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## **Endogenous Production and Excretion of Boldenone (17 $\beta$ -hydroxyandrosta- 1,4-dien-3-one), an Androgenic Anabolic Steroid**

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### **ABSTRACT**

**17 $\beta$ -Hydroxyandrosta-1,4-dien-3-one (boldenone)** is an anabolic steroid and banned as a doping substance in sports. The detection of boldenone **I** and/or one of its main metabolites, 17 $\beta$ -hydroxy-5 $\beta$ -androst-1-en-3-one **II**, and 3 $\alpha$ -hydroxy-5 $\beta$ -androst-1-en-17-one **III**, in the urine of an athlete is considered as a positive doping case. The detection of **I**, **II**, or **III** in urine samples of individuals who did not apply boldenone or a boldenone analogue has not been previously reported.

In urine samples from two laboratory staff members who were not treated with boldenone, boldenone **I** and its metabolites **II**, and **III** were detected in the routine screening procedure for anabolic steroids. For identification, **I**, **II**, and **III** were isolated from urine after enzymatic hydrolysis, further separated by high performance liquid chromatography (HPLC), derivatized with N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) / trimethyliodosilane (TMIS), and analyzed by gas chromatography/mass spectrometry (GC/MS). The measured GC retention times and mass spectra (low resolution and high resolution) of **I**, **II**, and **III** from the urine samples were identical with those obtained from authentic reference compounds.

Incubation of testosterone or androst-4-en-3,17-dione with feces of a person producing **I** yielded several reduced products as well as 1-dehydrogenated **androst-4-en-3,17-dione** (androsta-1,4-diene-3,17-dione **IV**) in low amount, indicating that this person has 1-dehydrogenase activity in the gut. Incubation of testosterone with feces of a person not producing **I** did not yield any 1-dehydrogenated product.

Conclusion for a possible origin of **I**, **II** and **III** is that testosterone or androst-4-en-3,17-dione may enter the intestine via the bile ("enterohepatic route") and be 1-dehydrogenated by seldomly distributed enteric microorganisms (bacteria or fungi) to androsta-1,4-diene-3,17-dione **IV**. Reabsorption of **IV** into the circulatory system followed by common metabolic pathways leads to boldenone **I** and its metabolites **II**, and **III**.

A comprehensive publication of the presented paper is still in preparation and will be published elsewhere.