Project review

"Proteomic analysis of serum exposed to GH: a future essay for detection of GH doping"

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Growth hormone (GH) is an anabolic hormone produced throughout life from the pituitary gland. The actions in adult subjects include promotion of muscle and bone growth and stimulation of lipid consumption. Numerous evidence from official bodies as well as the lay press and underground literature strongly indicate that GH is being used as a doping agent. Excess exposure to GH is associated with hypermetabolism, irreversible bone deformations, a risk of type 2 diabetes mellitus, and probably also cardiovascular morbidity. Growth hormone is therefore on the prohibited list of drugs and related compounds.

No approved or licensed method is so far available for the detection of GH doping. The GH preparations used for doping are not structurally different from naturally produced GH and injected GH is rapidly eliminated by the organism. Detection of GH in urine is also not a viable method. The strategies pursued thus far relates to the detection in the blood of growth factors known to be stimulated by long-term GH abuse. The specificity of these methods remain a matter of debate.

Proteomics or protein profiling by means of so-called two dimensional gel electrophoresis is a powerful and unique method to demonstrate the expression of proteins in human tissues. We have recently refined this method for the use of human serum, which can be obtained by a single blood sample. The protein profile, or proteome, in serum can thus be characterised and we have preliminary data to indicate that the proteome change significantly when the individual has been exposed to GH. Moreover, proteomics also allows the detection of novel GH-related proteins.

The project aims to further characterise the impact of GH exposure on serum proteomics. Serum obtained from individuals with known GH disturbances will be evaluated together with serum obtained after high dose GH administration to healthy adults. In addition, the impact of physical exercise on serum proteomics will be investigated.

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Results and Conclusions

The background for the project was that doping with GH is a significant problem in the world of sports and that viable novel methods for its detection are still needed. Our hypothesis was that serum proteomics may provide the basis for future GH doping detection strategies based on either global changes in the protein pattern or identification of new GH-dependent candidate proteins. To this end a collaboration was established between Aarhus University Hospital and Edison Biotechnology Institute, Ohio University in order to provide pertinent serum samples from clinical protocols involving patients and healthy subjects exposed to conditions with alterations in GH status, and to perform proteomic analysis on serum samples from these protocols. Our project has included several modes of GH exposure ranging from patients with overproduction of GH (acromegaly) before and after treatment with either surgery or medication (a GH antagonist = pegvisomant) to patients with GH-deficiency before and after substitution with GH. More importantly, we also included a study with high dose exogenous GH administration to young and healthy male subjects for 8 days (i.e. “doping”). We successfully completed serum proteomics in these protocols and identified several new biomarkers of GH activity that could serve as future targets for an anti doping strategy. Our study also revealed that many biomarkers exist as isoforms and that these isoforms may respond to GH in a reciprocal manner such that the total level of the protein remains unchanged. Several isoforms of apo A-1, which is a lipoprotein in serum, changed in all the studies and may as such serve as a sensitive (but perhaps unspecific) GH biomarker. By contrast, one isoform of α-1 antitrypsin only changed in the study involving exogenous GH administration. Thus, this biomarker seems specific to exogenous GH. Exercise alone was also studied and this was associated with changes in two of the GH-dependent biomarkers. In conclusion, our study has identified new GH biomarkers that merit further investigations from an anti doping perspective. We suggest a study focusing on high dose GH administration (to mimic GH doping) to a larger number of subjects of both sexes and including a wash-out period. The candidate proteins should include isoforms of apo A-1, α-1 antitrypsin and should be assayed by means of proteomics as well as with more conventional assays.

Publications

Ding J, List EO, Okada S, Kopchick JJ. Perspective: Proteomic approach to detect human growth hormone doping. Growth Hormone & IGF Research 2009;19:399-407

Diana Cruz-Topete, Britt Christensen, Lucila Sackmann-Sala, Shigeru Okada, Jens Otto L. Jorgensen , John J. Kopchick. Serum Proteome Changes in Acromegalic Patients following Transsphenoidal Surgery: Novel Biomarkers of Disease Activity. (Submitted)

Juan Ding, Shigeru Okada, Jens Otto L. Jorgensen, John J. Kopchick. Biomarkers of GH doping in healthy human subjects (in preparation)
Diana Cruz-Topete, Christensen B, Jorgensen JOL, Kopchick. Serum proteomic profiles in GH-deficient patients before and after GH substitution (in preparation)

Christensen B, Jorgensen JOL, Kopchick. Serum proteomic profiles before and after pegvisomant administration in patients with acromegaly as well as in healthy subjects (in preparation)