The aim of this review is to analyse the studies on nandrolone metabolism to determine if it is possible for an athlete to test positive for nandrolone without having ingested or injected nandrolone.

The anabolic androgenic steroid 19-nortestosterone, also called nandrolone, was first synthesised by Birch in 1950. Nandrolone has an anabolic effect, and is used in the treatment of certain chronic diseases. The use of nandrolone by athletes became popular in the late 1950s. Athletes use nandrolone in an oral or injectable form to increase muscle strength and improve performance. As a result of the potential performance enhancing benefits and potential health risks associated with anabolic steroid use, the International Olympic Committee (IOC) prohibited the use of nandrolone in sport in 1976.

“A doping offence for nandrolone was defined as a concentration of NA in human urine exceeding 2 ng/ml in men and 5 ng/ml in women.”

When nandrolone is ingested or injected by humans subjects, three metabolites of nandrolone can be isolated and measured in the urine by gas chromatography-mass spectrometry. These metabolites have been identified as 19-norandrosterone (NA; 3α-hydroxy-5α-oestrane-17-one), 19-nortestosterone (3α-hydroxy-5β-oestrane-17-one), and 19-norepiandrosterone (3β-hydroxy-5α-oestrane-17-one). These metabolites are isomeric compounds, having the same chemical composition and molecular mass but different chemical structure. NA is usually the most abundant urine metabolite of nandrolone. The presence of these metabolites in the urine forms the basis of doping analysis for the illegal use of nandrolone by athletes. This was based on the premise that these urine metabolites could only have been derived from exogenous nandrolone. A study in 1982 appeared to have found NA, or a similar compound, in the urine of athletes who had not used nandrolone. In 1996, the IOC stated that a critical concentration for nandrolone metabolites in the urine had been established. A doping offence for nandrolone was defined as a concentration of NA in human urine exceeding 2 ng/ml in men and 5 ng/ml in women.

“Recently, the possibility of false positive tests for nandrolone has been raised.”

Recently, the possibility of false positive tests for nandrolone has been raised. Explanations for false positive tests have included supplement contamination and endogenous production of nandrolone and regulation of metabolic pathways of nandrolone metabolism by various physiological factors and supplement interventions. The aim of this review is to analyse the studies on nandrolone metabolism with the overall goal of determining whether it is indeed possible for an athlete to test positive for nandrolone without having either ingested or injected nandrolone. The question of a positive test resulting from nutritional supplements and food contamination is beyond the scope of this review.

EVIDENCE FOR ENDOGENOUS NA

The origin of endogenous NA in the urine of athletes who have not knowingly ingested or injected nandrolone is central to resolving the question of whether it is possible to have a false positive test. The first study to suggest that NA could be found in the urine of people free of exogenous nandrolone was a study on laboratory staff (n = 14). Their urine was analysed using isotope dilution mass spectrometry, and NA or a similar compound was suspected. This suspicion was based on the detection of a small peak for the ion at m/z 256. In retrospect, this signal may have been caused by interference of other endogenous compounds (noise) and perhaps represents a false positive finding. The authors acknowledged the limitations of the study, the analytical technique lacking specificity and sensitivity.

Studies in 1988 and 1990 again raised the possibility of endogenous NA in the urine of humans. Kicman and Brooks used radioimmunoassay and measured NA in the urine of men and women, who were supposedly free of exogenous nandrolone, ranging from 3.8 to 49.4 ng/ml. However, these data should be interpreted with caution as it could be argued that the analytical technique again lacks both specificity and sensitivity. Debruyckere et al measured NA in the urine of three subjects at concentrations of 9, 14, and 37 ng/ml. These results were later attributed to nandrolone contaminated meat which the subjects may have eaten.

In 1996, the IOC declared that the presence of a small amount of NA in the urine was not considered to constitute a doping offence. This suggests that they acknowledged the possibility...
of endogenous NA production. It can only be assumed that this decision was reached on the basis of data collected by IOC laboratories during routine drug testing, as the scientific evidence at the time was equivocal. In the late 1990s, analytical procedures for the detection and quantification of steroid metabolites in urine had become increasingly sensitive. This may have accounted for an appreciable number of positive urine samples for NA being analysed in certain anti-doping laboratories. Many of the positive samples were from participants of sports that had previously not been associated with anabolic steroid use. Further research with more sensitive equipment was undertaken to determine if NA could be produced naturally by the human body. This research showed convincing evidence that NA was found in the urine of subjects free of exogenous nandrolone. The urine NA concentrations in these studies ranged from 0.01 to 1.79 ng/ml. In a study by Galan Martin et al., high NA concentrations in five sportspeople (4, 5, 6, 8, and 14 ng/ml) were measured. One woman in the study, who was postmenopausal, had a NA concentration of 22 ng/ml. It could be argued that these athletes had administered nandrolone. These results are difficult to explain and perhaps further investigation of these subjects is necessary before a definite opinion can be formed.

**METABOLISM OF NA**

**Aromatisation**

Metabolic pathways for the endogenous production of NA in the human body need to be considered. Under normal circumstances, testosterone is aromatised to oestrogen by the aromatase enzyme complex. Androstenedione, the direct precursor of testosterone, is also aromatised to oestrogen by the aromatase enzyme. The important step in this metabolic process is the removal of the methyl group from the 19th carbon of either testosterone or androstenedione. Nandrolone differs structurally from testosterone and androstenedione in lacking the methyl group at the 19th carbon, and it is additionally different from androstenedione in substitution of a ketone group for an hydroxy group at the 17th carbon. Is it feasible that 19-norsteroids (nandrolone and metabolites) are intermediates in the aromatisation process? (fig 1).

Animal studies, in vitro experiments, and observations in humans, particularly pregnant women, add support to the proposal that 19-norsteroids are intermediates in the aromatisation of androgens to oestrogen. Oestrogen concentrations in women increase significantly both at the time of ovulation and during pregnancy. Raised urine NA concentrations after soccer games were significantly higher than before games. For NA concentrations after games, 70% of the urine samples were below 0.1 ng/ml, and 20% were between 0.1 and 0.2 ng/ml. In four urine samples were above 1.0 ng/ml, the maximum value being 1.79 ng/ml. When urine is tested for banned substances and the specific gravity of the urine sample is measured above 1.020, urine metabolite concentrations are adjusted by a correction factor. This analysis is based on the premise that urine flow rate and urine metabolite excretion per se can also show considerable genetic variability in expression and activity in certain people, with increased activity of the aromatase enzyme producing larger amounts of oestrogen. This prompts the question of whether genetic upregulation of the aromatisation process in these people increases the production of 19-norsteroids.

**Exercise**

Intense exercise has been associated with raised levels of NA in the urine, Le Bizec et al. studied professional soccer players over 19 months and collected 385 urine samples. Urine NA concentrations after soccer games were significantly higher than before games. For NA concentrations after games, 70% of the urine samples were below 0.1 ng/ml, and 20% were between 0.1 and 0.2 ng/ml. NA in four urine samples were above 1.0 ng/ml, the maximum value being 1.79 ng/ml. When urine is tested for banned substances and the specific gravity of the urine sample is measured above 1.020, urine metabolite concentrations are adjusted by a correction factor. This analysis is based on the premise that urine flow rate and urine metabolite excretion remain constant during and directly after exercise. However, this is an erroneous assumption as it has been shown that excretion of pseudohedrine after exercise was increased in subjects in whom urine volume remained constant. Thus urine metabolite excretion may not remain constant during and directly after exercise, and
random urine sample collection after exercise may be unreliable. A more accurate measure would be to collect a urine sample over a 24 hour period, allowing the calculation of excretion rate of urine metabolites. However, this is not practical, particularly when testing for drug use in sport.

The serum androgen response to exercise in athletes can vary according to the type, duration, and intensity of the exercise task. Serum concentrations of testosterone, androstenedione, and dehydroepiandrosterone increase with short term, intense exercise as the result of increased testicular production by an unknown mechanism. An increase in serum testosterone after exercise may also be caused by a decrease in the plasma volume or a decrease in hepatic clearance. The effect of exercise on serum oestradiol is also extremely variable. A urine specimen collected after high intensity exercise could have a higher concentration of NA.

It is conceivable that the increase in circulating androgens in people participating in short duration, high intensity exercise could result in the stimulation of the aromatase enzyme complex, resulting in an absolute increase in the amount of NA in the urine. Therefore, there are sufficient data to suggest that a urine specimen collected after high intensity exercise could have a higher concentration of NA for reasons other than dehydration.

Trauma and hypoglycaemic stress

As yet, no study has investigated the possible effect that traumatic stress (musculoskeletal injury) may have on 19-norsteroid metabolism. Interestingly, two international male athletes, one an international rugby player and the other a paraOlympian (B Frasure, personal communication), recently tested positive for NA above 2 ng/ml, after both having suffered significant injuries just before passing a urine sample tested positive for NA. This raises the question of whether trauma and hypoglycaemic stress on the metabolism of dehydroepiandrosterone (DHEA) in these athletes is about 150. No specific mention is made in any of the studies of the age of the male subjects. This is relevant because testosterone production decreases with advancing age.

Further research is necessary.

Mineral cofactors and herbal products

There is also a theoretical argument that certain substances not prohibited in sport may alter nandrolone metabolism. For example, the trace element zinc is a cofactor in many enzymic processes in the body. An increase in serum testosterone in men who are marginally zinc deficient has been shown after zinc supplementation. Also, diets deficient in zinc resulted in a significant decrease in serum testosterone concentration.

As yet, no study has investigated the possible effect that trauma and hypoglycaemic stress on the metabolism of 19-norsteroids may have on the aromatisation process. However, inspection of their data shows that, in certain populations an important consideration. The amount of NA in the urine from the subjects did not exceed 1 ng/ml, except in the study of Galan Martin et al., where the urine from the subjects did not exceed 1 ng/ml, except in the study of Galan Martin et al., the concern is which in which have already been raised.

There is no explanation by the IOC of why the threshold concentration for NA is higher in women."
the reason for it is the higher circulating levels of oestrogen, particularly at the time of ovulation, is this not indirect support for the presence of 19-norsteroids as intermediates in the aromatisation of androgens to oestrogen. Bradford-Bill has stated: “It is the essence of science to disclose both the data upon which a conclusion is based and the methods by which the conclusion is obtained”.

The IOC has defended the status quo on nandrolone and confirmed these threshold values of 2 ng/ml in men and 5 ng/ml in women in Monaco in October 1999. The conditions of strict liability are currently applied in the case of any athlete contravening the above thresholds.

METHODS TO TEST FOR NA
A solution to the controversy surrounding nandrolone in sport is to develop a testing procedure that can accurately differentiate endogenous nandrolone metabolites from nandrolone that is ingested or injected. The technique of gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) to calculate the $^{13}C/^12C$ ratio is currently being developed as a method to fulfil this purpose. This is based on the principle that natural steroids have a different carbon isotopic signature from synthetic steroids. The $^{13}C/^12C$ ratio for synthetic nandrolone metabolites is lower than that for endogenous metabolites, therefore administering exogenous nandrolone will lower this ratio. This ratio has also been proposed as a method of detecting the use of synthetic testosterone as an alternative to the testosterone/epitestosterone ratio. However, a potential problem with GC-C-IRMS is the lack of reproducibility and sensitivity because of the low levels of endogenous nandrolone metabolites present in the body. At present, this method can only be applied to “high” concentrations of NA (60 ng/ml) in the urine.

Le Bizec et al.

has proposed examining the steroid conjugates as an additional criterion to distinguish between the endogenous or exogenous origin of nandrolone metabolites. Endogenous NA was found to be 30% sulpho-conjugated in contrast with administered nandrolone, which was found to be 100% conjugated to glucuronic acid when excreted in the urine.

Kintz et al. has proposed that analysis of hair samples from athletes is another option to consider for detecting the presence of exogenous nandrolone. The analysis of hair samples could be used to accurately verify positive results obtained by gas chromatography-mass spectrometry. Until the hair sample and GC-C-IRMS techniques have been validated on a large scale, a prudent approach after the detection of NA in urine samples above the cut off concentration is for the athlete to have further blood tests before the sample is declared positive, as is done for athletes with a high testosterone/epitestosterone ratio.

CONCLUSION
The abuse of the steroid testosterone presented a new problem for drug control in sport. Perhaps the same can now be said for nandrolone. According to the Olympic movement anti-doping code, NA is not a prohibited substance. However, should NA in the urine exceed a certain threshold concentration, the interpretation is that nandrolone has been ingested or injected. There is strong scientific evidence to suggest that NA can appear in the urine of people free of exogenous nandrolone. Evidence suggests that NA may occur as an intermediate in the aromatisation of testosterone to oestrogen. Recent evidence has shown that the amount of NA in the urine can be regulated by the administration of human chorionic gonadotrophin. Therefore, threshold concentrations for men (2 ng/ml) and women (5 ng/ml) as defined by the IOC are still open to debate because conclusive scientific evidence showing how these values may be altered by various physiological stimuli is lacking. In accordance with this, multicentre studies need to answer further specific questions on the current urine threshold concentrations for nandrolone metabolites and whether physiological stressors and permitted supplement interventions can alter NA excretion.

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