"Cobalt quantification from erythrocytes and urine: Complementation of the ABP and definition of contributions by Vitamin B12-derived cobalt"

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Project Overview

Due to the erythropoiesis-stimulating effects, the misuse of cobalt and cobalt salts in sports is prohibited both in- and out-of-competition. While total urinary cobalt levels can be determined by means of inductively coupled plasma-mass spectrometry (ICP-MS), there are currently no assays for the detection of inorganic cobalt which exclude cobalt-containing molecules such as Vitamin-B12. But especially in cases of atypical findings with elevated cobalt concentrations, the analysis of Vitamin-B12-depleted urine is required to provide accurate information on the ionic cobalt content of the sample. Therefore, a quantitative test method for inorganic urinary cobalt will be developed within this study by using different depletion approaches such as solid phase extraction (SPE) or liquid chromatography (LC) in combination with ICP-MS. In particular during prolonged exposure to high concentrations, cobalt was found to be irreversibly incorporated into red blood cells. As the determination of the cobalt content in erythrocytes could be highly relevant to uncover long-term cobalt exposure in a doping control context, an assay for the quantitative determination of cobalt from a defined amount of erythrocytes will be additionally set up. Both assays will eventually be used to analyze blood and urine samples collected within two administration studies with cobalt chloride and Vitamin-B12 (dose: 1 mg/day over a period of 14 days). The Vitamin B12 administration study will provide important insights into the influence of Vitamin-B12 supplementation – which is legitimately used by many athletes – on urinary cobalt levels.

Results and Conclusions

The manipulation of blood and blood components, commonly referred to as "blood doping" is one of the continuing challenges in the field of sports drug testing. For that purpose, specific and sensitive detection methods enabling the detection of prohibited substances and methods of doping are required. As a cheap an easy available alternative to illicit blood transfusions, erythropoiesis stimulating agents have been shown to be misused in sport. To illegally improve the athlete's aerobic capacity and endurance performance, the administration of ionic cobalt (Co^{2+}, e.g. $CoCl_2$) can be used to stimulate the endogenous erythropoietin (EPO) biosynthesis. By contrast, several organic Co-containing compounds such as cyanocobalamin (vitamin B12) are not prohibited in sports, and thus, the need of analytical differentiation of urinary Co-concentrations is desirable. To this end, an excretion study with daily applications of either 1 mg of $CoCl_2$ or 1 mg of cyanocobalamin was conducted with 20 volunteers over a period of 14 consecutive days where urine, plasma, and concentrated red blood cells were

analyzed. The samples were collected starting 7 days before the administration until 7 days after. For total cobalt analyses, inductively coupled plasma mass spectrometry (ICP-MS), which yielded significantly elevated levels exclusively after inorganic cobalt intake, was utilized. Moreover, a liquid chromatography (LC)-ICP-MS approach was established and employed for the simultaneous determination of organically bound and inorganic cobalt by chromatographic separation within one single run. Especially for illegal Co^{2+} supplementation in sports this approach can be complemented to a prospective detection method.

Finally, for adequate method characterization and quantitative analyses, one or more internal standards need to be implemented and the chromatographic separation of additional cobalt-containing organic species as well as the stability of different variants of cyanocobalamin, especially with regard to photolytic degradation and possible conversions, need to be clarified. Nevertheless, despite the fast and preparative chromatographic run, inorganic cobalt is clearly separated and Co^{2+} concentrations attributed to unbound cobalt and exceeding future threshold levels will be regarded as antidoping rule violations. With regard to routine doping controls the presented approach offers an initial testing tool in order to identify those doping control samples that justify subsequent accurate cobalt quantification.