PROJECT REVIEW

"Detection of doping with Ghrelin determination of blood profiles and urinary excretion, and stimulation of GH release after intravenous injection in athletes"

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Purified Ghrelin is a 28-amino-acid peptide with a Ser at the third residue. Ghrelin is produced in submucosal cells of the stomach. Small amounts of ghrelin are also produced in the hypothalamic arcuate nucleus. Ghrelin plasma concentrations depend on race and body fat, meal, and time of the day.

ghrelin stimulates GH release from pituitary cells in a dose-dependent manner. Intravenous injection of ghrelin induces potent GH release. Endogenous Ghrelin is secreted from the stomach to circulate in the bloodstream and act directly on the pituitary to release GH. Therefore, ghrelin will induce all the effects well known from OH. In skeletal muscle, IGFs are the only known mitogenes that stimulate both the proliferation and differentiation of skeletal muscle cells.

It seems very likely that, like GH, ghrelin may also be effective in improving training adaptation processes, especially increasing muscle mass and shortening regeneration processes.

Taken together, from the standpoint of anti-doping politics and research in sport, it is important to fully understand the physiological and endocrine effects of endogenous ghrelin in male and female athletes under different conditions of physical activities and eating patterns. It is furthermore important to understand the interactions between exogenous ghrelin, being intravenously injected, and the dependent endocrine parameters like GH and lGFs, in order to be able to differentiate between endogenous and exogenous ghrelin and the respective effects and thereby demonstrating misuse of the peptide. Up to now, no investigations on ghrelin in athletes have been published so far on ghrelin, since this peptide has only very recently be described.

The study will be conducted of two steps. In step one we will determine spontaneous action of ghrelin, dependent hormones like GH and lOFs and binding proteins under different conditions in athletes. We will investigate female and male athletes of different sports (especially endurance and power) and different body composition, at rest, during and after exercise (competition and training) and under several nutritional states. We will measure plasma concentrations and will work on establishing a method for measurement of urinary ghrelin concentrations as well.

In step two we will investigate effects of exogenous ghrelin administration on endogenous profiles of ghrelin, OH and related peptides. Again, we will investigate female and male athletes of different sports under the above mentioned conditions.

DETECTION OF DOPING WITH GHRELIN: DETERMINATION OF BLOOD PROFILES AND URINARY EXCRETION AND STIMULATION OF GH RELEASE AFTER INTRAVENOUS INJECTION IN ATHLETES

Results and conclusions

We included 44 endurance (E, n = 23) and power (P, n = 21) athletes of both gender (m: n = 24 , f: n = 20). All 24 subjects participated in the main part 1 of the study (analysis of physiologic profile of Ghrelin and corresponding parameters), 2 subjects additionally participated in part 2 (injection of exogenous Ghrelin).

The main results are:

1. Total Ghrelin values significantly declined over time in both active groups (endurance and strength), while values remained nearly constant in the inactive groups, independent from gender. Furthermore, values remained constant without carbohydrate intake but declined significantly over time under carbohydrate loading. This trend was also independent from gender and from kind of activity.
2. Active Ghrelin values significantly declined over time under carbohydrate loading in the active but not in the inactive groups, which was independent from kind of sport or gender. The differences against the first value did not show any clear time effect, but showed significantly lower values in the strength versus the endurance groups, and in the female athletes under carbohydrate loading compared to the low-carbohydrate condition.
3. Exogenous Ghrelin injection led to extremely high increases in total and active Ghrelin plasma concentrations with a maximum 15 min after the injection. Baseline values of total Ghrelin were not yet reached 180 min after the injection, while active Ghrelin baseline concentration was reached already after about 90 min.
4. HGH values declined significantly over time in the female group but remained constant in the male athletes. This effect was independent from type of sport and from carbohydrate intake.
5. Exogenous Ghrelin injection led to high increases in HGH serum concentration (15 – 30 ng/ml over baseline) 60 min after the injection. Values declined to baseline after 120 min. This effect was independent from carbohydrate intake, but was more pronounced during run exercise.

6. IGF1 declined significantly over time in the active but not in the inactive groups. This effect was independent from gender, type of sport, and carbohydrate intake.
7. In spite of high exogenous Ghrelin injection-induced HGH increments, we could not find any effect on IGF1 concentration 60 – 180 min after Ghrelin injection. In the active group, IGF 1 declined over time after Ghrelin, while values remained constant in the inactive situation.
8. Urinary total Ghrelin concentrations were measured 1.4 - 1.6 times higher after stabilizing with HCL or HCL plus PMSF as compared to untreated urine. 8 – 9 hrs overnight urinary total Ghrelin values were 1.3 – 1.6 time higher as compared to 3 hrs intervention-period daytime values. Neither carboloading, nor gender or physical activity did affect urinary total Ghrelin concentrations.
9. Urinary active Ghrelin concentrations were 20 – 30 times lower as compared to total Ghrelin concentrations. Urinary active Ghrelin concentrations were measured 1.1 - 1.4 times higher after stabilizing with HCL or HCL plus PMSF as compared to untreated urine. 8 – 9 hrs overnight urinary active Ghrelin values were not different from 3 hrs intervention-period daytime values. Neither carboloading, nor gender or physical activity did affect urinary active Ghrelin concentrations.
10. In spite of extremely high plasma total and active Ghrelin concentrations 60 min after exogenous Ghrlein injection, urinary values did not show any increase during the sampling period until 3 hrs after the injection.

To summarize, Ghrelin seems to be mainly independent from acute or chronic physical activity. This makes it unlikely that Ghrelin plays a major role in any physiological anabolic adaptation processes according to training. As the effect of exogenous Ghrelin on serum Ghrelin and hGH concentration is very short lasting, it seems to be unlikely that this substance might play a major role as a doping agent. However, it could be demonstrated, that the effect on hGH secretion was very high, so that Ghrelin should stay on the list of forbidden substances.

In the investigation plasma immunoreactive ghrelin levels will be measured by a radioimmunoassay (RIA) that uses ^{125}I-labeled bioactive ghrelin as a tracer and a rabbit polyclonal antibody raised against full-length octanoylated human ghrelin.