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$^{12}C/^{12}C$ ratios of endogenous steroids after oral boldenone administration

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Abstract

Boldenone (17β-hydroxy-androsta-1,4-dien-3-one, Bo) is an anabolic androgenic steroid included in the WADA prohibited list [1]. Bo is fundamentally a xenobiotic. However, sometimes it may be synthesized endogenously, where typically very low concentrations can be observed in urine [2,3]. $^{12}C/^{13}C$ analysis by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) is the method of choice to demonstrate the potentially exogenous origin of Bo and/or its main metabolite 17β-hydroxy-5β-androst-1-en-3-one (BM1) [3]. Although metabolism is well investigated by *Galetti et al.* [4], *Schänzer et al.* [5] and *Gomez et al.* [6] influence on endogenous steroids is not completely studied. For the present study, 6 male volunteers received a single oral dose of 50 mg Bo. Urine was collected over a period of 24h pre- and 48h post-administration. Bo, BM1 and several commonly endogenous steroids were analyzed by GC-C-IRMS. For this, the method described by *Piper et al.* [3] was modified. This included solid phase extraction, enzymatic hydrolysis, liquid-liquid extraction and several subsequent semi-preparative HPLC purification steps. 5β-Pregnane-3α,20β-diol (PD) served as endogenous reference compound (ERC). Bo, BM1, etiocholanolone (E), androsterone (A), testosterone (T), 5β-androstane-3α,17-diol (BD) and 5α-androstane-3α,17β-diol (AD) were analyzed as target compounds (TC). No influence on PD after Bo administration could be detected. $\delta^{13}C$-values for Bo and BM1 were depleted up to more than 48h. Compared to BM1, Bo changed to higher $^{13}C/^{12}C$ ratios. For BD a significant influence on $\delta^{13}C$-values was observed. In addition, E was depleted in ^{13}C over the whole time of sample collection. Additionally to the influence on 5β-steroids, which represents the favoured enzymatic metabolic pathway of Bo, also a short-term influence on 5α-steroids was observed. In the first samples after administration a strong depletion in ^{13}C for A and AD was detected. Additionally, T was strongly depleted in ^{13}C over the same interval. It can be concluded, that T is a direct metabolite of Bo. It can be assumed that Bo is metabolized via reduction of the double-bond between C1 and C2 to T. ^{13}C depletion of AD and A results out of the T-metabolism.
We could demonstrate that Bo-metabolism via 5β-reductase represents the preferred metabolic pathway. For E and BD, ^{13}C de- pleted values over the time of sample collection were found. In addition, T, AD and A were influenced following the administration of Bo. It can be assumed that these compounds also represent Bo-metabolites.

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

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