Yvonne Schrader, Mario Thevis and Wilhelm Schänzer

Quantitative Determination of Metabolic Products of 19-Norandrostenediol in Human Plasma using Gas Chromatography / Mass Spectrometry

Institute of Biochemistry, German Sport University, Cologne, Germany

Extended abstract*

Prohormones were available as nutritional supplements for over-the-counter (OTC) sales in the United States until the commencement of the *Anabolic Steroid Control Act of 2004*. In 1999 nandrolone prohormones 19-norandrostenedione (estr-4-ene-3,17-dione) and 19-norandrostenediol (estr-4-ene-3β,17β-diol, NDiol) have been added to the list of prohibited substances of the World Anti-Doping Agency as they are metabolized to the common nandrolone metabolites norandrosterone and noretiocholanolone. In 2001 results of a pilot study dealing with the *in vivo* conversion of 19-norandrostenedione were published[1]. But so far, no studies on the metabolism and *in vivo* conversion of 19-norandrostenediol have been reported, nor have quantified data on resulting plasma nandrolone levels.

To investigate the *in vivo* biotransformation of NDiol, an evaluation of plasma levels of 19-norsteroids, in particular of unconjugated nandrolone, was performed within an open label crossover phase I-clinical trial with eight healthy male volunteers. To gain information if the route of administration affects the metabolic rate of the compound, NDiol was tested formulated as capsules as well as sublingual tablets. Volunteers administered a 100 mg NDiol capsule and a 25 mg sublingual tablet, respectively, separated by a period of at least two weeks. Blood samples (10 mL) were collected immediately before supplementation and at 10, 20, 30, 45, 60, 90 minutes, 2 h, 3 h, 4 h, 6 h, 8 h and 24 h after administration. Plasma concentrations of 19-norandrostenediol, nandrolone, as well as major metabolites (19-norandrosterone and 19-noretiocholanolone) were determined using a validated assay based on gas chromatography/mass spectrometry (GC/MS).

*for further details please refer to:

Schrader *et al*. Drug Metab Dispos. 2006 Aug; 34(8): 1328-35

Mean values (± *SE*) of C_{max}, t_{max} and AUC were calculated for plasma total concentrations (i.e. conjugated plus unconjugated compounds) of 19-norandrostenediole ($NDiol_{tot}$), nandrolone (NL_{tot}), 19-norandrosterone (NA_{tot}), 19-noretiocholanolone (NE_{tot}), as well as unconjugated nandrolone (NL_{unc}) as obtained from eight volunteers (Table 1). The ingestion of 100 mg capsules of 19-norandrostenediol yielded maximum plasma total concentrations of 1.1 ng/mL (± 0.7) for 19-norandrostenediol, 4.0 ng/mL (± 2.6) for nandrolone, 154.8 ng/mL (± 130.8) for 19-norandrosterone and 37.7 ng/mL (± 6.9) for 19-noretiocholanolone. The use of 25 mg sublingual tables resulted in 3.3 ng/mL (± 1.0) for 19-norandrostenediol, 11.0 ng/mL (± 6.4) for nandrolone, 106.3 ng/mL (± 40.1) for 19-norandrosterone and 28.5 ng/mL (± 20.8) for 19-noretiocholanolone.

Table 1: Summary of pharmacokinetic evaluation

dosage form (dose/unit)	analyte	C_{max} (ng/mL)	t_{max} (h)	AUC $_{0\text{-}24}$ (h*ng/mL)
capsule (100 mg)	$NDiol_{to}$	1.1 (± 0.7)	4.3 (± 2.7)	11.4 (± 13.6)
	NL_{tot}	4.0 (± 2.6)	2.6 (± 2.6)	33.5 (± 24.2)
	NA_{tot}	154.8 (± 130.8)	2.8 (± 2.3)	1371.9 (± 1365.0)
	NE_{tot}	37.7 (± 6.9)	3.8 (± 2.6)	529.1 (± 185.7)
	NL_{unc} [a]	-	-	-
sublingual tablet (25 mg)	$NDiol_{to}$	3.3 (±1.0)	0.4 (± 0.2)	4.4 (± 3.3)
	NL_{tot}	11.0 (± 6.4)	0.6 (± 0.3)	15.3 (± 7.6)
	NA_{tot}	106.3 (± 40.1)	0.9 (± 0.3)	277.9 (± 122.9)
	NE_{tot}	28.5 (± 20.8)	0.8 (± 0.3)	49.5 (± 31.0)
	NL_{unc} [a]	4.4 (± 1.0)	0.4 (± 0.1)	5.0 (± 1.2)

[a] only one volunteer generated quantifiable amounts; mean values were not calculated

After ingestion of 100 mg NDiol capsules only very low amounts of pharmacologically active NL_{unc} were detected in plasma samples of the volunteers. In fact, values of only one volunteer exceeded the limit of quantification of 0.5 ng/mL as determined at 0.7 ng/mL and 0.6 ng/mL 45 and 60 minutes after administration, respectively (Fig 1A). Most interestingly, ten minutes after sublingual administration of 25 mg NDiol sublingual tablets considerable amounts of unconjugated nandrolone in plasma samples of all volunteers were quantified. Plasma concentrations of each volunteer peaked within 30 minutes after application and were determined between 3.2 and 5.7 ng/mL (Fig. 1B). These results demonstrate the importance of prohibiting prohormones such as 19-norandrostenediol, in particular as plasma concentrations of unconjugated nandrolone between 0.3-1.2 ng/mL have been reported to influence endocrinological parameters.

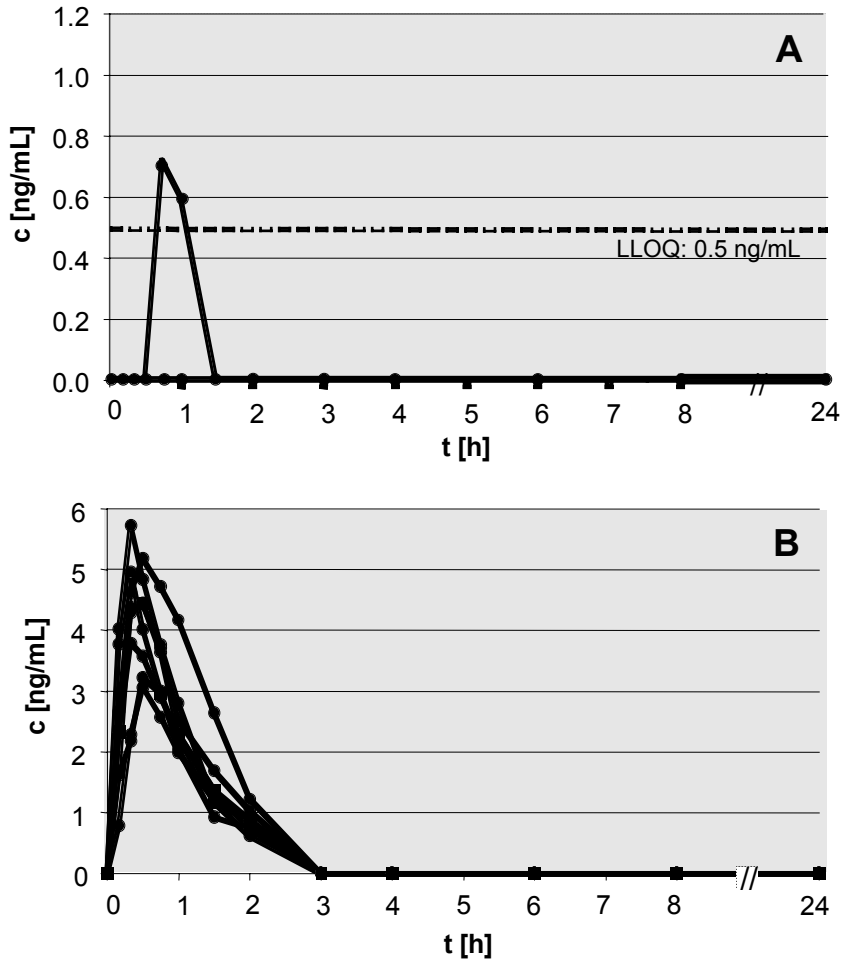

Figure 1: Plasma concentrations of unconjugated nandrolone after administration of a 100 mg NDiol capsule (A) and use of a sublingual tablet containing 25 mg of NDiol (B), respectively (eight volunteers)

Acknowledgments

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References

[1] Machnik M, Schrader Y, Schänzer W (2001) Plasma Levels of 19-Norsteroids after Oral and Buccal Administration of Norandrostenedione, in: *Proceedings of the 19th Cologne Workshop on Dope Analysis* (Schänzer W, Geyer H, Gotzmann A and Mareck-Engelke U eds), pp 125-131, Sport & Buch Strauss, Edition Sport, Cologne.