacylated Ghrelin (human) asy Sampling ELISA kit (cat# A05319)

PROTOCOL

Venous blood was collected into three separate EDTA vacutainers from eigh individuals who fasted overnight. Treatment of the blood was performed immediately after collection. For EDTA + AEBSF samples, AEBSF stock solution was added at 1:100 to whole blood. After gentle mixing by inversion (x3) of the recapped tubes, the whole blood was centrifuged at 1500g, 4°C for 5 min. The plasma was then rapidly aliquoted and immediately frozen on dry ice. Samples were stored at either -20°C or -80°C until the time of assay.

Plasma samples were thawed on ice, centrifuged for 1 min at 1500g, 4°C, and kept on ice before transferring to the assay plates. Samples were measured in duplicate (50 µl per well) for AG and UAG using double-antibody sandwich ELISAs from Bertin Bioreagent.

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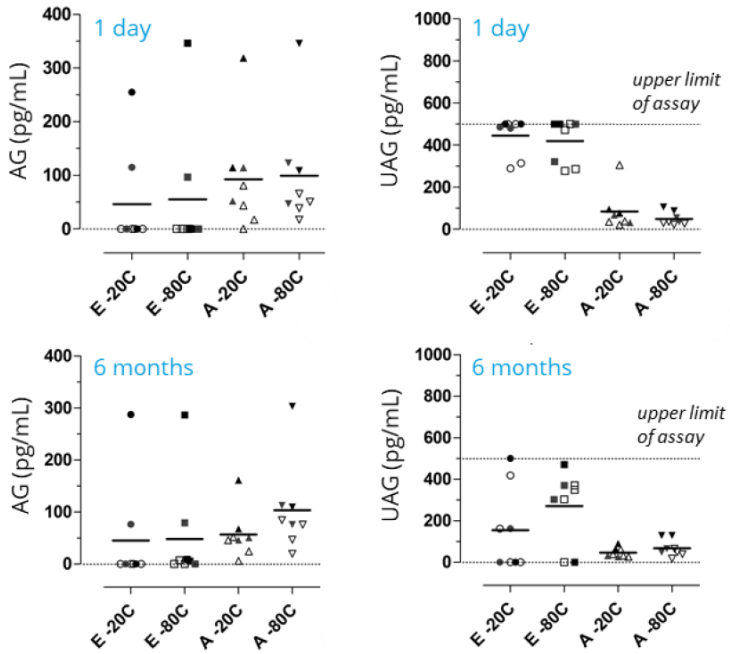
RESULTS

Non-AEBSF plasma contained low AG and high UAG, indicating rapid conversion of AG to UAG. However, AEBSF plasma, stored at -80°C and measured at 0 and 6 months, contained AG and UAG ranges of 12–350 and 17–170 pg/ml, respectively. Mean (SEM) AG/UAG ratios were 1.7 (0.3) and 1.8 (0.5) at each time point, with no significant effect of storage period.



AG and UAG levels measured in samples collected in EDTA tubes without [E] or with the addition of AEBSF [A] after 1 day or 6 months of storage at either -20°C or -80°C.

CONCLUSION

This study highlights the importance of **immediately stabilizing blood samples on collection for the determination of both AG and UAG concentrations** and provides a valuable tool for their measurement in physiological and interventional studies. The easy sampling ELISA kits from Bertin Bioreagent kits allow accurate measurement of levels of acylated ghrelin and unacylated ghrelin in AEBSF-stabilized blood samples.