Human chorionic gonadotrophin and sport

A. T. Kicman, R. V. Brooks and D. A. Cowan

Drug Control Centre, King's College, London, UK

Human chorionic gonadotrophin (hCG) is a glycoprotein hormone which is produced in large amounts during pregnancy and also by certain types of tumour. The biological action of hCG is identical to that of luteinizing hormone, although the former has a much longer plasma half-life. Some male athletes use pharmaceutical preparations of hCG to stimulate testosterone production before competition and/or to prevent testicular shutdown and atrophy during and after prolonged courses of androgen administration. Testosterone administration can be detected by measuring the ratio of concentrations of testosterone to epitestosterone (T/E). An athlete is often considered to have failed a drug test if the urinary T/E ratio is greater than 6. In contrast, hCG administration stimulates the endogenous production of both testosterone and epitestosterone without increasing the urinary T/E ratio above normal values. Although the administration of hCG was banned by the International Olympic Committee (IOC) in 1987, no definitive test for hCG has been approved by the IOC. Currently, the only way of measuring small concentrations of hCG is by immunoassay, and this does not have a discriminating power as great as gas-liquid chromatography with mass-spectrometry which is necessary to satisfy IOC requirements. Extraction procedures and chromatographic steps could be introduced before using a selected immunoassay for hCG to meet these requirements.

Keywords: hCG, sport, immunoassay, detection, doping

With the introduction of 'peptide hormones and their analogues' as a new doping class by the International Olympic Committee (IOC), attention has focused on the problem of human chorionic gonadotrophin (hCG) administration. Although there is a vast amount of literature on hCG, very little to date has been published in relation to hCG and sport. This review attempts to address this problem by giving a concise but general account on hCG with particular emphasis on its detection and misuse in sport.

Origin and function

Human chorionic gonadotrophin is a glycoprotein which is produced in large amounts during pregnancy and also by certain types of tumour. It is also

secreted in very small amounts, probably by the pituitary gland, in normal men and non-pregnant women¹. Small concentrations of hCG have been measured in the serum of normal subjects (less than 5 IU/l), and hCG or hCG-like material has been found in tissues, pituitary and urine extracts²⁻⁴.

Immunoassay for hCG is generally used as a diagnostic tool for pregnancy, trophoblastic tumour (hydatidiform mole, choriocarcinoma and germ cell tumour) and non-trophoblastic tumour (common sites being the testis, ovary, stomach and pancreas).

In pregnancy, hCG is secreted into the maternal blood shortly after conception (within 9 days) by the fused outer layer of cells surrounding the implanted blastocyst called the syncytiotrophoblast cells (*syn*, together; *kytos*, cell; *trophein*, to nourish; *blastos*, germ). The rate of secretion of hCG from the developing placenta, which is called the chorion (trophoblast cells with a lining of mesoderm), rises rapidly. Maximum concentration is reached in maternal blood and urine at approximately 8-10 weeks gestation (100 kIU/l). This is followed by a gradual decline to a nadir at 17-20 weeks, which is maintained at approximately 20 kIU/l, followed by a secondary much smaller peak around weeks 32-33.

The major role of hCG in pregnancy is considered to be the maintenance of the corpus luteum for the production of progesterone and to a lesser extent of oestradiol. It may also play an important part in male sexual development, by stimulating fetal Leydig cells, as there is a fall of fetal testosterone production during the second and third trimester corresponding to that of maternal hCG.

Hydatidiform mole is the end stage of a degenerating pregnancy, in which the trophoblastic tissue of the chorion has undergone hypertrophy, leading to absorption of the fetus. The villi of the mole may become locally invasive with some metastasis. Alternatively, the mole could progress to choriocarcinoma which is widely metastatic.

In the USA, the incidence of testicular tumour is 2-3 per 100 000 men. Nevertheless, it is the second most common malignancy after leukaemia in 20-35-year-old men⁵. Non-trophoblastic tumours of the testes are far rarer than germ cell tumours (which are of trophoblastic origin), accounting for only 1-2% of testicular neoplasms. Trophoblastic tumours can lead to the urinary excretion of several million international units of hCG per day, whereas non-trophoblastic tumours do not generally exceed a few hundred international units per day⁶.

Address for correspondence: D. A. Cowan, Drug Control Centre, King's College, Manresa Rd, London SW3 6LX, UK

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In the male, hCG-secreting tumours can give rise to elevations in testosterone and oestradiol secretion because of the action of hCG in stimulating normal steroidogenesis.

Structure

The structure and properties of hCG have been reviewed extensively by Birken and Canfield⁷ and Pierce and Parsons⁸.

Thyroid-stimulating hormone (TSH), together with the gonadotrophins, luteinizing hormone (LH), follicle-stimulating hormone (FSH) and hCG, forms a family of glycoprotein hormones which are composed of two dissimilar non-covalently linked subunits. The subunits, designated α and β , are linked by electrostatic and hydrophobic interactions. The strength of this binding is demonstrated by the fact that the subunits are not easily separated in a high concentration of urea (10M) at neutral pH but are separated in acidified urea (0.001 M HCl, 40°C, 1 h).

In humans, the approximate molecular weights of hCG, FSH, LH and TSH are 37 000, 33 000, 28 000 and 28 000 respectively. The α -subunit is very similar in primary structure within this family but the β -subunits differ. The hCG α -subunit consists of 92 amino acids and has two carbohydrate moieties which are branch chained to asparagine residues⁹, giving an average total molecular weight of 14 500.

Although there is some homology between the various β -subunits, in particular between LH and hCG, there are variant amino acid sequences which account for different target receptor recognition. FSH and TSH have very different variant sequences in comparison to hCG and LH. The homology between hCG and LH accounts for their identical target cell receptor recognition and action in stimulating steroidogenesis.

Importantly, the β -subunit of hCG consists of 145 amino acid residues, having 30 additional residues at the C-terminal region compared with the β -subunit of LH. It is within this region that a specific antigenic determinant is located which allows for immunological distinction as opposed to biological distinction between hCG and LH.

Attached to the β -hCG subunit are six carbohydrate moieties¹⁰, giving an average total molecular weight of 22 000. Two branch chain moieties are linked to asparagines and four are linked to serines within the unique C-terminal peptide region (Figure 1).

α - subunit																
H ₂ N -	<u>ALA</u>	-	PRO -	<u>ASP</u>	-	<u>VAL</u>	-	CHO ASN	-	CHO ASN	-	SER -	COOH			
	1		2	3		4		52		78		92				
β - subunit																
H ₂ N -	SER	-	CHO ASN	-	CHO ASN	-	CHO SER	-	CHO SER	-	CHO SER	-	CHO SER	-	GLN -	COOH
	1		13		30		121		127		132		138		145	

Figure 1. Position of carbohydrate (CHO) attachments to the α - and β -subunits of hCG. The α -subunit N-terminus is heterogeneous and begins at one of the three amino acids underlined (summarized from figures by Birken and Canfield⁷)

Figure 2. Structure of *N*-acetylneuraminic acid. The asterisk indicates the point of attachment to the rest of the carbohydrate moiety (2→3 linkage)

The studies by Kessler *et al.*^{9,10} showed that the complete hCG molecule has eight carbohydrate moieties in total, making it approximately 30% carbohydrate content by weight. Each moiety contains two attached *N*-acetylneuraminic acid groups, which is a sialic acid (Figure 2), giving rise to 16 sialic acids per hCG molecule. However, as there is microheterogeneity in the carbohydrate content of the gonadotrophins, and as these forms have not yet been fully characterized, it is important to appreciate that the total number of sialic acid groups per hCG molecule will only represent an average amount and this number will vary between reported studies. The greater number of sialic acid groups on hCG leads to a greater ionization constant and hence lower pI value when compared with the other gonadotrophins. The pI of hCG is about 3.5, FSH about 4.5 and that of LH about 5.5.

Differences in hCG carbohydrate content lead to observable differences in electrophoresis and isoelectric focusing. These differences are probably due to losses in isolation procedures as well as biological variation, together with very minor N-terminal heterogeneity in the α -subunit amino acid sequence.

The higher carbohydrate content of hCG leads to a larger Stokes' radius compared to FSH, LH and TSH (Stokes' radius is defined as the radius of a perfect unhydrated sphere having the same rate of passage through a size-exclusion column as the unknown protein in question). This causes the hormone to elute in the 65–70 000 MW region instead of its true weight of 37 000.

International Standards and units

The first International Standard (IS) for hCG was established in 1938 having a potency of only 10 IU/mg of crude fraction isolated. Using hCG isolated from pregnant female urine, a second IS (code 61/6) was established in 1964¹¹. Its potency was assessed in terms of bioassay as compared with the first IS. Amongst the bioassays applied were increases in the weight of the rat or mouse ventral prostate and seminal vesicles as a result of hCG stimulation of testosterone production (interstitial cell stimulating activity). Even though the second IS was considerably purer than the first, there was only approximate-

ly 20% (w/w) of pure hCG present in the material isolated.

With the introduction of radioimmunoassays for hCG in the 1970s, it became obvious that the second IS was not suitable for immunoassays because of its impurity and heterogeneity. This led to the preparation by the Center for Population Research, Washington DC, of a new reference compound specifically for radioimmunoassay. Part of this material, coded CR119, was used by the World Health Organization (WHO) to prepare an International Reference Preparation (IRP) for immunoassay^{12, 13}.

The IRP (code 75/537) was very pure, having less than 1% (w/w) of contaminants. Its unitage was expressed in terms of the second IS using bioassay calibration. This was necessary to allow continuity in clinical laboratories' results. Each ampoule supplied by WHO contained 650 IU of hCG with 1 IU being equivalent to 108 ng of hCG.

When the supplies of the second IS ran out, WHO logically renamed the pure IRP, calling it the third IS, but kept the designated code number of 75/537. Although the third IS is very pure, quantities of standard are normally expressed in terms of international units rather than weight. This is because hCG is microheterogeneous in structure and new biological standards are rarely identical to the ones that they replace.

Standards of the α - and β -subunits of hCG are also expressed in international units, where 1 IU = 1 μ g. Here the unitage is defined in terms of mass alone as the isolated subunits of hCG have no biological activity.

Biological action at the cellular level

The biological action of hCG at the cellular level is identical to that of LH. A full account of the receptor interaction and transducing systems involved is beyond the scope of this brief review and for further detailed information the review by Cooke and Romerts¹⁴ is recommended.

In the male, LH and hCG interact with specific target receptors on the surface of Leydig cells in the testes. The hormone-bound receptors activate cyclic AMP and calcium ion secondary messenger systems, which in turn activate various protein kinases, finally stimulating steroidogenesis and protein synthesis. There is evidence to suggest that phospholipid metabolites, in particular the leukotrienes, may also act as secondary messengers in LH/hCG induced steroidogenesis.

Initially, the interaction of hCG with the receptor is by low affinity binding with the β -subunit. This leads to a second and much higher affinity interaction with the α -subunit^{15, 16}. The binding of LH and hCG to the receptors is not completely reversible, as some internalization and degradation of the receptor-bound complex occurs¹⁷.

Stimulation of testicular steroidogenesis by hCG in healthy adult men is very rapid. A 50% increase in plasma testosterone concentration has been measured 2 h after an intramuscular injection of 6000 IU of hCG¹⁸. However, there is no direct correlation between plasma concentrations of hCG and testoster-

Figure 3. Effects of a single injection of 6000 IU hCG intramuscularly on plasma levels of hCG (a) and testosterone (b). Values are the mean (s.e.m.) of the three subjects (hCG) or seven subjects (testosterone). (Reproduced with permission from Saez JM, Forest MG. Kinetics of human chorionic gonadotropin-induced steroidogenic response of the human testis. I. Plasma testosterone: implications for hCG stimulation test. *J Clin Endocrinol Metab* 1979; **49**: 278–83)

one, the rise in plasma testosterone being biphasic (Figure 3). The favoured hypothesis for the biphasic response is that oestradiol plays an intratesticular role in androgen production¹⁹. A big dose of hCG is known to induce Leydig cell aromatase which causes a large increase in testicular oestradiol secretion.

The rising oestradiol partially suppresses the cytochrome P450c17* enzyme²⁰ activities leading to a dampening effect on the hCG-induced increase in testosterone synthesis. Importantly, the increase in aromatase activity would not significantly deplete the testosterone formed in the testes as the proportion converted by aromatization to oestradiol would still be very low. As the plasma hCG concentration begins to fall there is an accompanying decrease in aromatase activity and hence oestradiol synthesis, which relieves the partial enzymatic block. A maximum in plasma testosterone concentration is then attained around 3–4 days after hCG administration.

*Previously known as C17,20-lyase and 17 α -hydroxylase (see Miller²⁰)

In adult men with hypogonadotrophic hypogonadism due to the administration of anabolic-androgenic steroids, the increase in plasma testosterone is attenuated following hCG stimulation and there is no significant change in plasma oestradiol concentrations. The steroidogenic enzymes cannot be fully reactivated with a single dose of hCG²¹. However, if chronic administration of hCG is continued (e.g. 5000 IU, 3 times per week) during and/or after the administration of supraphysiological doses of testosterone, then testosterone secretion and spermatogenesis can be stimulated and maintained²².

Evidence suggests that hCG binds with low affinity to FSH receptors in rodents and has an intrinsically weak follicle-stimulating activity and thyroid-stimulating activity due to its general structural homology with FSH and TSH^{23,24}. This secondary biological activity is borne out in humans by the ability of large plasma concentrations of hCG to stimulate and maintain spermatogenesis in hypogonadotrophic hypogonadism and by the hypothesis that the thyrotoxicosis, which is associated in patients with trophoblastic diseases, is caused by the exceedingly high concentrations of serum hCG.

Metabolic clearance rate and half-life

About 20–30% of hCG administered intravenously or intramuscularly is excreted in the urine during the succeeding 5–6 days^{25,26}. The metabolic clearance rate (MCR) is 3.4 and 3.9 ml/min in men and women respectively.

Although the MCR of the β -subunit of hCG is about tenfold greater than that of hCG, less than 1% of the β -subunit is excreted in urine²⁶. Evidence suggests that β -hCG is metabolized in the kidney to a smaller component, of molecular weight 12 000–17 500. This β -core component is devoid of the carboxy-terminal peptide immunological determinant which is also necessary for biological activity. The β -core fragment is present in large quantities in the urine of pregnant women²⁷ and crude commercial hCG preparations²⁴.

The difference in the degree of sialylation between the gonadotrophins (hCG > FSH > LH) influences hepatic uptake and hence MCR and plasma half-life. A comparative study of half-lives of LH following complete hypophysectomy and hCG after removal of the placenta showed the distribution of both hormones to be in at least two compartments. There was a fast initial process of disappearance of $t_{1/2}$ of approximately 21 min for LH compared with 11 h for hCG and a slower process of disappearance of $t_{1/2}$ of approximately 235 min for LH compared with 23 h for hCG²⁸. The plasma half-life values quoted for hCG differ somewhat from those found by Nisula and Wehmann²⁶ but nevertheless the comparative study demonstrates more than adequately the marked difference in the disappearance rates of LH and hCG. Administrations of hCG injected intravenously and intramuscularly have demonstrated that there is a slightly longer half-life of the hormone for the latter route, probably because of the slower release into the circulation²⁹. Desialylated hCG has a very short plasma half-life, due to the exposure of free galactose

groups within the carbohydrate moieties. These groups are specifically recognized by hepatic receptors leading to the uptake, internalization and destruction of asialo-hCG.

Renal clearance

The renal clearance of hCG approximates 1 ml/min²⁶. This means that in 1 min the equivalent mass of hCG in 1 ml of plasma is secreted into the bladder from the volume of glomerular filtrate formed (average rate is 125 ml/min).

The renal clearance (C_R) of hCG may be defined by the equation:

$$C_R = [U]V/[P]$$

where $[U]$ is the concentration of hCG in urine; V is the volume (ml) of urine secreted per minute, and $[P]$ is the concentration of hCG in plasma.

As the normal volume of urine secreted into the bladder approximates 1 ml/min over a 24 h period and the clearance rate of hCG approximates to 1 ml/min, it follows that plasma and 24 h urinary concentrations of hCG are of similar nature. Measurements of hCG concentrations in first morning-voided urine samples can also give an approximation of hCG plasma concentrations simply because of less fluctuation in the renal clearance of hCG during the sleeping hours (Table 1). However, a number of assumptions have been made in all of the above and there is a need to characterize more fully the possible relationships between urinary filtration rate and hCG disposition.

Immunoassay and detection

An excellent general introduction to the principles of immunoassay techniques has been written by Edwards³⁰.

Initially, polyclonal antibodies for use in hCG immunoassays were raised by immunization with the intact molecule. With the postulate of conformational similarities between the native hormones³¹, there was up to 100% cross-reaction of these antisera with LH. This problem was overcome to a large degree by the introduction of antibodies raised to antigenic sites on either the intact β -subunit of hCG³² or its carboxy-terminal region. These antibodies normally recognize the corresponding antigenic sites on the whole hCG molecule as well as the free β -subunit.

Specificity was further improved by the introduction on a commercial scale of monoclonal antibodies raised to hCG and/or the free β -subunit. Typical cross-reactions of monoclonal antibodies with the structurally related glycoprotein hormones are much less than 1%^{33–35}.

Although the problem of immuno-crossreactivity between the gonadotrophins has been solved, there remains the general problem of measuring any polypeptide or protein analyte with a microheterogeneous structure^{36,37}. The heterogeneity of the gonadotrophins has been studied by Stockell Hartree *et al.*³⁸ using ion-exchange chromatography. The elution pattern of these hormones showed that there was no separation of these components as sharp peaks, which indicates considerable heterogeneity.

Table 1. Concentrations of hCG in early morning plasma and first morning-voided urine after intramuscular injection of hCG (5000 IU) on day 1. Measurements were performed using immunoradiometric (Miaclone) and immunoenzymetric (Serozyme) kits⁵⁴ (Serono Diagnostics Ltd, Windsor, UK)

Subject	Day	hCG (IU/l)			
		Serum		Urine	
		Miaclone	Serozyme	Miaclone	Serozyme
1	Pre-injection	<0.1	<1.0	2.2	<1.0
	3	164	152	149	84
	5	55	43	146	121
	8	18	10	49	28
2	Pre-injection	<0.1	<1.0	0.8	<1.0
	3	164	145	330	247
	5	51	38	57	47
	8	13	9	20	12
	10	5	3	14	7
3	Pre-injection	<0.1	<1.0	2.6	<1.0
	2	244	223	251	216
	4	83	74	77	75
	8	13	11	30	21
	10	6	5	3	2

Such heterogeneity could be due to artefacts generated mainly by desialylation and other carbohydrate losses during the isolation procedures used, although undoubtedly a number of naturally occurring isoforms do exist. Evidence suggests that the carbohydrate moieties influence the structural conformation of the glycoprotein hormones³⁹. Heterogeneity of gonadotrophin standards and immunogens is generally accepted as accounting for the discrepancies in results between different competitive immunoassays for hCG and also between those methods and 'two-site' immunometric methods. These different results are apparent in the UK external quality assessment scheme. In the future, it may be possible to resolve this problem by characterizing the differences in structures between the isoforms and only to use monoclonal antibodies with particular epitope specificity.

At the Drug Control Centre, hCG administration in sportsmen is currently detected by analysing untimed urine samples using immunometric assays which incorporate highly specific monoclonal antibodies. The principle of the immunometric assay is that labelled antibody is added in excessive amounts to the antigen being measured. With 'two-site' immunometric assays the antigen-antibody complex is then coupled to another antibody which is linked to a solid phase. The solid-phase linked antibody is also added in excess and the overall antigen-antibody complex is commonly called a 'sandwich'. The way the sandwich is then isolated depends on the type of solid phase used, e.g. precipitation or magnetic separation. End-point measurement depends on the type of label being used, e.g. radioactive, enzymatic, fluorometric.

There are two limitations with the two-site immunometric assay. With very large concentrations of hCG such as found with trophoblastic tumours, a biphasic response known as the 'hook effect' can be elicited (Figure 4). This occurs with certain methods

because the labelled antibody and solid-phase linked antibody are present together in a milieu of antigen excess. Increasing amounts of antigen excess results in decreasing values of analyte measured because of the decreasing amounts of antigen-antibody sandwiches formed. In these cases, results due to a small concentration of analyte measured may not be distinguished from those of very large concentration.

Certain commercial assay kits which are used to quantitate hCG show 'hook effect' characteristics with concentrations in excess of 500 000–1 000 000 IU/l, far higher concentrations than could ever be achieved in the urine as a result of hCG administration. Other immunometric assay procedures overcome the problems of a biphasic response by incorporating a wash step between the addition of the solid-phase linked antibody and the labelled antibody. Hence, any unbound antigen is removed before the addition of labelled antibody. This will

Figure 4. Biphasic response ('hook effect') in the two-site immunometric assay

result in concentrations of antigen greater than the largest calibrant concentration appropriate for that assay, giving results equal to or greater than the largest calibrant but smaller than their true value.

A second limitation is due to the presence of antibodies in the blood of certain individuals, which are directed against the same immunoglobulins as those which are used in the assay, e.g. sheep, rabbit or mouse⁴⁰. In the two-site immunometric assays, this results in cross-linking between the labelled antibody and the solid-phase linked antibody, giving rise to false positives. There is no known presence of these interfering antibodies in urine. In clinical laboratories which analyse blood samples for hCG by using immunometric assays, cases of false positives have arisen due to the presence of these heterophilic antibodies⁴¹⁻⁴³. It follows that caution must be exercised, if in the future blood samples are to be used for drug testing in sport.

Misuse of hCG in sport

There is little conclusive evidence from the many scientific studies performed that the use of synthetic anabolic-androgenic steroids in physiological or supraphysiological concentrations compared with that of testosterone, has any appreciable direct anabolic effect on skeletal muscle growth (hyperplasia and/or hypertrophy) in healthy adult males^{44, 45}. Nevertheless, there is some evidence to suggest that any enhancement in athletic performance may be due to the androgenic effect of these steroids upon the central nervous system resulting in increased aggression and competitiveness⁴⁶⁻⁴⁸. The experienced male athlete may gain an anabolic effect, albeit indirectly, by knowing how to capitalize on the behavioural effects resulting from androgen administration to enable him to train harder. The improvement in performance may be so small as to be virtually impossible to prove with statistical significance. However, a tiny improvement in performance may be all the sportsman requires to win a better place in a closely fought competition. In any case, anabolic steroids continue to constitute the greatest percentage of total positive samples detected by the IOC-accredited laboratories year after year (e.g. 49% in 1989).

Other possible mechanisms by which the use of anabolic steroids may enhance performance are by stimulating the production of erythropoietin and also by direct action on the stem cells in the bone marrow⁴⁹. This would cause an increase in erythrocyte formation and therefore an increase in oxygen uptake, resulting in a possible improvement in endurance capacity. Also some athletes claim that the administration of synthetic anabolic steroids enables faster recovery from fatigue during training. This may be due to a general anticatabolic effect on subjects with large concentrations of endogenous corticosteroids due to the stress of severe training schedules⁵⁰.

Consequently it is possible that, if the action of anabolic and androgenic steroids is by the effects discussed rather than by any direct myotrophic action then there may be no advantage in taking synthetic

anabolic steroids in preference to testosterone. Indeed, the many undesirable effects of synthetic anabolic steroids, in particular the risks of hepatotoxicity, hepatoma and prostatic carcinoma associated with 17 α -alkylated steroids, combined with the knowledge of efficient gas-liquid chromatography with mass-spectrometry (GC-MS) as a detection system for these drugs, may prompt the abuse of testosterone as the steroid of choice. Alternatively, some athletes may switch from a course of synthetic anabolic steroids to administering testosterone in anticipation of testing. Now that testing is being introduced at training periods as well as at competitions in the UK and a number of other countries, the logical temptation of administering testosterone as the chosen steroid of abuse may be very strong. Indeed in 1989, the IOC-accredited laboratories found that testosterone was the second most common anabolic steroid detected after nandrolone.

Pharmaceutical preparations of hCG can be used to stimulate testosterone production in men before competition. In addition, it may be used to prevent testicular shutdown and atrophy during and/or after prolonged courses of natural or synthetic androgen administration. The alternative use of LH preparations for these purposes is not practical due to the small amount supplied per ampoule (e.g. 75 IU) and its much shorter plasma half-life compared to hCG. A possible undesirable effect of continued hCG administration in the healthy adult male is that of gynaecomastia, probably as a result of raised oestrogen secretion from the testes. This effect can be counteracted by concomitant testosterone administration²².

In non-pregnant women, it is difficult to see any advantages to be gained in athletic performance from the administration of hCG. It may be that timed hCG stimulation could be used to mimic early pregnancy by prolonging the luteal phase of the menstrual cycle, thus enabling the administration of a course of anabolic-androgenic steroids without accompanying ovarian atrophy. Nevertheless, as it is currently not possible to distinguish between hCG administration and hCG production in early pregnancy, female urine samples are not analysed for hCG.

In 1979, a prototype assay for the detection of testosterone administration using the ratio of urinary concentrations of testosterone to LH was published⁵¹. Only untimed urines are collected for drug analysis, so this ratio was used because it is independent of the volume of water excreted. However, this method was not fully explored by other countries, who were very reliant on GC-MS techniques which cannot measure protein hormones such as LH.

In 1982, an alternative method, which has been adopted by the IOC, using the ratio of urinary total testosterone to the 17 α -isomer, epitestosterone, was developed by the IOC-accredited laboratory in Cologne⁵². As epitestosterone is only a very minor product of testosterone metabolism (less than 1%), the detection of testosterone administration can be determined by an increase in the ratio of urinary testosterone to epitestosterone (T/E). This evidence led to a ban based on the T/E ratio by the IOC in 1983.

Action is often taken against an athlete if the urinary T/E ratio exceeds 6.

The administration of hCG to normal adult males is known to stimulate endogenous epitestosterone secretion as well as testosterone secretion, thus increasing the blood testosterone concentration without altering the urinary T/E ratio⁵³. Initial results from this work led to the testing of 740 samples from male athletes for hCG. Of these samples, 21 were found to have unnaturally large concentrations of urinary hCG⁵⁴. These results led to a ban on hCG administration by the IOC in November 1987.

However, the only practical way for testing hCG is by immunoassay. Although there are a number of commercial immunoassay kits on the market which use highly specific monoclonal antibodies to detect the presence of hCG in urine, the singular use of immunoassay is currently not an acceptable definitive test for hCG by the IOC Medical Commission. Although polypeptide and protein hormone administration is banned under the doping class of 'peptide hormones' there are no IOC-approved tests for them. The rationale for this unacceptability is that these assays do not have the discriminating power, equivalent to GC-MS, sufficient to satisfy the IOC requirements.

With GC-MS, the banned drug is separated from other potentially interfering substances in the urine by high-resolution chromatography before being identified by the mass-spectrometer. A similar sort of approach could be taken with testing for hCG administration where it is separated from other possible interfering solutes present in the urine before confirmation by immunoassay. Extraction procedures and/or size-exclusion chromatography could be employed for this purpose.

The problem of microheterogeneity together with possible immunoreactive fragments could be overcome by standardization, using either one particular commercially supplied hCG immunoassay kit or using a number of kits which give concordant results. An external quality assessment scheme has been suggested between the IOC-accredited laboratories to monitor consistencies in results⁵⁵. A normal range for healthy adult men, together with a suitable cut-off point for the determination of hCG administration, would have to be established. Of course, the failure of an hCG drug test due to an hCG-secreting tumour would be a piece of singular good fortune in causing the athlete to seek medical advice.

Another challenging problem to be overcome is the effect of possible changes in the renal clearance rate of hCG such as might result from prolonged vigorous exercise. As only single untimed urine samples are collected from athletes, a proposed cut-off level would have to be well in excess of normal values for urinary hCG obtained from individuals after exercise. In addition, an hCG to LH ratio would give supplementary information which might not be subject to the effects of changes in renal clearance rate.

Conclusion

Drug control in sport has evolved from the comparatively simple task of detecting foreign substances in

urine. Now that 'peptide' hormones have been added as a doping class, all the IOC-accredited laboratories have a responsibility to implement suitable detection methods for hCG administration.

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