

Señor Superintendente
SUPERINTENDENCIA DE INDUSTRIA Y COME
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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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Referencia : Expediente No. **11.156.685**
Asunto : Solicitud de patente para: **"COMPOSICIÓN FARMACÉUTICA QUE COMPRENDE UN ESTEROIDE ANABÓLICO Y UN COMPUESTO DE VITAMINA D PARA ADMINISTRACIÓN PARENTAL PARA LA PREVENCIÓN Y/O TRATAMIENTO DE LA FRAGILIDAD"**
Solicitante : **ORGANEXT RESEARCH B.V.**
Fecha de solicitud : 17 de noviembre de 2011

I. RECURSO VÍA GUBERNATIVA

Yo, Luz Clemencia DE PÁEZ, en mi condición de apoderada de la sociedad **ORGANEXT RESEARCH B.V.** solicitante de la patente de la referencia, por el presente escrito atentamente manifiesto a usted que debidamente notificada de la Resolución No. **86139** del 26 de diciembre de 2013, interpongo en su contra el recurso de **REPOSICIÓN**, para que sea revocada y en consecuencia se conceda el privilegio de patente de invención para **"COMPOSICIÓN FARMACÉUTICA QUE COMPRENDE UN ESTEROIDE ANABÓLICO Y UN COMPUESTO DE VITAMINA D PARA ADMINISTRACIÓN PARENTAL PARA LA PREVENCIÓN Y/O TRATAMIENTO DE LA FRAGILIDAD"** a favor de **ORGANEXT RESEARCH B.V.**

II. OPORTUNIDAD DE LA IMPUGNACIÓN

La Resolución No. **86139** del 26 de diciembre de 2013, con fecha de publicación listado 31 de diciembre de 2013, fecha de vencimiento 31 de enero de 2014, y fecha del término legal 7 de febrero de 2014.

"EXCELENCIA LEGAL EN UN MUNDO SIN FRONTERAS"

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III. LA DECISIÓN RECURRIDA

- 3.1 Mediante la Resolución objeto de la presente impugnación, el Superintendente de Industria y Comercio decidió:
- 3.1.1 Denegar la patente de invención a la creación titulada: **“COMPOSICIÓN FARMACÉUTICA QUE COMPRENDE UN ESTEROIDE ANABÓLICO Y UN COMPUESTO DE VITAMINA D PARA ADMINISTRACIÓN PARENTAL PARA LA PREVENCIÓN Y/O TRATAMIENTO DE LA FRAGILIDAD”**.
- 3.2 En la parte motiva de la providencia, se mencionaron los siguientes fundamentos:
- 3.2.1 “las nuevas reivindicaciones 1 a 6 no superan la objeción sobre falta de unidad de invención, de acuerdo a las siguientes apreciaciones”:
- 3.2.2 “La solicitante argumenta que los dos grupos inventivos identificados por el examinador en la solicitud corresponden a composiciones farmacéuticas para administración parenteral y para administración intramuscular. Continúa la solicitante señalando que dichas composiciones para administración intramuscular han sido eliminadas del nuevo capítulo reivindicatorio y que de esta manera la objeción por falta de unidad de invención se ve superada”.
- 3.2.3 “Al respecto, este Despacho reitera a la Solicitante que el oficio No. 9646 explícitamente señalaba:
- “Los grupos inventivos están definidos por las siguientes características esenciales:*
- 1º composición para administración parenteral que comprende un esteroide anabólico y un compuesto de vitamina D.*
- 2º dispositivo de inyección para la administración intramuscular de un líquido”*”.
- 3.2.4 “Por lo tanto, contrario a la argumentación de la Solicitante, el segundo grupo inventivo (reivindicación 6, incluida en el radicado 11-156685-00005-0000) no corresponde a composiciones para administración intramuscular sino a un dispositivo de inyección para la administración de un líquido, para el cual las características técnicas, mecánicas y de funcionamiento, así como sus elementos

y la relación de los mismos no se encuentran sustentados en la divulgación inicial; por lo que se concluye, que la eliminación de la frase “administración intramuscular” no subsana la falta de unidad de invención en la solicitud, tal como se indicó en el mencionado oficio”.

- 3.2.5 “Que el primer grupo inventivo definido en la(s) reivindicación(es) 1 a 5, no cumple(n) con el requisito de nivel inventivo”.
- 3.2.6 “En el presente caso, la composición reclamada en la solicitud no tiene nivel inventivo, teniendo en cuenta que la reivindicación 1 se refiere a: “una composición farmacéutica que comprende un esteroide anabólico y un compuesto de vitamina D en un portador líquido para administración parenteral””.
- 3.2.7 “La diferencia entre la invención, definida en la reivindicación 1 y la composición de D1 consiste en que la combinación de la anterioridad D1 no se encuentra en una sola forma farmacéutica”.
- 3.2.8 “El efecto que logra el hecho de incluir el esteroide anabólico y un derivado de la vitamina D en una composición de administración parenteral es un efecto sinérgico sobre la masa muscular y la función muscular”.
- 3.2.9 “Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: cómo modificar la combinación divulgada en D1 que comprende nandrolona decanoato, vitamina D3 alfa-hidroxilada y calcio para proveer una composición de administración parenteral de nandrolona decanoato y un derivado de la vitamina D”.
- 3.2.10 “Sin embargo, la utilización de una composición farmacéutica para administración parenteral que emplea soluciones de un esteroide y un derivado de la vitamina D en aceite de sésamo y maní o araquís está divulgada en el documento D2, que enseña una composición farmacéutica para administración parenteral que contiene un esteroide de tipo estrógeno y un derivado de vitamina D3 (Pág. 1, Párr. [007]) en un vehículo que comprende aceite de sésamo, maní o araquís, o soluciones acuosas de propilenglicol (Pág. 8, Párr. [088])”.
- 3.2.11 “En consecuencia, la persona del oficio normalmente versada en la materia incluiría un vehículo farmacéuticamente aceptable para administración parenteral revelado en el documento D2 en la combinación divulgada en el documento D1

para llegar así al objeto de la reivindicación 1 de la solicitud. Por lo cual se considera obvia”.

3.2.12 “En conclusión, la reivindicación 1 no cumple con el requisito de nivel inventivo (Art. 18)”.

3.2.13 “Las reivindicaciones dependientes 2 a 5 no mencionan alguna característica que haga inventiva la composición de la reivindicación 1, por lo cual se considera que no son inventivas”.

3.2.14 “La Solicitante argumenta que partiendo de las enseñanzas divulgadas en D1, no es posible extraer información respecto a los componentes de la composición farmacéutica reclamada en la solicitud, toda vez que la formulación de D1 comprende tres ingredientes activos, por lo que una persona normalmente versada en la materia no se vería motivada a reducir la cantidad de dichos ingredientes en vista de que se esperaría que disminuyera la funcionalidad de la formulación farmacéutica”.

3.2.15 “Al respecto este Despacho se permite señalar que contrario a lo señalado por la Solicitante, el estudio clínico divulgado en D1 evalúa en una población de mujeres operadas por fractura de cadera la respuesta clínica de la intervención con una combinación de nandrolona decanoato, vitamina D3 alfa-hidroxilada y calcio frente a un grupo control (calcio 0.25µg V.O diariamente). Los resultados del estudio permiten establecer claramente a una persona versada en la materia que luego de 6 y 12 meses de administración de la intervención anabólica existe una diferencia significativa ($p < 0.01$) en el volumen muscular y la masa muscular relativa, tanto de la pierna operada como de la no operada frente al grupo control (Pág. 500, Tabla 4), lo que significa que el calcio por sí solo no genera un efecto positivo en la respuesta clínica. Por tanto, una persona versada en la materia tendría la expectativa razonable de obtener los mismos resultados al eliminar de la combinación el calcio, es decir, emplear la intervención anabólica de nandrolona decanoato y vitamina D3 alfa-hidroxilada, toda vez que el calcio no proporciona ningún efecto en la respuesta clínica evaluada tal como se evidencia en el grupo control del estudio D1”.

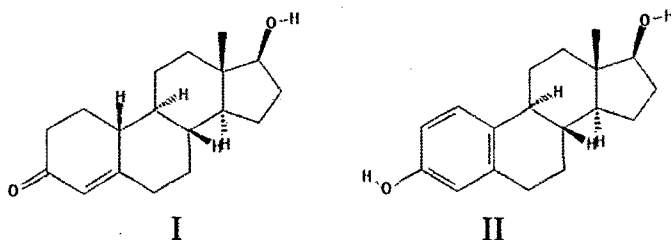
3.2.16 “Continúa señalando la Solicitante que en la presente invención los dos compuestos activos están íntimamente asociados y por tanto se administran al mismo tiempo, tal y como se demuestra en los ejemplos de la descripción, donde

al administrar el esteroide anabólico y el derivado de vitamina D en una sola dosis presenta un efecto sinérgico sobre la masa muscular del paciente (tabla 1). Y concluye señalando que dicha sinergia no se enseña ni sugiere en D1”.

3.2.17 “Al respecto este Despacho aclara a la Solicitante que los ejemplos divulgados en la descripción corresponden únicamente a ensayos in vitro, en los cuales no es correcto emplear los términos “administración” o “dosis”, toda vez que estos hacen referencia a la terapia farmacológica en estudios in vivo, por lo que los términos apropiados para el tipo de ensayos divulgados en la presente solicitud son co-tratamiento y concentración. Asimismo, es importante resaltar que los ejemplos divulgados son realizados en células hSkMC adquiridas de PromoCell (Pág. 15, Párr. 004), por lo que no se puede hablar de un efecto sinérgico sobre la masa muscular del paciente, toda vez que lo que se evalúa es la proliferación celular de una línea en cultivo. En relación a las enseñanzas reveladas en D1, es claro que la anterioridad únicamente presenta los cambios en tejidos suaves luego de 6 y 12 meses de tratamiento con la combinación del esteroide anabólico y la vitamina D3 y aunque estos no se administran en una sola forma farmacéutica (25mg vía IM cada tres semanas y 0.25µg V.O diariamente), los parámetros farmacocinéticos del decanoato de nandrolona (tiempo de vida $t_{1/2}$ 7-12 días²) y la administración diaria de la vitamina D3 permiten establecer a una persona medianamente versada en la materia que las concentraciones plasmáticas de los fármacos divulgados en D1 se encuentran dentro de la ventana terapéutica y en relación constante con una cinética similar a la de administrar una sola composición como la de la presente solicitud que incluye los dos fármacos en el mismo rango de concentración que el divulgado en D1”.

3.2.18 “Continúa la Solicitante argumentando que el documento D2 hace referencia a composiciones que comprenden derivados de vitamina D y un estrógeno, el cual no es un esteroide anabólico y por tanto no está en capacidad de enlazarse con un receptor andrógeno. Señala la Solicitante que la actividad biológica de los estrógenos es muy diferente a la de los esteroides, por lo que al tener claras las diferencias biológicas y estructurales entre un esteroide y un estrógeno anabólico se evidencia que la combinación entre un compuesto de vitamina D y un estrógeno nunca va a coincidir con el que se obtiene a partir de la combinación de vitamina D con un esteroide anabólico. Y concluye señalando que la composición reivindicada y sus características esenciales no son derivadas de forma evidente a partir de los documentos citados por el examinador”.

3.2.19 “Tal como lo indica la Solicitante los estrógenos presentan propiedades biológicas bien diferentes de los esteroides anabólicos, sin embargo, contrario a lo señalado en el memorial, no existen mayores diferencias estructurales entre la nandrolona – fórmula I -(esteroide anabólico) y el β -estradiol – fórmula II - (uno de los estrógenos divulgados en D2) ya que ambos corresponden a derivados de ciclopentano perhidrofenantreno, por lo que presentan propiedades químicas similares en relación a la solubilidad y la estabilidad. Por ejemplo, es bien sabido que los núcleos esteroidales que presentan el grupo 3-ceto son inestables en presencia de grupos funcionales ácidos presentes en adhesivos y otros excipientes empleados a nivel farmacéutico, por lo que una persona versada en la materia enfrentada a la necesidad de formular una composición estable de un esteroide como nandrolona, resolvería dicho problema empleando un portador líquido como el divulgado en D2, es decir, usando aceite de sésamo, maní o araquís (Pág. 8, Párr. [088]) como vehículo para la formulación”.



3.2.20 “Por tanto, más allá de las diferencias en la respuesta biológica entre los estrógenos y los esteroides anabólicos, la lectura del documento D2 indicaría claramente a una persona versada en la materia que el aceite de sésamo, maní o araquís empleado como vehículo en D2 resulta apropiado para formular una solución estable de nandrolona y un derivado de vitamina D como la divulgada en D1 dadas las características de solubilidad y estabilidad de dichos compuestos, por lo que se considera que la composición de la presente invención carece de nivel inventivo”.

3.2.21 “En consecuencia, la materia reclamada en la presente solicitud carece de nivel inventivo, y por ende, no cumple lo dispuesto en el artículo 18 de la Decisión 486”.

IV. OBJETO DEL RECURSO

De acuerdo con los argumentos que expongo a continuación, solicito atentamente a su Despacho lo siguiente:

1. Revocar la Resolución No. **86139** del 26 de diciembre de 2013.

2. Conceder el privilegio de patente de invención para **“COMPOSICIÓN FARMACÉUTICA QUE COMPRENDE UN ESTEROIDE ANABÓLICO Y UN COMPUESTO DE VITAMINA D PARA ADMINISTRACIÓN PARENTAL PARA LA PREVENCIÓN Y/O TRATAMIENTO DE LA FRAGILIDAD”**, a favor de **ORGANEXT RESEARCH B.V.**

V. FUNDAMENTOS DEL RECURSO

Respetuosamente me permito disentir de lo expuesto por ese Despacho en la Resolución objeto del presente recurso, toda vez que la presente solicitud sí cumple con el requerimiento de nivel inventivo exigido en la Decisión 486, como se demostrará a continuación:

5.1 Antecedentes

De acuerdo a los fundamentos establecidos en la resolución pendiente, se tiene que la presente solicitud no presenta unidad de invención y tampoco presenta nivel inventivo, éste último en vista de las enseñanzas de los documentos **D1**: “Positive effects of anabolic steroids, vitamin D and calcium on muscle mass, bone mineral density and clinical function after a hip fracture”, Hedström, M., et al., J Bone Joint Surg, 84, 497-503, Mayo 2002 y **D2**: US2005063992.

5.1.1 Sobre la Unidad de Invención

El artículo 25 de la Decisión 486, hace referencia a:

“Artículo 25.-*La solicitud de patente sólo podrá comprender una invención o un grupo de invenciones relacionadas entre sí, de manera que conformen un único concepto inventivo”.*

5.1.2 Sobre la Jurisprudencia en relación al análisis de nivel inventivo

El Tribunal Andino de Justicia se refiere a la forma de evaluar Nivel Inventivo en la Interpretación Prejudicial 36-IP-2010 en los siguientes términos:

(...) Del nivel inventivo:

“De conformidad con el artículo 18 de la Decisión 486, una invención goza de nivel inventivo al no derivar de manera evidente del estado de la técnica, ni resultar obvia para una persona entendida o versada en la materia. Este requisito ofrece al examinador la posibilidad de determinar si con los conocimientos técnicos que existían al momento de la invención, se hubiese podido llegar de manera evidente a la misma, o si el resultado hubiese sido obvio para un experto medio en la materia de que se trate, es decir, para una persona del oficio normalmente versada en el asunto técnico correspondiente”.

Con respecto a lo anterior, el Tribunal de Justicia de la Comunidad Andina ha manifestado lo siguiente:

“En este punto conviene advertir que uno es el examen que realiza el técnico medio respecto de la novedad y otro el que se efectúa con respecto al nivel inventivo; si bien en uno y otro se utiliza como parámetro de referencia el ‘estado de la técnica’, en el primero, se coteja la invención con las ‘anterioridades’ existentes dentro de aquella, cada uno (sic) por separado, mientras que en el segundo (nivel inventivo) se exige que el técnico medio que realiza el examen debe partir del conocimiento general que él tiene sobre el estado de la técnica y realizar el cotejo comparativo con su apreciación de conjunto, determinando si con tales conocimientos técnicos existentes ha podido o no producirse tal invención”. (Proceso N° 12-IP-98. Interpretación

prejudicial de 20 de mayo de 1998, publicada en Gaceta Oficial del Acuerdo de Cartagena N° 428, de 16 de abril de 1999)".

Ahora bien, para los efectos del artículo 18 de la Decisión 486 resulta pertinente definir qué se entiende por "obvio" y por "evidente" y, en consecuencia, determinar qué se debe entender por nivel inventivo.

Lo obvio es "que se encuentra o pone delante de los ojos; muy claro o que no tiene dificultad. Mientras que lo evidente "certeza clara y manifiesta de la que no se puede dudar" [1]. Como se deduce, algo que resulte obvio no es necesariamente evidente; empero lo que es evidente, es también obvio.

En este orden de ideas, se puede concluir que una invención goza de nivel inventivo cuando a los ojos de un experto medio en el asunto de que se trate, se necesita algo más que la simple aplicación de los conocimientos técnicos en la materia para llegar a ella, es decir, que de conformidad con el estado de la técnica el invento no sea consecuencia clara y directa de dicho estado, sino que signifique un avance o salto cualitativo en la elaboración de la regla técnica.

5.1.3 La presente invención sí posee unidad de invención.

En este respecto, se le informa al señor Examinador que la reivindicación 6 del último pliego reivindicatorio que fue presentado ante este Despacho, ha sido eliminada, con lo cual se restringe el objeto inventivo de la presente solicitud exclusivamente a la composición de administración parental que corresponde al primer grupo inventivo identificado por el señor Examinador. De tal manera que, la objeción formulada a este respecto se debe tener por superada toda vez que el nuevo pliego reivindicatorio comprende un único concepto inventivo.

5.1.4 La presente invención si posee Nivel Inventivo

Adicionalmente a la eliminación de la reivindicación 6, se modificó la reivindicación 1 del actual pliego reivindicatorio, restringiendo el esteroide anabólico a un éster de nandrolona, el cual se encuentra debidamente soportado en la página 10, último párrafo del capítulo descriptivo de la presente solicitud tal como fue originalmente presentada. Así mismo, el compuesto relativo de la vitamina D, se restringió a un grupo de compuestos predeterminado y soportado en la página 11, último párrafo del capítulo descriptivo y en la reivindicación original 3. En consecuencia, la nueva reivindicación 2, ahora se refiere

únicamente el decanoato de nandrolona y la reivindicación 3 ha sido eliminada del nuevo pliego reivindicatorio.

En lo que respecta ahora a las objeciones hechas con base en las enseñanzas de los documentos D1 y D2, mi representada presenta a continuación los siguientes argumentos que demuestran que la presente invención, tal como se encuentra recitada en el nuevo pliego reivindicatorio SI es inventiva frente a las enseñanzas del estado de la técnica.

Con respecto al documento D1, el Examinador afirma que a partir de la tabla 4, página 500, es posible concluir que el calcio por sí solo, no produce ningún efecto positivo. A este respecto, mi representada respetuosamente desea poner de presente que no comparte la opinión del Señor Examinador toda vez que en D1 no se enseñan los efectos del calcio en el organismo a partir de no otorgarles éste mineral a pacientes bajo control clínico. De hecho, en dicha tabla 4 de D1, se muestra el efecto de la combinación de un tratamiento anabólico en presencia de calcio, el cual se compara con un tratamiento basado solamente en calcio. Bajo este ensayo es imposible sacar conclusiones del efecto del tratamiento anabólico si el calcio no está dado en adición al tratamiento anabólico, tal y como lo reconocen los mismos autores de D1, en la página 502, columna izquierda en el último párrafo: *"el presente estudio sugiere que una pequeña dosis de decanoato nandrolona en combinación con una pequeña dosis de alfacalcidol y calcio, presenta efectos positivos en la composición del cuerpo, en la densidad mineral de los huesos (DMH) y en la función clínica de señoras de edad, después de haber sufrido una fractura de cadera"*.

Sin embargo, es bien sabido en el arte común de ésta técnica, que el calcio presenta en realidad un efecto sobre los huesos y los músculos, lo cual lo reconoce oficialmente Los Centros estadounidenses para el Control y Prevención de Enfermedades (US Centers for Disease Control and Prevention): *"El calcio es un mineral necesario para el cuerpo, para tener huesos y dientes sanos y un apropiado funcionamiento del corazón, los músculos y los nervios"*. De lo anterior se tiene que D1, reafirma lo establecido por los centros americanos de control y prevención de enfermedades, al mencionar: *"Una baja dosis de calcio fue seleccionada para el grupo bajo control, debido a que presenta un menor efecto sobre la DMH, que cuando se da solo"* (pág. 498, col. Derecha, líneas 7-9, D1).

Es así, cómo basados en los resultados que expone D1, no se puede concluir cuál va a ser el efecto del tratamiento anabólico, si el tratamiento no incluye calcio y la

comparación tendría que ser hecha con un grupo de pacientes bajo control, que no reciben tratamiento exclusivamente a base de calcio. Contrario a lo que afirma el señor Examinador en la presente solicitud, se tiene que una persona del oficio normalmente versada en la materia, concluiría igualmente que no se pueden sacar conclusiones, sobre sí los mismo resultados se obtendrían al remover o no el calcio en el tratamiento. Bajo éste contexto, es como los inventores de ésta solicitud, mantienen su posición con respecto a que las enseñanzas de D1 en un régimen de fármacos que contienen expresamente calcio, no permite obtener ningún tipo de conclusiones o expectativas, sobre efectos sinérgicos atribuidos a la combinación de compuestos específicos, tal y como los comprendidos en la composición farmacéutica recitada en la reivindicación 1 de la presente solicitud, que se fundamenta principalmente, en la combinación de un éster de nondrolona y un compuesto determinado de vitamina D, que puede ser seleccionado entre ergocalciferol, colecalciferol, calcidiol y calcitriol.

En lo que concierne al efecto sinérgico de la invención, ante el cual el Examinador, contraargumenta que se trata de un ensayo in-vitro, se exponen a continuación los siguientes argumentos:

En primera medida y como se puede observar en al actual reivindicación 1 del nuevo pliego reivindicatorio de la presente solicitud, se tiene que ésta reclama una composición farmacéutica a base de un éster de nandrolona, en donde la nandrolona es el compuesto activo que es empleado en el ejemplo 3 de ésta solicitud, en el que se evidencia el efecto sinérgico de éste compuesto en combinación con $1\alpha,25\text{OH}$ -vitamina D3. Los ésteres de nandrolona después de haber sido administrados parentalmente en un individuo, se adhieren rápidamente a la circulación a través de esterases que están presentes en la sangre, para producir el compuesto activo nandrolona (ver Kicman, British Journal of Pharmacology 2008, pag. 505, col. Izquierda, líneas 2-5). Por lo tanto y en lo que concierne al esteroide anabólico, las presentes reivindicaciones están limitadas exclusivamente a aquellos compuestos que tiene forma de nandrolona, después de haber sido administrados en un individuo.

La nandrolona acciona efectivamente su efecto sinérgico, cuando se enlaza con un receptor andrógeno (ver, Bergink et al., Acta Endocrinológica, 1985 y pág. 36, columna derecha, 2do párrafo, y ver. Kicman, 2008). Obviamente, el ejemplo 3, hace referencia a un ensayo in-vitro, en donde la nandrolona alcanza su efecto sinérgico, únicamente bajo la combinación con el compuesto $1\alpha,25\text{OH}$ -vitamina D3, en respuesta a que los ésteres de nandrolona, tales como el decanoato nandrolona in-vitro, no se adheriría al compuesto activo de nandrolona, ya que éstos están in-vivo, mediante esterases en la circulación.

En lo que respecta a los compuestos seleccionados de vitamina D, los cuales son ergocalciferol, colecalciferol, calcidiol y calcitriol, que son en sí vitamina D2, vitamina D3, 25-hidroxivitamina D3 y 1 α ,25-hidroxivitamina D3, respectivamente, es importante aclarar que la vitamina D2 (ergocalciferol) y la vitamina D3 (colecalciferol), se convierte *in-vivo* en un proceso de dos etapas en 1 α ,25-hidroxivitamina D2 y 1 α ,25-hidroxivitamina D3, mediante hidroxilación. Estas son las formas activas de la vitamina D, las cuales ambas se enlazan al mismo receptor, como por ejemplo lo es el receptor vitamina D y tienen la misma función. La primera etapa de esta conversión, para la vitamina D3, es a partir de una vitamina D3 a 25-hidroxivitamina D3 (calcidiol), que es seguidamente convertida a 1 α ,25-dihidroxivitamina D3 en una segunda etapa. Lo anterior, lo describe perfectamente el estudio de Schuster, *Biochimica et Biophysica Acta* 1814 (2011): 186-189 (ver introducción, en el pág. 187, seguido del encabezado: "*La cascada de la vitamina D*"). De esta manera y en lo que concierne a los compuestos de vitamina D, el actual pliego reivindicatorio se refiere exclusivamente a las formas activas 1 α ,25-dihidroxivitamina D3 (calcitriol) y el compuesto que *in-vivo* se convierte en su compuesto activo colecalciferol, calcidiol, o en su forma activa 1 α ,25-dihidroxivitamina D2 (ergocalciferol o vitamina D2).

Es así, como ambas formas activas de la vitamina D (D2 y D3), se enlazan a un mismo receptor y presentan por lo tanto la misma función. Igualmente a lo que ocurre con la nandrolona en el ejemplo 3, que como ya se discutió se refiere a un experimento *in-vitro*, la forma activa 1 α ,25-dihidroxivitamina D3, alcanza su efecto sinérgico únicamente en combinación con la nandrolona, en vista de que el ergocalciferol, colecalciferol y calcidiol no se convertirían, bajo un experimento *in-vitro* en su forma activa bajo condiciones *in-vitro*, cuando solo y exclusivamente ocurre *in-vivo*.

Con la anterior aclaración, se tiene que el actual pliego reivindicatorio reclama sustancias que *in-vivo* se convierten en el esteroide activo anabólico de nandrolona y en la forma activa del compuesto de vitamina D, tal y como lo es el 1 α ,25-dihidroxivitamina D3, que se exponen en el ejemplo 3 de esta solicitud, y donde se pudo demostrar la presencia de un efecto sinérgico o también con el compuesto 1 α ,25-dihidroxivitamina D2. Como se mencionó anteriormente, este último compuesto se enlaza al mismo receptor y presenta la misma funcionalidad que el compuesto 1 α ,25-dihidroxivitamina D3, con lo cual se concluye que las sustancias activas que se reclaman en las presentes reivindicaciones, tienen la misma función y efecto que la nandrolona y el 1 α ,25-dihidroxivitamina D3, tal y como se expone en el ejemplo 3.

Adicionalmente a lo anterior, se tiene que en la acción oficial pendiente el Examinador afirma que una persona del oficio versada en la materia establecería fácilmente que el tratamiento divulgado en el documento D1, el cual consiste en la administración intramuscular del decanoato nandrolona cada tercera semana y una administración diaria oral de alfacalcidol y la simultánea administración parental de la composición de la presente invención, presentan cinéticas similares. Ante lo anterior, mi representada se encuentra en completo desacuerdo, ya que la farmacocinética del alfacalcidol que se administra diariamente y oralmente, hace relación a un primer orden farmacocinético, mientras que la administración parental de alguno de los compuestos reclamados por ésta invención, siendo estos ergocalciferol, colecalciferol, calcidiol y calcitriol, hace referencia a un orden-cero farmacocinético. Con la administración parental de la composición de la reivindicación 1, es claro que esta se alternadiariamente con una baja y alta concentración del compuesto de vitamina D en el plasma, lo cual no es enseñado ni sugerido en D1 y por lo tanto, contrario a lo afirmado por éste Despacho, la cinética y los niveles de alfacalcidol en el plasma de los individuos de D1, difieren completamente de los compuestos de vitamina D, reivindicados en esta solicitud.

Ahora en lo que respecta a la diferencia entre los esteroides anabólicos y los estrógenos que divulga el documento D2, se tiene que en primera medida, los estrógenos y los ésteres de nandrolona tienen un esqueleto estructural compuesto por 18 carbonos C18, pero se diferencia en que los estrógenos comprende un anillo aromático A que carece de nandrolonas y ésteres. Esta diferencia es clave, ya que los ésteres de nandrolona presentan propiedades específicas que a su vez se ven reflejadas en propiedades químicas diferentes, ejemplo de ellos es la alta solubilidad de los estrógenos en agua y que pueden ser administrados oralmente (ver parr. [0008] de D2), mientras que los ésteres de nandrolona son insolubles en agua y no pueden ser administrados oralmente. Sin embargo, es claro que para una persona del oficio versada en la materia, que exista o no diferencias en la estructura y propiedades química de las sustancias en cuestión es irrelevante, en vista de que lo que conlleva a combinar los ésteres de nandrolona con compuestos de vitamina D, no tiene que ver con la estructura química, sino con la actividad farmacocinética de dichos compuestos, que al estar formulados bajo una composición farmacéutica para administración parental, se obtendrá un efecto sinérgico. Por esta razón, los inventores se mantienen en su posición de que suponiendo que un estrógeno y un compuesto de vitamina D, conformen una sola composición para administración parental, igualmente no motivaría a una persona del oficio versada en la materia, a combinar los compuestos específicos de vitamina D, reclamados en esta solicitud con un éster de nandrolona, en vista de que en D1, se enseña claramente que los compuestos de vitamina D y los esteroides anabólicos deben ser administrados por

separado, siendo así, intramuscularmente para el esteroide anabólico y oralmente para la vitamina D en diferentes intervalos.

Así entonces, teniendo en cuenta los anteriores argumentos y que el efecto sinérgico de una composición farmacéutica reivindicada en una solicitud de patente es indicio del nivel inventivo de la misma, se demuestra entonces la altura inventiva de la composición farmacéutica de la presente solicitud, frente a los documentos del estado previo de la técnica.

5.4 Concesión de una solicitud foránea:

Aunque compartimos la opinión de su Despacho, en cuanto a que la concesión de una solicitud de patente en otros países, equivalente a la solicitud Colombiana, no obliga a su Despacho a otorgar el privilegio de patente para la misma invención, sí constituye un antecedente favorable para el estudio de patentabilidad que respetuosamente solicitamos tener en cuenta. Por lo tanto, y como ya lo informamos, la presente invención ha sido otorgada por la Oficina de Patentes de Europa: EP2470214 (B1) (ver anexo), de España: ES2425095 (T3) y por la oficina de patentes Mejicana.

A este respecto, deseamos poner de presente que si bien cada país es autónomo de conceder o negar una solicitud luego de la realización del estudio de fondo, es claro que el hecho de que la novedad y el nivel inventivo de una invención hayan sido reconocidos por varias oficinas a nivel mundial, es un indicio de la patentabilidad de la misma toda vez que (i) el estudio de novedad y nivel inventivo en todas las Oficinas de Patentes del mundo es realizado por personas con conocimientos técnicos en el área de interés que son consideradas personas idóneas para emitir conceptos sobre la patentabilidad de una invención y que (ii) los resultados de los exámenes de novedad o de patentabilidad efectuados respecto a solicitudes extranjeras pueden ser reconocidos como suficientes para acreditar el cumplimiento de las condiciones de patentabilidad de la invención, tal como se señala en el artículo 46 de la Decisión 486:

“Artículo 46.- La oficina nacional competente podrá requerir el informe de expertos o de organismos científicos o tecnológicos que se consideren idóneos, para que emitan opinión sobre la patentabilidad de la invención. Asimismo, cuando lo estime conveniente, podrá requerir informes de otras oficinas de propiedad industrial.

De ser necesario, a efectos del examen de patentabilidad y a requerimiento de la oficina nacional competente, el solicitante proporcionará, en un plazo que no excederá de 3 meses, uno o más de los siguientes documentos relativos a una o más de las solicitudes extranjeras referidas total o parcialmente a la misma invención que se examina:

- a) copia de la solicitud extranjera;*
- b) copia de los resultados de exámenes de novedad o de patentabilidad efectuados respecto a esa solicitud extranjera;*
- (...)*

*La oficina nacional competente podrá **reconocer los resultados de los exámenes referidos en el literal b) como suficientes para acreditar el cumplimiento de las condiciones de patentabilidad de la invención***" (Negrilla fuera del texto).

Teniendo en cuenta lo anterior, el solicitante respetuosamente solicita que se revoque la resolución impugnada y en su lugar se otorgue la patente solicitada para todas las reivindicaciones constitutivas del último pliego reivindicatorio.

5.3 Conclusiones

Mediante un nuevo pliego reivindicatorio y argumentos técnicos y teóricos, se superan las objeciones inicialmente establecidas, por falta de nivel inventivo.

De esta forma, se considera que la presente solicitud cuenta con todos los requisitos necesarios para que le sea concedida la patente.

VI. PETICIÓN

Por todo lo anterior, solicito a ese Despacho lo siguiente:

6.1 Que al considerar las razones precedentes se revoque la decisión contenida en la Resolución No. **86139** del 26 de diciembre de 2013, en cuanto resuelve negar el privilegio de patente de invención.

6.2 Que se otorgue el privilegio de patente de invención para: que sea revocada y en consecuencia se conceda el privilegio de patente de invención para **“COMPOSICIÓN FARMACÉUTICA QUE COMPRENDE UN ESTEROIDE ANABÓLICO Y UN COMPUESTO DE VITAMINA D PARA ADMINISTRACIÓN PARENTAL PARA LA PREVENCIÓN Y/O TRATAMIENTO DE LA FRAGILIDAD”** a favor de **ORGANEXT RESEARCH B.V**

VII. NOMBRE Y DIRECCIÓN

El nombre y la dirección de la solicitante recurrente son los siguientes:

ORGANEXT RESEARCH B.V

De La Reijstraat 2,
NL-6814 AE Arnhem,
Países Bajos

VIII. NOTIFICACIONES

Atentamente manifiesto a usted que recibiré notificaciones en la Secretaria de su Despacho o en mi oficina de abogada situada en la Carrera 4ª. No. 72-35, piso 4º, de esta ciudad de Bogotá, D.C

IX. ANEXOS

- Formulario de modificaciones en solicitud pendiente
- Nuevo pliego reivindicatorio conformado por 4 reivindicaciones
- Comprobante de pago por concepto de modificaciones voluntarias, por un valor de \$128.000.

- Carta de aceptación de la patente Europea
- Reivindicaciones concedidas pro la oficina de patentes Europea

Señor Superintendente,

LUZ CLEMENCIA DE PAEZ
LUZ CLEMENCIA DE PAEZ
C.C. 35.456.344 de Usaquén
T.P.A. No. 23.555

mm COLO-18885-0841-001-5
AHAVAHA\ID-259721\WPC18885
No. M-2014-259721VAHA



Industria y Comercio
SUPERINTENDENCIA

Espacio reservado para el adhesivo de radicación

DIRECCIÓN DE NUEVAS CREACIONES
MODIFICACIONES Y/O CORRECCIONES

1. Identificación del Trámite

☐ Error material

☒ Modificación a una Solicitud en trámite

☒ Patente de Invención

☐ Patente de Modelo de Utilidad

☐ Registro de Diseño Industrial

☐ Esquema de Trazado de Circuitos Integrados

2. Datos del solicitante

Persona Natural ☐

Persona Jurídica ☒

ORGANEXT RESEARCH B.V.

Nombre o denominación / Nombre o razón social completa

Nombre representante legal

Tipo de empresa

Micro ☐

Pequeña ☐

Mediana ☐

Otra ☐

Documento de identificación:

☐ C.C.

☐ C.E.

☐ NIT

☐ Otro Número

Nacionalidad/País de constitución	Dirección y domicilio del titular	Ciudad
Países Bajos	De La Reijstraat 2, NL-6814 AE Arnhem, Países Bajos	Arnhem
Dirección electrónica	No. Fax	Número telefónico
clientes@cavelier.com	2 11 86 50	3 47 36 11



Autorizo expresamente a la Superintendencia de Industria y Comercio para que todas las comunicaciones sean enviadas a través de la dirección de correo electrónico

3. Datos del apoderado:

Apellidos y Nombre

De Páez Luz Clemencia

Documento de identificación

C.C. 35.456.344

Tarjeta profesional

T.P.A. 23.555

Dirección y domicilio		Ciudad
Carrera 4ª No. 72 –35		Bogotá
Dirección electrónica	No. Fax	Número telefónico
cavelier@cavelier.com	2 11 86 50	3 47 36 11
En caso de haber presentado poder general para asuntos que se adelanten ante la Delegatura de Propiedad Industrial, sírvase indicar el número de su radicación o protocolo de poder general		

4. Datos del trámite Corrección: Expediente No 11.156.685
Aparece Debe ser Modificación: ☒ Modificación al capítulo reivindicatorio. Presento nuevo capítulo reivindicatorio compuesto por 4 reivindicaciones y pido respetuosamente que el examen de patentabilidad sea realizado sobre estas nuevas reivindicaciones presentadas. Respetuosamente manifiesto que a pesar de eliminar materia del pliego reivindicatorio originalmente presentado, mi representada no renuncia a la protección que pueda obtener sobre dicha materia y además se reserva el derecho de presentar una solicitud divisional que la contenga durante el trámite de la presente solicitud, beneficiándose de la fecha de prioridad inicialmente reivindicada. ☐ Modificación al capítulo descriptivo. ☐ Modificación al resumen. ☐ Modificación a los dibujos.

5. Anexos ☒ Comprobante de pago de la tasa para la presentación de la corrección y/o modificación por \$128.000. ☐ Poder, si fuere el caso. ☒ Documento que contiene las modificaciones o correcciones. ☐ Copia de la solicitud y sus anexos en formato magnético.

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mem

6. Firma	
Nombre y apellidos	Firma
LUZ CLEMENCIA DE PAEZ	<i>Luz Clemencia De Paez</i>
C.C. 35.456.344 DE Usaquén	Tarjeta Profesional 23.555

REIVINDICACIONES MODIFICADAS

1. Una composición farmacéutica, comprende un éster de nandrolona y un compuesto de vitamina D, seleccionado del grupo que consiste de ergocalciferol, colecalciferol, calcidiol y calcitriol, en un portador líquido para la administración parenteral.
2. La composición farmacéutica de acuerdo con la reivindicación 1, en donde el éster de nandrolona es decanoato de nandrolona.
3. La composición farmacéutica de acuerdo con la reivindicación 1 o 2, comprende de 5 a 600 mg de decanoato de nandrolona, y 17,5 µg a 15 mg de colecalciferol.
4. La composición farmacéutica de acuerdo con cualquiera de las reivindicaciones de la 1 a 3, el líquido portador es seleccionado entre el grupo que consta de aceite de araquís, aceite de semilla de algodón y aceite de sésamo.

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(11) **EP 2 470 214 B1**

(12) **EUROPEAN PATENT SPECIFICATION**

- (45) Date of publication and mention of the grant of the patent:
15.05.2013 Bulletin 2013/20

(21) Application number: 10747526.1

(22) Date of filing: 23.08.2010
- (51) Int. Cl.:
A61K 45/06 (2006.01) A61K 31/56 (2006.01)
A61K 31/592 (2006.01) A61P 19/10 (2006.01)
A61P 21/06 (2006.01)

(86) International application number:
PCT/NL2010/050524

(87) International publication number:
WO 2011/025368 (03.03.2011 Gazette 2011/09)

(54) **METHOD OF TREATING FRAILTY**
VERFAHREN ZUR BEHANDLUNG VON GEBRECHLICHKEIT
PROCÉDÉ DE TRAITEMENT DE LA FRAGILITÉ

- | | |
|--|---|
| <p>(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB
GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO
PL PT RO SE SI SK SM TR</p> <p>(30) Priority: 24.08.2009 EP 09168522</p> <p>(43) Date of publication of application:
04.07.2012 Bulletin 2012/27</p> <p>(73) Proprietor: OrgaNext Research B.V.
6811 AA Arnhem (NL)</p> <p>(72) Inventors:
• PRINS, Marjanna
NL-6814 AE Arnhem (NL)
• KLOOSTERBOER, Helenius Jan
NL-5345 AV Oss (NL)</p> <p>(74) Representative: Jansen, Cornelis Marinus
VEREENIGDE
Johan de Wittlaan 7
2517 JR Den Haag (NL)</p> <p>(56) References cited:
• HEDSTRÖM M ET AL: "Positive effects of anabolic steroids, vitamin D and calcium on muscle mass, bone mineral density and clinical function after a hip fracture. A randomised study of 63 women." THE JOURNAL OF BONE AND JOINT SURGERY. BRITISH VOLUME MAY 2002, vol. 84, no. 4, May 2002 (2002-05), pages 497-503, XP002574974 ISSN: 0301-620X cited in the application</p> | <ul style="list-style-type: none">• LYRITIS G P ET AL: "Effect of nandrolone decanoate and 1-alpha-hydroxy-calciferol on patients with vertebral osteoporotic collapse. A double-blind clinical trial" BONE AND MINERAL, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 27, no. 3, 1 January 1994 (1994-01-01), pages 209-217, XP022641723 ISSN: 0169-6009 [retrieved on 1994-01-01]• SHEFFIELD-MOORE MELINDA ET AL: "Androgen therapy induces muscle protein anabolism in older women" JOURNAL OF CLINICAL ENDOCRINOLOGY & METABOLISM, vol. 91, no. 10, October 2006 (2006-10), pages 3844-3849, XP002574975 ISSN: 0021-972X cited in the application• SCHROEDER E TODD ET AL: "Six-week improvements in muscle mass and strength during androgen therapy in older men" JOURNALS OF GERONTOLOGY, SERIES A, BIOLOGICAL SCIENCES AND MEDICAL SCIENCES, WASHINGTON, DC, US, vol. 60, no. 12, 1 December 2005 (2005-12-01), pages 1586-1592, XP009131317 ISSN: 1079-5006 cited in the application• CAMPBELL STEPHEN ET AL: "Pharmacological Treatment of Frailty in the Elderly" JOURNAL OF PHARMACY PRACTICE AND RESEARCH, vol. 39, no. 2, June 2009 (2009-06), pages 147-151, XP9131388 ISSN: 1445-937X |
|--|---|

Note: Within nine months of the publication of the mention of the grant of the European patent in the European Patent Bulletin, any person may give notice to the European Patent Office of opposition to that patent, in accordance with the Implementing Regulations. Notice of opposition shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

EP 2 470 214 B1



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Jansen, Cornelis Marinus
VEREENIGDE
Johan de Wittlaan 7
2517 JR Den Haag
PAYS-BAS

For any questions about
this communication:
Tel.: +31 (0)70 340 45 00

Date
18.04.13

Reference P89215EP10	Application No./Patent No. 10747526.1 - 1456 / 2470214
Applicant/Proprietor OrgaNext Research B.V.	

Decision to grant a European patent pursuant to Article 97(1) EPC

Following examination of European patent application No. 10747526.1 a European patent with the title and the supporting documents indicated in the communication pursuant to Rule 71(3) EPC dated 13.12.12 is hereby granted in respect of the designated Contracting States.

Patent No. : 2470214
Date of filing : 23.08.10
Priority claimed : 24.08.09/EPA 09168522

Designated Contracting States
and Proprietor(s) : AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI
LT LU LV MC MK MT NL NO PL PT RO SE SI SK SM TR
OrgaNext Research B.V.
Jansbuitensingel 7
6811 AA Arnhem/NL

This decision will take effect on the date on which the European Patent Bulletin mentions the grant (Art. 97(3) EPC).

The mention of the grant will be published in European Patent Bulletin 13/20 of 15.05.13.

Examining Division

Laffargue-Haak T

Merckling-Ruiz V

Stoltner A



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ANMERKUNG ZUR ENTSCHEIDUNG ÜBER DIE ERTEILUNG
EINES EUROPÄISCHEN PATENTS (EPA Form 2006)

1. **EPA Informationsbroschüre "Nationales Recht zum EPÜ"**
Diese Broschüre enthält nützliche Informationen zu den formalen Erfordernissen und den Handlungen, die vor den Patentbehörden der Vertragsstaaten vorzunehmen sind, um Rechte in diesen Staaten zu erlangen. Da diese Handlungen einem ständigen Wandel unterworfen sind, sollte immer nur die neueste Ausgabe der Broschüre benutzt werden. Nachträgliche Informationen werden im Amtsblatt veröffentlicht.
2. **Übersetzung der europäischen Patentschrift nach Artikel 65 (1) des Europäischen Patentübereinkommens**
Sie werden erneut darauf hingewiesen, dass bestimmte Vertragsstaaten nach Artikel 65 (1) EPÜ eine Übersetzung der europäischen Patentschrift verlangen; hierauf wird in der Mitteilung gemäß Regel 71 (5) EPÜ verwiesen. Die Nichteinreichung dieser Übersetzung kann zur Folge haben, dass das Patent in dem betreffenden Staat/in den betreffenden Staaten als von Anfang an nicht eingetreten gilt. Weitere Einzelheiten entnehmen Sie bitte der oben genannten Broschüre.
3. **Zahlung von Jahresgebühren für europäische Patente**
Nach Artikel 141 EPU können "nationale" Jahresgebühren für das europäische Patent für die Jahre erhoben werden, die an das Jahr anschließen, in dem der Hinweis auf die Erteilung des europäischen Patents im "Europäischen Patentblatt" bekanntgemacht wird. Weitere Einzelheiten entnehmen Sie bitte der oben genannten Broschüre.

NOTE RELATING TO THE DECISION TO GRANT A
EUROPEAN PATENT (EPO Form 2006)

1. **EPO Information Brochure "National law relating to the EPC"**
This brochure provides useful information regarding formal requirements and the steps to be taken before the patent authorities of the Contracting States in order to acquire rights in those states. Since the necessary steps are subject to change the latest edition of the brochure should always be used. Subsequent information is published in the Official Journal.
2. **Translation of the European patent application under Article 65(1) of the European Patent Convention**
Your attention is again drawn to the requirements regarding translation of the European patent specification laid down by a number of Contracting States under Article 65(1) EPC, to which reference is made in the communication under Rule 71(5) EPC. Failure to supply such translation(s) may result in the patent being deemed to be void "ab initio" in the State(s) in question. For further details you are recommended to consult the above-mentioned brochure.
3. **Payment of renewal fees for European patents**
Under Article 141 EPC "national" renewal fees in respect of a European patent may be imposed for the years which follow that in which the mention of the grant of the European patent is published in the "European Patent Bulletin". For further details you are recommended to consult the above-mentioned brochure.

REMARQUE RELATIVE A LA DECISION DE DELIVRANCE
D'UN BREVET EUROPEEN (OEB Form 2006)

1. **Brochure d'information de l'OEB "Droit national relatif à la CBE"**
Cette brochure fournit d'utiles renseignements sur les conditions de forme requises et sur les actes à accomplir auprès des offices de brevet des Etats contractants aux fins d'obtenir des droits dans les Etats contractants. Étant donné que les actes indispensables sont susceptibles de modifications, il serait bon de toujours consulter la dernière édition de la brochure. Toute information ultérieure est publiée au Journal Officiel.
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Votre attention est de nouveau attirée sur l'obligation faite par certains Etats contractants, en vertu de l'article 65(1) CBE, de fournir une traduction du fascicule du brevet européen, à laquelle il est fait référence dans la notification établie conformément à la règle 71(5) CBE. Si la(les) traduction(s) n'est(ne sont) pas fournie(s), le brevet européen peut, dès l'origine, être réputé sans effet dans cet(ces) Etat(s). Pour plus de détails, nous vous renvoyons à la brochure susmentionnée.
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REVIEW

Pharmacology of anabolic steroids

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Athletes and bodybuilders have recognized for several decades that the use of anabolic steroids can promote muscle growth and strength but it is only relatively recently that these agents are being revisited for clinical purposes. Anabolic steroids are being considered for the treatment of cachexia associated with chronic disease states, and to address loss of muscle mass in the elderly, but nevertheless their efficacy still needs to be demonstrated in terms of improved physical function and quality of life. In sport, these agents are performance enhancers, this being particularly apparent in women, although there is a high risk of virilization despite the favourable myotrophic–androgenic dissociation that many xenobiotic steroids confer. Modulation of androgen receptor expression appears to be key to partial dissociation, with consideration of both intracellular steroid metabolism and the topology of the bound androgen receptor interacting with co-activators. An anticatabolic effect, by interfering with glucocorticoid receptor expression, remains an attractive hypothesis. Behavioural changes by non-genomic and genomic pathways probably help motivate training. Anabolic steroids continue to be the most common adverse finding in sport and, although apparently rare, designer steroids have been synthesized in an attempt to circumvent the dope test. Doping with anabolic steroids can result in damage to health, as recorded meticulously in the former German Democratic Republic. Even so, it is important not to exaggerate the medical risks associated with their administration for sporting or bodybuilding purposes but to emphasize to users that an attitude of personal invulnerability to their adverse effects is certainly misguided.

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Keywords: anabolic steroids; clinical; designer; health; mechanism; performance; receptor; SARMs; sport

Abbreviations: AF, activation function; BALCO, Bay Area Laboratory Co-operative; DHEA, dehydroepiandrosterone; DHT, 5 α -dihydrotestosterone; FDA, Food and Drug Administration; Hsp, heat-shock protein; LC-MS/MS, liquid chromatography–mass spectrometry/mass spectrometry; MENT, 7 α -methyl-19-nortestosterone; SARM, selective androgen receptor modulator; THG, tetrahydrogestrinone; UCLA, University of California, Los Angeles; WADA, World Anti-Doping Agency

Introduction

Androgens

Androgens exert their effects in many parts of the body, including reproductive tissues, muscle, bone, hair follicles in the skin, the liver and kidneys, and the haematopoietic, immune and central nervous systems (Mooradian *et al.*, 1987). The androgenic effects of these hormones can be generally considered as those associated with masculinization and the anabolic effects as those associated with protein building in skeletal muscle and bone.

In the male foetus, androgens stimulate the development of the Wolffian ducts (epididymis, vas deferens, the seminal vesicles and ejaculatory duct) and the male external genitalia (penis, urethra and scrotum) (Wilson *et al.*, 1981). During puberty, the androgenic effects resulting from increased

testicular steroidogenesis are manifested by growth of the testes, external genitalia and the male accessory reproductive glands (prostate, seminal vesicles and bulbourethral), and secretory activity begins. Further, the secondary sexual characteristics manifested during puberty can be divided into those that are a result of androgenic and anabolic effects. The androgenic effects are the enlargement of the larynx causing a deepening of the voice, the growth of terminal hair (in the pubic, axillary and facial regions; in other regions such growth depends on a number of factors), an increase in sebaceous gland activity (can lead to acne), and CNS effects (libido and increased aggression). Anabolic effects are the growth of skeletal muscle and bone, the stimulation of linear growth eventually ceasing due to the closure of the epiphysis. In men, androgens are essential for sustaining reproductive function, and they play an important role in maintaining skeletal muscle and bone, cognitive function and a sense of well-being.

The most important androgen secreted is testosterone; in the eugonadal man, the Leydig cells in the testes produce

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~95% of the testosterone in the body. The ovaries and the adrenal glands (in both sexes) produce very little testosterone but secrete weaker androgens; in particular, dehydroepiandrosterone (DHEA; and its sulpho-conjugate) and androstenedione are of physiological importance in the women, not least because they can undergo peripheral conversion to more potent androgens, for example to testosterone and 5 α -dihydrotestosterone (DHT). Another weaker endogenous androgen, androstenediol, also binds to oestrogen receptors.

The effects of androgens are modulated at cellular level by the steroid-converting enzymes within the particular target tissue (Figure 1). In reproductive target tissues, testosterone can be considered to be a prohormone, being readily converted by 5 α -reductase to the more potent androgen DHT. In other tissues, such as adipose tissue and parts of the brain, testosterone is converted by aromatase to the oestrogen, oestradiol. In bone, the mechanism of action of the anabolism of androgens has not been entirely elucidated but both a direct effect of testosterone and a mediated effect by aromatization to oestradiol are important (Orwoll, 1996; Zitzmann and Nieschlag, 2004). In the human skeletal muscle (collected less than 12 h post-mortem), 5 α -reductase activity (either type 1 or 2) is not detectable (Thigpen *et al.*,

1993), so testosterone itself is chiefly binding to the androgen receptor (as supported also by a number of animal studies, mainly in the rat). Aromatase expression and activity is significant in human skeletal muscle (Larionov *et al.*, 2003) but whether the conversion of androgens to oestrogens within this tissue is physiologically important for mediating some of the myotrophic effect of androgens is yet to be determined.

Modulation of the effects of androgens may also occur at the molecular level due to differences in the distribution of androgen receptor coregulators in various tissues, these coregulators being proteins that affect the transcriptional activity of the androgen receptor (Heinlein and Chang, 2002b; Wolf and Obendorf, 2004). This is a developing field and the comparative importance of many of these coregulators is yet to be established for any particular cell type, let alone their relative *in vivo* importance in examining tissue differences in androgen action. It is envisaged that genetic manipulation of the mouse will assist in elucidating their physiological relevance.

With structural modifications to testosterone, the anabolic effects of androgens can be enhanced but, even so, these cannot be divorced entirely from their androgenic effects. Hence, a more accurate term for anabolic steroids is anabolic-androgenic steroids, but, for simplicity, the shorter term is used within this paper. The disassociation of anabolic from androgenic effects can be at cellular level, depending on the intracellular metabolism of the anabolic steroid in different tissues, with the activity of 5 α -reductase being particularly important (see the section 'Intracellular metabolism and the myotrophic-androgenic index'). An appealing hypothesis is that anabolic-androgenic dissociation can also occur as a result of anabolic steroids inducing specific conformational changes of the androgen receptor complex, which then affects subsequent interaction with various coregulators in different tissues (see the section 'Androgen receptor expression and the importance of coregulators'). There is little data, as yet, to support such a hypothesis, but it is known that the androgen co-activator FHL2 is expressed predominantly in the heart (Muller *et al.*, 2000; Wolf and Obendorf, 2004), and it is possible that a number of other androgen receptor coregulators could be tissue specific. How an anabolic steroid may affect androgen receptor conformation and interaction with particular coregulators is of obvious interest, as such knowledge may eventually offer an additional mechanism for anabolic-androgenic dissociation.

The development of nonsteroidal selective androgen receptor modulators (SARMs) may offer better dissociation of biological effects than anabolic steroids and possibly even permit the therapeutic targeting of specific tissues and organs. Potential therapeutic modalities could then be specific agonists for restoration of fat-free muscle mass and strength in those with chronic illnesses such as HIV and specific antagonists for the treatment of prostate cancer in men or hirsutism in women (Wolf and Obendorf, 2004; Bhasin *et al.*, 2006). In anticipation of the potential of such agonists for performance enhancement in sport, SARMs have been added to the World Anti-Doping Agency's (WADA's) 2008 list of prohibited substances in sport, despite none yet being available on the market.

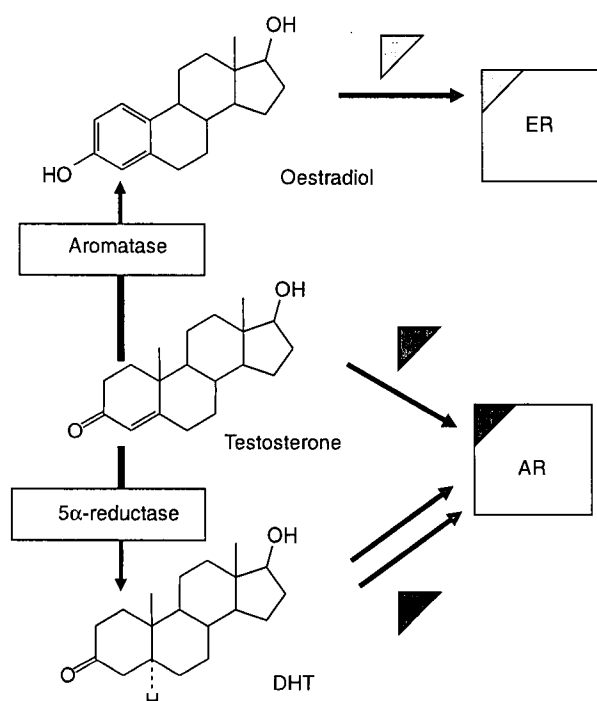


Figure 1 Testosterone can bind directly with the androgen receptor (AR). In target tissues where intracellular enzymes are present, the action of testosterone is mediated by metabolism. Testosterone is irreversibly converted by the enzyme 5 α -reductase to 5 α -dihydrotestosterone (DHT), which binds with greater affinity to the androgen receptor (AR), or by aromatase to oestradiol, which binds to the oestrogen receptor (ER). Testosterone and DHT can be also converted to weaker androgens (not displayed), again being dependent on whether the target tissue has the necessary enzyme activity, e.g., 3 α -hydroxysteroid dehydrogenase, 17 β -hydroxysteroid dehydrogenase.

Control of anabolic steroids

Anabolic steroids are controlled substances in several countries, including Australia, Argentina, Brazil, Canada, the United Kingdom and the United States. Even so, there is a readily available supply of steroids worldwide for non-medicinal purposes, because, in most countries, anabolic steroids can be sold legally without a prescription (Hermansson, 2002; Cramer, 2005). Thus, many foreign distributors do not violate the laws of their own country when they sell these substances to customers overseas via the Internet and by e-mail orders. The majority of the hormone products in the European market come from countries within the European Union and Russia, but also sometimes from Thailand, Turkey, Egypt, India and Pakistan (Hermansson, 2002). In the United States, significant quantities of anabolic steroids come from Mexico, as well as other countries such as Russia, Romania and Greece (Cramer, 2005).

In the United Kingdom, anabolic steroids are controlled under Schedule IV Part 2 of the Misuse of Drugs Act; the Act includes most of the anabolic steroids, together with clenbuterol (adrenoreceptor stimulant) and human growth hormone. There is no restriction on the possession of these substances when they are part of a medicinal product and are for self-administration. However, prosecutions of intent to supply have been made of individuals found in possession of large quantities of these substances without a prescription for them. A Home Office licence is required for importation and exportation of anabolic steroids, except in cases of small quantities for legitimate purposes.

As to doping control in human sport, the International Olympic Committee (IOC) Medical Commission introduced anabolic steroids as a banned class in 1974 (Kicman and Gower, 2003b). The name of this banned class was amended to anabolic agents in the 1990s to incorporate out-of-competition testing for clenbuterol and other β_2 -agonists, which are also considered to have anabolic activity. In 1999, WADA was set up as a foundation under the initiative of the IOC with the support and participation of intergovernmental organizations, governments, public authorities, and other public and private bodies fighting against doping in human sport. Under WADA, the rules and technical documents concerning anabolic steroids (and other drugs) are constantly evolving and for up to date information the reader is strongly advised to access the WADA web site (<http://www.wada-ama.org/en/>).

Misuse of anabolic steroids in sport and society

The use of anabolic steroids for cosmetic benefits among both adults and adolescents in society may be incorrectly regarded as a comparatively harmless pharmacological manipulation that can aid the development of bulging muscles and a well-toned figure. Surveys of anabolic steroid abuse by gymnasium users found that, overall, around 5% were using such drugs (Korkia and Stimson, 1993), whereas among people attending gyms equipped for competitive bodybuilding, the proportion of current or previous users was around 25–50% (Lenehan *et al.*, 1996; Korkia and Stimson, 1997). Nevertheless, it is difficult to estimate the true number of anabolic steroid users in the whole of the

United Kingdom but these drugs are used on a nationwide basis, as discussed in depth by the report from the British Medical Association (BMA, 2002). Similar surveys indicate a high prevalence of use in the United States (Yesalis *et al.*, 1993, 1997; Yesalis and Bahrke, 2000).

For drug control in sport, anabolic steroids are regarded (correctly) as performance enhancers, as well as harmful to health. Of the 198 143 urine samples analysed in 2006 by 34 WADA-accredited laboratories, 4332 (2%) were found to contain a prohibited substance ('A-sample'), of which 1966 (45% of all the adverse findings) were positive for anabolic steroids. Comparison of the adverse findings for worldwide testing for over a decade show that there has been little change year after year, the most common steroids being testosterone, nandrolone, stanozolol and methandienone. Testosterone has an unfavourable anabolic-androgenic dissociation compared with other anabolic steroids, but it is more difficult to prove its administration, as it is also produced endogenously. Some consider that the WADA statistics do not reflect the real extent of doping with anabolic steroids, particularly within top-level athletics but few would dispute that the urge to succeed and the rewards of success, both financial and otherwise, have provided powerful incentives to some competitors to look for every possible means of improving their performance, despite the risk of denunciation and penalties.

Chemical structures and activity*Common anabolic steroids*

Some of the structural modifications that have been introduced into the testosterone in an attempt to maximize the anabolic effect and minimize the androgenic are shown in Figure 2, and examples of anabolic steroids are given in Figure 3. Many of these steroids have been withdrawn as licensed products in numerous countries worldwide but they continue to be available as pharmaceutical preparations in others, for example, methandienone, methyltestosterone, oxandrolone and stanozolol. The only preparations currently available as licensed products for human use within the United Kingdom are testosterone and its esters, nandrolone (as the decanoate ester), mesterolone and oxymetholone (named patient basis only). Boldenone and trenbolone are restricted to veterinary purposes only in some countries, but, nonetheless, sports competitors and body-builders have been known to administer these anabolic steroids.

Oral activity can be conferred by substitution of the 17α -H on the steroid nucleus with a methyl or ethyl group to make the 17α -alkylated anabolic steroids. Alkyl substitution prevents deactivation of the steroid by first-pass metabolism by sterically hindering oxidation of the 17β -hydroxyl group. A methyl group attached to C-1 can also confer oral activity, as in methenolone or mesterolone, but these two anabolic steroids are considered to be relatively weak in pharmacological activity.

Parenteral preparations do not require a 17α -alkyl group but usually the 17β -hydroxyl group is esterified with an acid moiety (van der Vies, 1993) to prevent rapid absorption from

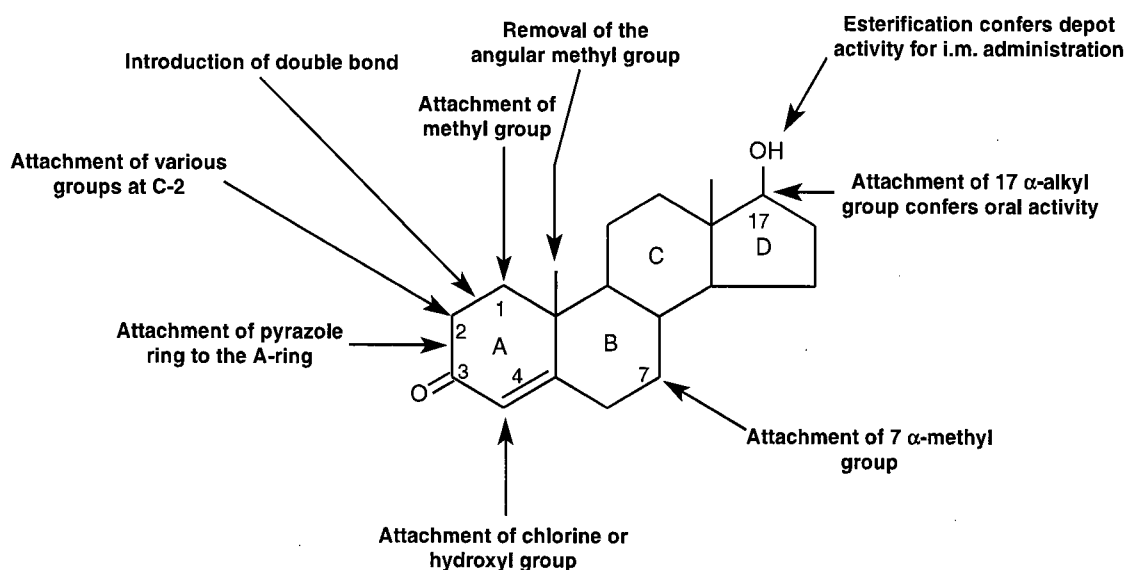


Figure 2 Structural modifications to the A- and B-rings of testosterone that increase anabolic activity; substitution at C-17 confers oral or depot activity (i.m.). Figure from Kicman and Gower (2003b), a commissioned article by the Analytical Investigations Standing Committee, reproduced with permission from the Association of Clinical Biochemists.

the oily vehicle, usually arachis oil plus a small amount of benzyl alcohol. Once in the circulation, hydrolysis rapidly occurs by the action of blood esterases to yield the active compound. The esters include cyclohexylpropionate, decanoate, laurate and phenylpropionate for nandrolone; acetate, cypionate, decanoate, enanthate, isocaproate, phenylpropionate, propionate and undecanoate for testosterone, undecylenate for boldenone and acetate for trenbolone. The mechanism of action of the nandrolone esters and other anabolic steroids, and the effect of drug delivery systems on their biological activity have been studied by van der Vies (1993). The duration of action of the esters depends upon the rate of absorption from the site of administration. This is dependent on the chain length of the acid moiety and also the formulation, being related to the partition coefficient of the derivatives between the oil used in the formulation and plasma. In general, the longer the chain length, the more slowly the preparation is released into circulation, thus prolonging the duration of action. Furthermore, testosterone undecanoate is also orally active, the 11 carbon chain ester making the molecule so lipophilic that its route of absorption is partially shifted from the hepatic portal vein to the lymph system, thus bypassing first-pass metabolism to some extent, it being released into the circulation via the thoracic duct (Coert *et al.*, 1975; Horst *et al.*, 1976).

Non-pharmaceutical water-based testosterone suspensions for injection are advertised on bodybuilding web sites and cheats in sport may find these attractive as, in theory, these should be relatively short acting. Non-pharmaceutical-based preparations, whether oil or water based, may be a particular hazard to health as the contents may not have been prepared under sterile conditions.

Transdermal formulations are invariably testosterone based, legitimately designed for replacement therapy, and include the 'patch' and hydroalcoholic gels, to be applied on

a daily basis. Other short-acting testosterone preparations include those that are designed to be administered by the sublingual or buccal route. Such short-acting formulations are of particular concern in sport, as the exogenous source of testosterone is rapidly eliminated following cessation of treatment. Increased out-of-competition testing helps to combat the cheat who is using short-acting preparations and ceasing administration prior to competition in anticipation of testing. It is of interest that an illicit preparation called 'The Cream' was designed for transdermal application (see the section 'Designer steroids').

Steroid dietary supplements

A current cause for concern is the recent manufacture of analogues of established anabolic steroids to tap into the bodybuilding market. To avoid the statutory controls of countries regarding the manufacture and supply of drugs, these compounds are often widely marketed as nutritional/dietary supplements, examples being DHEA, androstenedione, androstenediol, and their 19-nor equivalents (these steroids are prohormones), and analogues of testosterone and stanozolol called 1-testosterone and prostanazolol, respectively (Figure 4). It is a consequence of their widespread availability that a minority of athletes will also use these steroids in an attempt to improve sporting performance, and because they are structurally related to mainstream anabolic steroids, sports antidoping laboratories are made to incorporate such compounds into their drug screens under the WADA rules. These steroids are supplied for oral administration, and are therefore subject to first-pass metabolism, a very important factor as to the extent the steroid is deactivated or converted to a more active form. Some of the putative metabolites of dietary supplements have been identified by mass spectrometry, but not by other

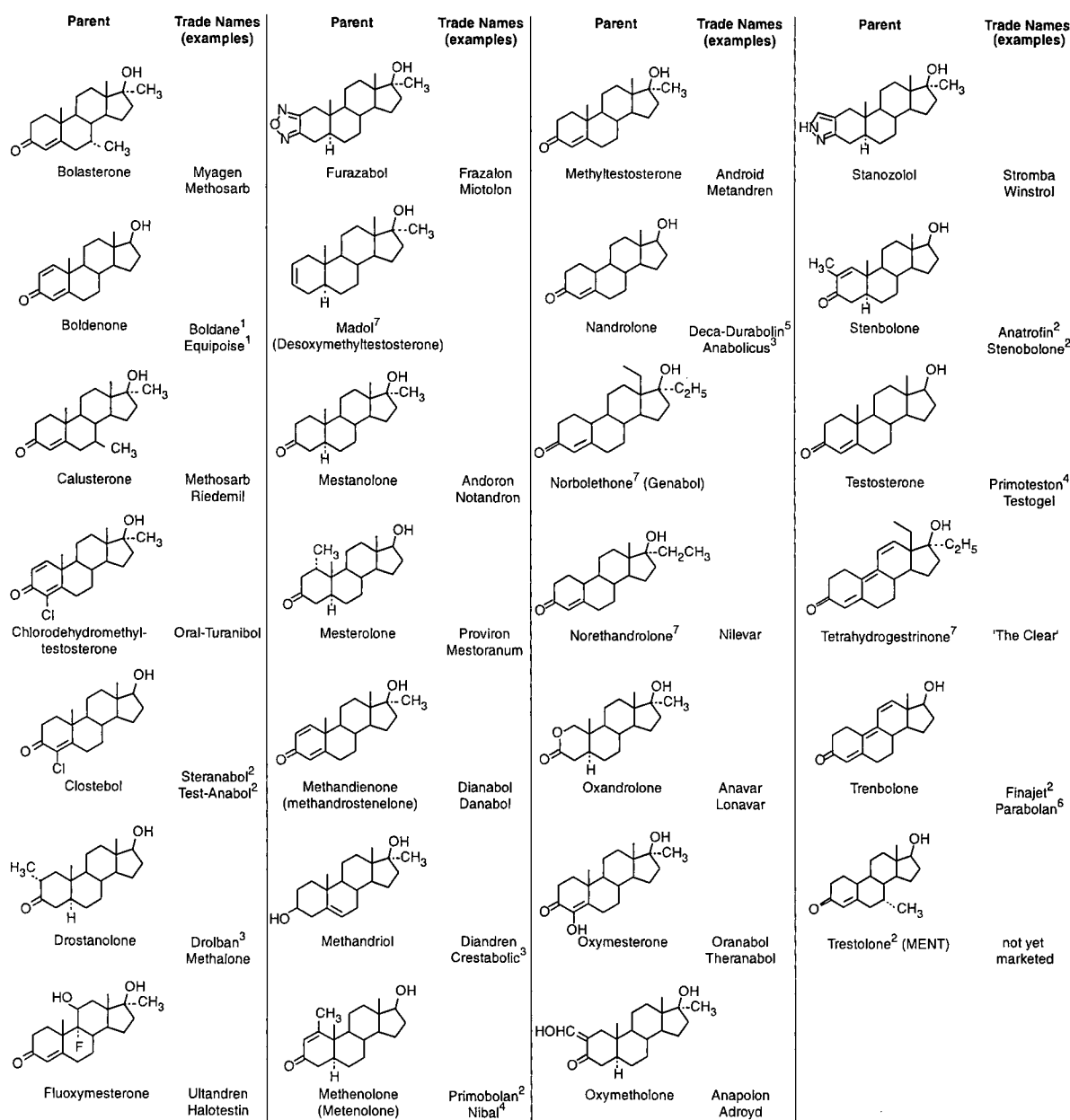


Figure 3 Structures of anabolic-androgenic steroids with corresponding diagnostic metabolites and examples of registered trade names. Superscripts (¹⁻⁶) refer to 17 β -hydroxyl-esterified preparations: ¹undecylenoate; ²acetate; ³propionate; ⁴heptanoate; ⁵decanoate; ⁶hexahydrobenzylcarbonate. Superscript⁷—see the section on 'Designer steroids'.

analytical techniques such as nuclear magnetic resonance spectroscopy to confirm configuration of the structure; the interested reader is referred to the extensive review by Van Eenoo and Delbeke (2006).

With respect to prohormone supplements of testosterone, as recently reviewed by Brown *et al.* (2006), these are modelled on steroids that are endogenously produced, that is, androstenedione, androstenediol and DHEA. However, supplements of the weaker androgens DHEA or androstenedione may be of little or no benefit to healthy young men who wish to improve their strength and sporting performance if, as would be expected, any anabolic effect is

primarily mitigated through peripheral conversion to testosterone. Ingestion of DHEA can result in an increase in circulating DHEA and androstenedione, but it is not resolved as to whether there is an increase in plasma testosterone, see for example Brown *et al.* (1999). This is not surprising because in the adult men the overall peripheral contribution of these precursor steroids to circulating testosterone is small. Any contribution from exogenous DHEA or androstenedione will be largely moderated by the large amount of testosterone contributed by the testis. In women, an increase in performance may be possible following ingestion of these supplements, as circulating testosterone would be expected

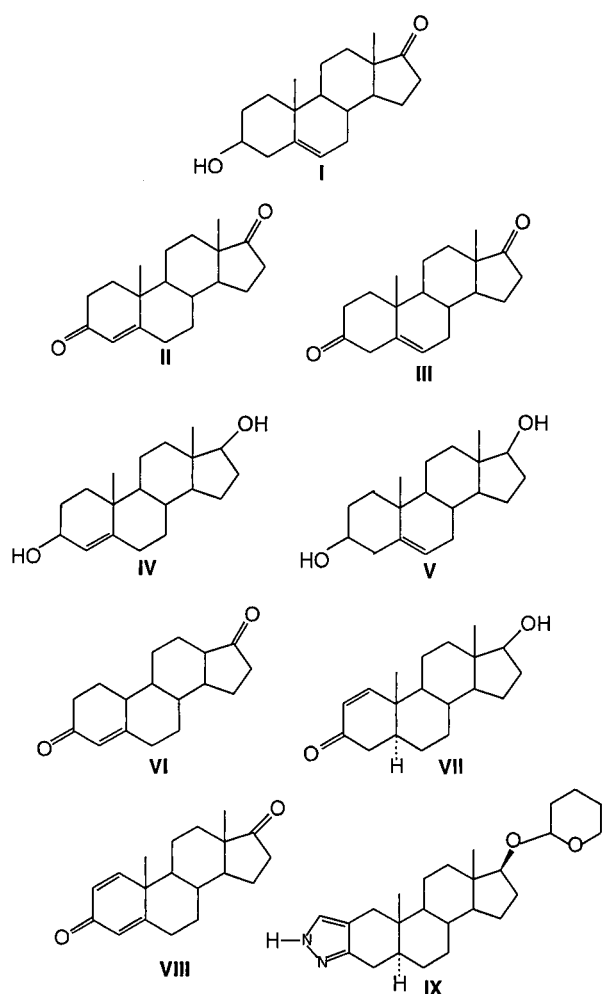


Figure 4 The 'supplements' (I) dehydroepiandrosterone (DHEA), (II) and (III) androstenedione ($\Delta 4$ and $\Delta 5$, versions respectively), (IV) and (V) androstenediol ($\Delta 4$ and $\Delta 5$ versions, respectively), (VI) 19-norandrostenedione (only $\Delta 4$ version displayed), (VII) 1-testosterone, (VIII) boldione and (IX) prostanazolol.

to increase. The plasma concentration of endogenous testosterone is approximately 1/10th that found in men and the relative proportion arising from peripheral conversion of weaker androgens is much greater. Even though only 12–14% of androstenedione is converted peripherally to testosterone (Horton and Tait, 1966; Bardin and Lipsett, 1967), this amount accounts for about half the circulating testosterone in the women. As the peripheral contribution to blood testosterone is far greater in the young adult women than the men, ingestion of modest amounts of androstenedione, DHEA or androstenediol (the natural steroid or the $\Delta 4$ analogue) is likely to significantly raise circulating testosterone. There are modest-to-large increases in circulating testosterone following androstenedione administration to women (Leder *et al.*, 2002; Kicman *et al.*, 2003a; Bassindale *et al.*, 2004; Brown *et al.*, 2004). Women who chronically administer large doses of weaker androgens that can be converted to more potent steroids would be expected to suffer from virilizing effects. In 2004, the FDA (Food and

Drug Administration), as part of its public health mission, sent warning letters to 23 companies in the United States requesting them to cease distributing androstenedione as dietary supplements (FDA, 2004).

Designer steroids

Designer anabolic steroids are considered as ones that are manufactured specifically to circumvent doping tests in human sport, and, therefore, for obvious reasons, they are supplied in a clandestine fashion. There are few examples to draw on. Classified documents (Franke and Berendonk, 1997) saved after the collapse of the German Democratic Republic revealed that, since 1983, a pharmaceutical company had produced preparations of epitestosterone propionate exclusively for the governmental doping programme. Epitestosterone, an epimer of testosterone, is a steroid with no anabolic activity but its administration with testosterone simultaneously or sequentially enables an athlete to manipulate the test for testosterone administration if the test is based solely on determination of the urinary testosterone/epitestosterone (T/E) ratio. Recently, a company in California called BALCO (Bay Area Laboratory Co-operative; Burlingame, CA, USA) attracted much media attention due to the high profile of the athletes involved, not least because of the supply of a transdermal preparation coded as 'The Cream' containing testosterone and epitestosterone, as well as a sublingual preparation of a new anabolic steroid coded as 'The Clear', which was identified from the contents of a spent syringe as tetrahydrogestrinone (THG) by the WADA-accredited laboratory within the University of California, Los Angeles (UCLA) (Catlin *et al.*, 2004).

Tetrahydrogestrinone can be easily manufactured by the catalytic hydrogenation of the ethynyl group of the progestogen gestrinone (Figure 5). This relatively simple synthetic step hides the thinking that probably lay behind the design of THG. Given the close homology of their receptors, there is an overlap between the activity of progestogens and androgens, especially those xenobiotic steroids that lack the C-19 methyl group, but which activity predominates depends on whether the alkyl substituent at carbon-17 is ethynyl or ethyl. Substitution of the 17α -H with an ethynyl group on nandrolone, a 19-nor anabolic steroid with some progestational activity, will result in a potent orally active progestogen, this being called norethisterone (norethindrone), a steroid that is still used in some contraceptives today. The synthetic route is described in a seminal paper by Djerassi *et al.* (1954). However, substitution with an ethyl group on nandrolone rather than ethynyl group results in another anabolic steroid known as norethandrolone, which also has oral activity. Gestrinone, is a pharmaceutically available progestogen that lacks the C-19 angular methyl group but has a 17α -ethynyl group, and it follows that reduction of this ethynyl group to the tetrahydro product should make THG a 'potent' androgen. This is indeed the case, as subsequently THG was found to be a highly potent androgen (and progestogen) in an *in vitro* bioassay system expressing human steroid receptors (Death *et al.*, 2004), and it promotes muscle accretion in orchidec- tomized male rats (Jasuja *et al.*, 2005). Despite the presence

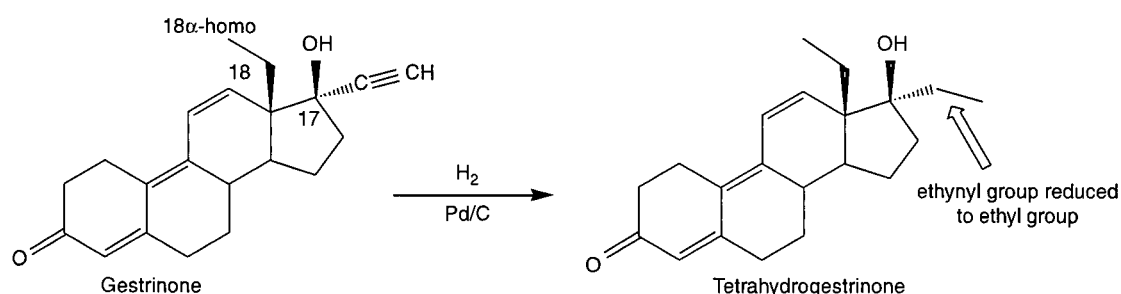


Figure 5 Catalytic hydrogenation of gestrinone to form tetrahydrogestrinone (THG). An example of a catalyst is palladium on carbon (Pd/C), as described in a procedure employed by Catlin *et al.* (2004).

of the 17 α -alkyl function, which should make the steroid resistant to first-pass metabolism, it is of interest that the instructions from BALCO Laboratories were to place a few drops of the liquid preparation under the tongue, that is, a sublingual route of administration. THG was invisible on the routine gas chromatography–mass spectrometry screen employed by the WADA-accredited laboratories and necessitated the development of a liquid chromatography–mass spectrometry/mass spectrometry (LC–MS/MS) screen for its detection; for a current and detailed review on the analysis of anabolic steroids see Kicman *et al.* (2008).

Underground chemists appear also to be accessing information concerning other steroids that were synthesized several decades ago by pharmaceutical companies but were never marketed. Such steroids that have been detected until recently are norbolethone (Catlin *et al.*, 2002), which was reputed to have been the active ingredient of 'The Clear' before being replaced by THG, and madol (Sekera *et al.*, 2005), which is also referred to as desoxymethyltestosterone (by the WADA-accredited laboratory in Montreal, who identified this steroid around the same time as the accredited laboratory at UCLA). Although the extent of this activity appears to be limited, as screening procedures rely on targeting selecting ions for monitoring by mass spectrometry, unknown steroids may escape detection. To demonstrate how this problem may be addressed, Thevis *et al.* (2005) developed an LC–MS/MS screening method based on common fragmentation pathways and Nielsen *et al.* (2006) used a combination of androgen bioassay detection and electrospray quadrupole time-of-flight mass spectrometric identification.

Mechanisms of action

Anabolic steroids are thought to exert their actions by several different mechanisms. These mechanisms include modulating androgen receptor expression as a consequence of (i) intracellular metabolism and by (ii) directly affecting the topology of the androgen receptor and thus subsequent interaction with co-activators and transcriptional activity. Other mechanisms include (iii) an anticatabolic effect by interfering with glucocorticoid receptor expression; and (iv) by non-genomic, as well as by genomic pathways, in the CNS resulting in behavioural changes. These mechanisms are discussed herein.

As an adjunct, much of the physiological importance of non-genomic actions of androgens is still to be elucidated, not least with respect to androgen-induced cell-cycle progression. The induction of second messenger signal transduction cascades by steroids commonly occurs within seconds to a few minutes, in contrast to genomic activity of the classic steroid receptors that takes 30–60 min. Regarding androgens, several non-genomic mechanisms appear to be involved, including mediation by the membrane-bound sex hormone-binding globulin receptor and also a putative G-protein-coupled receptor that androgens directly bind with, as well as through stimulation of nonreceptor tyrosine kinase c-SRC. The complexity of these mechanisms is described in detail elsewhere (Cato *et al.*, 2002; Heinlein and Chang, 2002a; Losel *et al.*, 2003). Ultimately, gene transcription may be modulated by these 'non-genomic' pathways but a well-recognized exception is the rapid elevation of calcium ion influx by a pathway that is confined to the cytoplasm. It is not currently known whether non-genomic actions of androgens at physiological concentrations are important in skeletal muscle growth, let alone what the non-genomic effects may be evoked by the administration of anabolic steroids. For the sake of brevity, this review will only very briefly touch again on non-genomic pathways under 'Behavioural Effects' (see the section 'Behavioural mechanisms').

Intracellular metabolism and the myotrophic–androgenic index

The structural changes to testosterone by medicinal chemists were designed to enhance the protein anabolic effect relative to the androgenic effect. Unfortunately, the anabolic effects could not be divorced entirely from the androgenic effects, although some synthetic steroids present a remarkable dissociation, at least based on the myotrophic–androgenic index. It may be argued that by today's standards this *in vivo* approach, which was developed over 50 years ago, is unsophisticated given the huge developments in molecular biology since that time. Despite the criticism that this approach has attracted, it is of note that anabolic steroids with high myotrophic activity and favourable index values, for example, nandrolone (esterified), oxymetholone, methandienone and stanozolol are still available as medicines in many countries. These steroids remain desirable as a doping agent to enhance sporting performance (as evident by the statistics collated by WADA) and for

bodybuilding purposes. For this reason, it is logical to summarize this approach, based on growth of a particular skeletal muscle called the levator ani relative to that of androgenic target tissue, usually the prostate gland, and attempt to explain the underlying mechanism of dissociation of the growth of the two tissues (compared with controls).

Eisenberg and Gordan (1950) proposed the use of the rat levator ani muscle as a bioassay of protein anabolic activity; the anatomical drawings from the dissection of the male rat, displaying the location of this muscle, the prostate and seminal vesicles are displayed in this paper. The rat levator ani muscle is part of the perineal complex of striated muscles that envelope the rectum. This muscle was chosen because previous workers had reported that testosterone propionate stimulated the growth of the perineal complex in infantile rats, and, additionally, this complex was easily separated from other tissues. Eisenberg *et al.* demonstrated that the levator ani muscle in castrated, immature rats responded well to the administration of various steroids such as testosterone propionate, 17 α -methyltestosterone and pituitary growth hormone (extracted from the anterior lobes of ox pituitaries). In contrast, there was a much smaller unparalleled increase in the weight of the seminal vesicles.

The foundation of the commonly used procedure of the myotrophic-androgenic index was based on a modification of the Eisenberg and Gordan method by Hershberger *et al.* (1953). Hershberger and co-workers preferred the use of the ventral part of the prostate rather than the seminal vesicles as a measure of tissue androgenic response in immature gonadectomized rats. They proposed a measure of hormonal myotrophic-to-androgenic activity using the following ratio:

Index ratio = (experimental levator ani weight–control levator ani weight)/(experimental ventral prostate weight–control ventral prostate weight) = increase in levator ani weight/increase in ventral prostate weight

Many investigators employed the approach proposed by Hershberger *et al.* (1953), but some made their own

modifications to it, and others still used the seminal vesicles as a bioassay of androgenicity.

Like the index value, the myotrophic or androgenic effects were themselves expressed as ratios to other reference steroids, for example, 17 α -methyl testosterone or testosterone for oral routes and testosterone propionate for parenteral routes. A comprehensive comparison of the anabolic and androgenic activities of many anabolic steroids and their dissociation index is given elsewhere (Potts *et al.*, 1976) but some examples are displayed in Table 1.

Kruskemper (1968) discusses the many failings of the procedures used for determining the myotrophic-androgenic index, for example, the seminal vesicles react more slowly to certain androgens, so that with short test administration, distortions can arise in favour of the myotrophic effect. The harshest criticism of this index was given by Nimni and Geiger (1957), Scow and Hagan (1957) and Hayes (1965). Testosterone administration for 56 days to young gonadectomized rats (castrated at 20–23 days of age) had no effect on the growth of the thigh muscle compared with controls, yet there was considerable growth in the perineal musculature (Scow, 1952; Scow and Hagan, 1957). Testosterone propionate or norethandrolone (17 α -ethyl-19-nortestosterone; also an anabolic steroid) administration promoted the growth of the levator ani muscle even in young normal or castrated rats on a protein-free diet, that is, a local anabolic effect proceeding at the expense of catabolic processes in other organs. Hayes (1965) stated that the rat levator ani muscle is not homologous to this muscle in other species, that is, it is not a typical sphincter muscle and does not lift the anus in rodents but is part of the male reproductive system. Thus, Hayes renamed the levator ani muscle, calling it the dorsal bulbocavernosus. All three groups of workers showed that the levator ani muscle reflects a general genitomyotrophic response rather than an overall response to androgens. Later, Hervey (1982) claimed that the male rat's characteristics are determined shortly after birth (due to a brief secretion of testosterone), and, thereafter, any increase in body mass is not affected by androgens.

Table 1 Comparison of myotrophic and androgenic activities of anabolic steroids—examples were drawn from a much more comprehensive table (with referenced papers) presented by Potts *et al.* (1976)

Steroid	Route	Reference steroid	Activity		Index value
			Myotrophic	Androgenic	
Chloromethyl T	p.o.	17 α -MeT	0.5	0.10–0.15	3–5
Methandienone	p.o.	17 α -MeT	0.60	0.20	3
Methenolone acetate	p.o.	17 α -MeT	0.86	0.12	7
Nandrolone decanoate	par.	T propionate	3.29–4.92	0.41–0.31	12.1–10.6
Norbolethone ^a	par.	T propionate	3.44	0.15–0.17	20
Norethandrolone	par.	T propionate	0.77–1.0	0.06–0.38	2–16
Oxandrolone ^b	par.	17 α -MeT	3.22	0.24	13
Oxymesterone	p.o.	17 α -MeT	1.34	0.42–0.61	2.2–3.2
Oxymetholone	p.o.	17 α -MeT	3.20	0.45	7.1
Stanozolol	p.o.	17 α -MeT	2.0–3.7	0.33–0.52	6–10.6
T	p.o.	17 α -MeT	0.36	0.28–0.50	0.7–1.3

Abbreviations: par., parenteral; T, testosterone.
^aNorbolethone was developed in 1966 but it was never commercially marketed. It was detected in two urine samples from an athlete (August 2001, March 2002) by Catlin *et al.* (2002). A US Government Investigation revealed that a chemist based in Champaign (IL, USA) synthesized this steroid and it was distributed by a company called BALCO Laboratories (see also the section 'Designer steroids').
^bOnly the activity for the parenteral route available.

Contrary to the opinions described above, there is nonetheless biochemical evidence that suggests that the genito-myotrophic response of the levator ani muscle may serve as an indicator of the general myotrophic responses in the developing rat for the following reasons. The same classic androgen receptor can be characterized in the prostate, the bulbocavernosus/levator ani muscle and typical skeletal muscles of the rat (Krieg and Voigt, 1977). Nandrolone (19-nortestosterone) and 5 α -DHT have a higher binding affinity than testosterone with the receptor. The prostate has 7 times the concentration of androgen receptors than the bulbocavernosus/levator ani muscles which in turn has 10 times more than other skeletal muscle. *In vitro* studies by Gloyna and Wilson (1969) and Massa and Martini (1974) have shown that 5 α -reductase activity is very high in rat sexual tissue such as the prostate and seminal vesicles but negligible, if at all, in skeletal muscle such as the levator ani and thigh muscle. Intracellular DHT is, therefore, low in skeletal muscle, and it is worth emphasizing that its presence is further diminished because of the high activity of the enzyme 3 α -hydroxysteroid-dehydrogenase in this tissue (and cardiac tissue as well), the enzyme that converts DHT irreversibly to 3 α -androstanediol (Massa and Martini, 1974; Smith *et al.*, 1980). The very low activity of 5 α -reductase in skeletal (and cardiac) muscle was subsequently confirmed by other investigators (Krieg *et al.*, 1976; Bartsch *et al.*, 1980), and although the enzymatic activity within the levator ani appears to be significantly higher, it still represents only 5% of that within the prostate. The rat levator ani may be a somewhat atypical striated muscle because of its greater concentration of androgen receptors, but, due to its very low 5 α -reductase activity, it can also be argued that it is not a typical part of target tissues associated with the reproductive system. Celotti and Cesi (1992), in their review of possible mechanisms of action of anabolic steroids, discuss that the peculiar androgen sensitivity of this muscle is intermediate between that present in the skeletal muscles and that of the prostate. The myotrophic effect of anabolic steroids may be reflected by the amplified response of the levator ani muscle due to its higher concentration of androgen receptors, an effect that is not apparently sufficient in other (typical) rat skeletal muscles to be observed using differences in weight (compared with controls) as the measurand.

A possible basis for increasing the myotrophic-to-androgenic ratio may be by exploiting the fundamental difference between the 5 α -reductase concentrations in skeletal muscle and androgenic tissue. One way of increasing the anabolic-androgenic dissociation is to administer a steroid that has a greater binding affinity for the androgen receptor but upon reduction to a 5 α -metabolite has a lesser affinity. Among the anabolic steroids, 19-nortestosterone (nandrolone) was one of the first synthesized, the most used and probably the best studied. Although DHT has a greater binding affinity for the androgen receptor than its parent steroid testosterone, by contrast the 5 α -reduced form of 19-nortestosterone, 5 α -dihydro-19-nortestosterone, has a lesser binding affinity than its parent steroid 19-nortestosterone (Toth and Zakar, 1982). Hence, in androgenic tissue, testosterone is converted to a more potent metabolite, whereas 19-nortestosterone is converted to a less potent one. As 5 α -reduction occurs

readily in androgenic tissue but is negligible in skeletal muscle, this explains why 19-nortestosterone has a greater myotrophic-to-androgenic ratio when compared with testosterone (Figure 6). If the model is correct, such a diminishment in androgenic activity should not be confined to the accessory reproductive tissues in the human such as the prostate, but also in non-genital target tissues where clear roles for the metabolism to DHT have been defined such as the male patterns of facial and body hair growth, thus allowing more muscle per whisker. Moreover, even where testosterone rather than DHT appears to stimulate other secondary sexual characteristics, such as voice deepening, with the discovery of two isozymes of 5 α -reductase (for review see Russell and Wilson, 1994), it cannot be ruled out that some of these actions attributed to testosterone need to be re-evaluated, the results of which may have relevance to the applicability of anabolic steroids with a high myotrophic-androgenic index. Much of the knowledge of the separate roles of testosterone and DHT came from 5 α -reductase deficiency syndrome, but these effects are all ascribed to mutations in the type 2 isoenzyme (Randall, 1994) and the biological role of the 5 α -reductase type 1 is harder to ascertain as there is no recognized type 1 deficiency. For example, type 2 5 α -reductase appears not to be necessary for the sebaceous gland response to androgens and the development of acne, but it is now known that the principal isoenzyme in this gland is the type 1 form (Thiboutot *et al.*, 1995; Sato *et al.*, 1998). As an adjunct, dutasteride (Avodart; manufactured by GlaxoSmithKline), which inhibits both type 1 and type 2 5 α -reductases and is used in the treatment of benign prostatic hyperplasia (Clark *et al.*, 2004) and male pattern hair loss (Olsen *et al.*, 2006), appears not to be helpful in the treatment for acne vulgaris (Leyden *et al.*, 2004). This suggests that further work at the molecular level is required to better understand the action of androgens on sebaceous gland function.

This mechanism of myotrophic-androgenic dissociation does not explain why other anabolic steroids that do not undergo 5 α -reduction, for example, those with an extra double bond in the A-ring, such as chlorodehydromethyltestosterone and methandienone (Schanzer, 1996), have a favourable myotrophic-androgenic index. Even so, it is possible that that myotrophic-androgenic dissociation may occur, simply because the effect of the particular steroid cannot be amplified by 5 α -reduction in androgenic target tissues, in common with the hypothesis proposed for the differential action of a steroidal SARM (see the section 'Selective androgen receptor modulators' for an explanation of the term) called MENT (7 α -methyl-19-nortestosterone; trestolone) (Agarwal and Monder, 1988; Kumar *et al.*, 1992), as reviewed by Sundaram and Kumar (2000).

Recently, as part of investigations to assess whether the designer steroid THG had anabolic and androgenic properties (see also next section), three papers report the effects of its administration on the growth of the levator ani, prostate and seminal vesicles compared with control steroids (Jasuja *et al.*, 2005; Labrie *et al.*, 2005; Friedel *et al.*, 2006a). Notwithstanding the possible differences in pharmacokinetics and bioavailability between THG and the control steroids administered, there appeared to be little myotrophic-androgenic

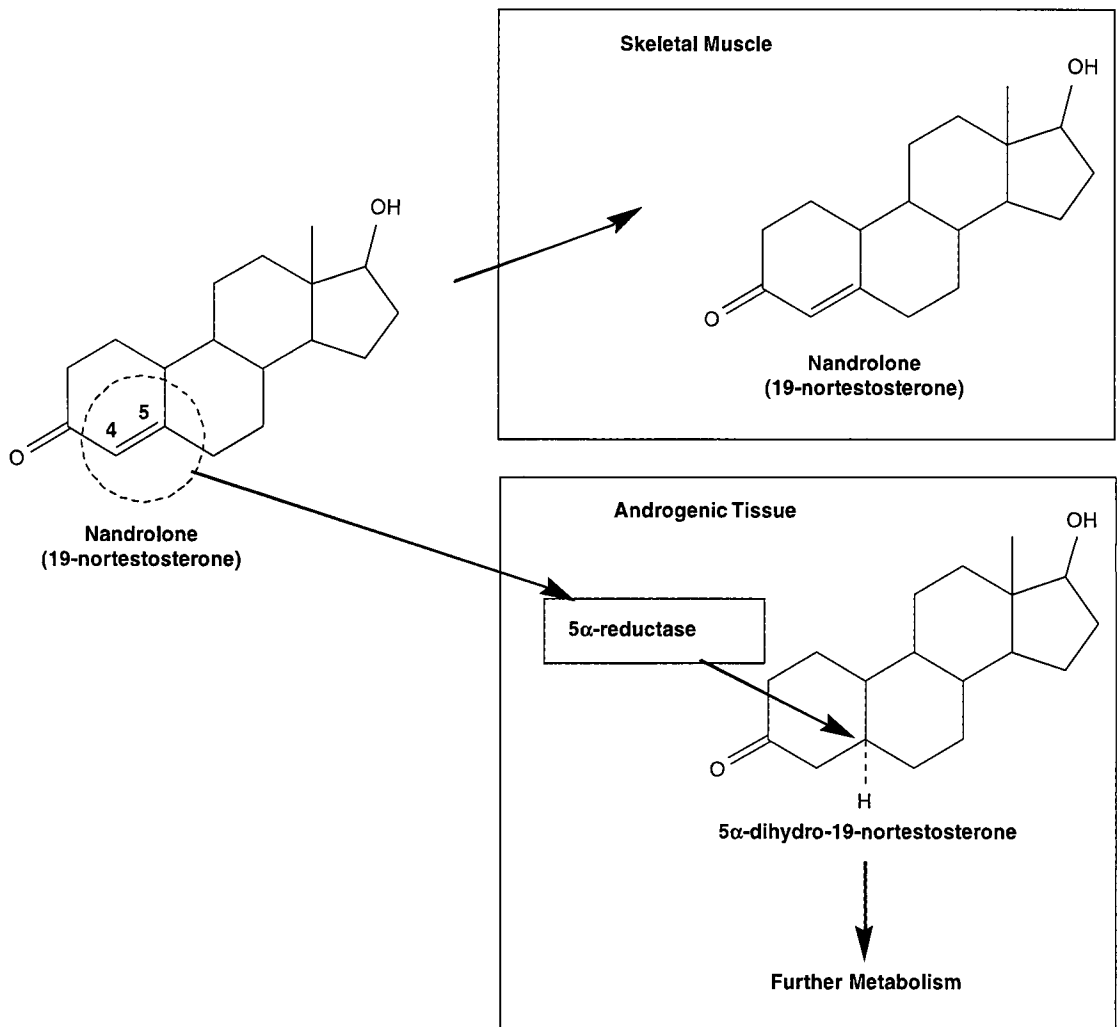


Figure 6 In androgenic tissues, nandrolone (19-nortestosterone) is readily converted by the enzyme 5 α -reductase into 5 α -dihydro-19-nortestosterone, i.e., the double bond between C4 and C5 is reduced. This metabolite binds with weaker affinity to the androgen receptor compared with the parent steroid. Further metabolism can occur because of the high activity of the enzyme 3 α -hydroxysteroid-dehydrogenase (which reduces the 3-oxo group) in androgenic tissue. In skeletal muscle, 5 α -reductase activity is negligible and, therefore, the parent steroid itself binds with strong affinity to the androgen receptor. It follows that there is a favourable disassociation of the myotrophic effects from the androgenic effects of nandrolone and also that there is a greater myotrophic-to-androgenic ratio when compared with testosterone.

dissociation, but, nonetheless, the bioassays clearly demonstrated that THG had anabolic and androgenic activity *in vivo*, and, therefore, belonged within the banned doping class of anabolic agents in sport, as defined by WADA.

As a final and very important point, it is of note that complete dissociation has not been achieved with any anabolic steroid synthesized, and, therefore, the chronic administration of these drugs, even those with a very high myotrophic-androgenic index value, such as found with nandrolone (19-nortestosterone), will result in hirsutism and, eventually, virilization of women and children.

Androgen receptor expression and the importance of coregulators
The androgen receptor belongs to the family of nuclear receptor superfamily (Mangelsdorf *et al.*, 1995), these intracellular receptors eliciting so-called 'classical' or genomic, actions by interacting with DNA and modulating

transcription. A DNA-binding domain, a ligand-binding domain and at least two transcriptional activation domains, characterize these receptors. Apart from binding with the steroid, the ligand-binding domain also functions in dimer formation and mediates transcriptional activation. The DNA-binding domain targets the receptor to specific DNA sequences known as steroid (or hormone) response elements. On the receptor, the DNA-binding domain consists of two subdomains called 'zinc-fingers'; each subdomain contains four cysteine residues that coordinate with a zinc atom, thus, stabilizing the 'finger' structure. The zinc-fingers are inserted between specific grooves of the DNA helix, thus, allowing maintenance of DNA-binding activity.

A general model of steroid receptor action is displayed in Figure 7. In the absence of hormone, it is by and large accepted that steroid receptors exist as an inactive oligomeric complex, being sequestered by the heat-shock protein (Hsp), Hsp90, which acts as a molecular chaperone. Hsps are

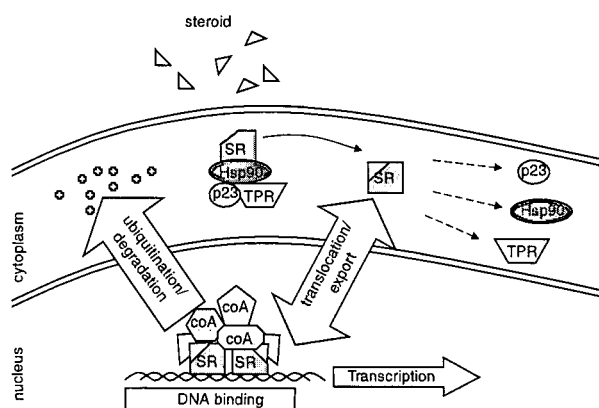


Figure 7 In the absence of hormone, the steroid receptor exists as an inactive oligomeric complex with the molecular chaperone heat-shock protein, Hsp90, and p23, and co-chaperones utilizing tetratricopeptide repeat (TPR) motifs. After hormone binding, the receptor–Hsp90 complex disassociates and the activated receptor is translocated into the nucleus. Activated receptors interact as homodimers with the steroid response element on the chromatin, triggering the formation of a transcription complex, a cluster of coregulators resulting in gene activation, transcription of the gene, protein translation, and a resultant alteration in cell function, growth or differentiation. This figure is redrawn in the own author's style but was based on part of the figure in the article by Weigel and Moore (2007).

so-called because they were discovered to accumulate under stress conditions including within heat-traumatized cells, but many are present and functionally important under normal conditions; they are named according to their molecular weight in kilodaltons. Another chaperone called p23 stabilizes the aporeceptor complex by blocking Hsp90 in the ATP-bound substrate conformation. Co-chaperones utilizing tetratricopeptide repeat motifs are necessary for docking of the Hsp90. As an adjunct, other chaperones, called Hsp40 and Hsp70 and an organizing protein called Hop (heat-shock organizing protein) are important in the assembly of the steroid receptor–Hsp90 complex. Picard (2006) gives a clear overview of molecular chaperones and cofactors that are relevant to steroid receptor action. Phosphorylation of the receptors is also important in regulation of receptor function (Weigel and Moore, 2007).

The steroid receptor–Hsp90 complex appears to be necessary for the receptor to stabilize in a conformation for binding to the ligand with high affinity and also to maintain its solubility in the cell. It is generally accepted that, although the receptor is held in this complex, it is inactive as a transcription factor, that is, the Hsp90 complex acts as a repressor of transcriptional activity by preventing one or several of the following: nuclear localization, dimerization, DNA binding and interaction with transcriptional co-activators.

Steroids are relatively small molecules, for example, testosterone has a molecular weight of 288, and they can passively diffuse into cells. In target tissues, that is, the cells that contain steroid receptors, the hormone binds to the receptor ligand-binding domain, causing dissociation of the receptor–Hsp90 complex, the resultant conformational (allosteric) change making the receptor active. In the case of the androgen (and glucocorticoid) receptor, the

chaperone complex resides in the cytoplasm, and following dissociation from the chaperone the activated receptor is translocated into the nucleus. Activated receptors interact as homodimers with the steroid response element on the chromatin, the effect of two receptors binding being cooperative (greater affinity and stability). This attachment to the DNA, in turn, triggers the formation of a transcription complex, a cluster of coregulators (also called comodulators) that fit around the receptors like 'pieces in a jigsaw puzzle'. Coregulators can be either positive or negative regulatory proteins, referred to as co-activators or corepressors, respectively (Perissi and Rosenfeld, 2005). Co-activator and corepressor complexes are required for nuclear receptor-mediated transcriptional regulation, generally liganded receptors recruiting co-activators resulting in gene activation, transcription of the gene, translation and a resultant alteration in cell function, growth or differentiation.

The function of the transcriptional activation domains on the receptor is to mediate the binding of the receptor to the comodulators. The receptor has an N-terminal activation function-1 (AF-1) and a second activation function-2 (AF-2) in the C-terminal ligand-binding domain. The mechanisms of AF-1 and AF-2 gene activation, with emphasis on AF-1 and AF-2 conformation and co-activator binding, have been reviewed by Warnmark *et al.* (2003). The mechanism of AF-1 gene activation is not well understood due to the lack of conformational information but, by contrast, many crystal structures of the ligand-binding domain of different nuclear receptors have been achieved, allowing a fuller understanding of AF-2-mediated transcriptional activation. AF-2 is dependent on ligand binding to the receptor for its activity, which causes the folding of a C-terminal helix (helix-12), acting as a lid over the ligand pocket upon ligand binding. This folding creates the activation surface/AF-2 domain, allowing the docking of AF-2 co-activators and the formation of a charge clamp that stabilizes co-activator interaction, these co-activators having the leucine-X-X-leucine-leucine (LXXLL) motif necessary for such interaction (X is any amino acid).

The molecular biology of the androgen receptor has been reviewed by Klocker *et al.* (2004). In contrast to other steroid receptors, most of its transcriptional activity is mediated through the N-terminal AF-1 domain, there being a reduced capacity of AF-2 in the androgen receptor to recruit LXXLL-containing co-activators. Instead, it has been suggested that the AF-2 of the androgen receptor acts primarily as an interaction platform for the recruitment of co-activators to the N-terminal region, this regulation of gene expression through the intradomain interaction and communication being unique to this receptor.

To date, several families of co-activator proteins have been identified but only two direct inhibitors of androgen receptor function have been identified *in vivo*, SHP and DAX-1, these being atypical orphan receptors that lack DNA-binding domains. Using X-ray crystallography, the interaction between peptide segments of SHP containing LXXLL-like motifs and the ligand-binding domain on the androgen receptor was investigated, and it was found that the LKKIL motif formed a complex, binding with a hydrophobic groove on the androgen receptor (Jouravel *et al.*, 2007). It was

suggested that this transcriptional activity of androgen receptors might be inhibited by SHP competing for binding to androgen receptor co-activators. The binding motif to the androgen receptor by DAX-1 is still to be elucidated. Another corepressor, FoxG1, appears to be a likely candidate for interaction with the androgen receptor *in vivo* but studies are necessary to prove whether this is the case (Obendorf *et al.*, 2007).

Anabolic steroids bind to the androgen receptor with different affinities. Saartok *et al.* (1984) compared the relative binding affinity of the anabolic steroids ethylestrenol, fluoxymesterone, mesterolone, methandienone, methenolone, 17 α -methyltestosterone, nandrolone and oxymetholone to that of tritiated methyltrienolone (17 β -hydroxy-17 α -methyl-4,9,11-estratrien-3-one; a steroid resembling trenbolone but with a 17 α -alkyl substituent) to androgen receptors in cytosol isolated from skeletal muscle and the prostate gland of the rat and skeletal muscle of the rabbit. The order of relative binding affinities in comparison with methyltrienolone, which had the strongest affinity, was nandrolone > methenolone > testosterone > mesterolone; a group which had relatively high and generally similar affinity for the androgen receptor in all three tissues. These investigators did not rank 17 α -methyltestosterone, but it had a relative binding affinity of 0.1, which made it an efficient competitor. The relative binding with fluoxymesterone, methandienone and stanozolol was much weaker and that with oxymetholone and ethylestrenol was too low to be determined. There is a large discrepancy as to what is known about the *in vivo* activities of these steroids compared with their *in vitro* activity, even taking into account possible differences in the bioavailability and clearance of these steroids (not least determined by the affinity to sex hormone-binding globulin in the blood circulation). For example, oxymetholone and stanozolol have low relative binding affinity compared with 17 α -methyltestosterone in the *in vitro* study, but, conversely, these steroids have a relatively high myotrophic activity compared with the same steroid when administered to the castrated rat (see Table 1). Furthermore, Feldkoren and Andersson (2005) found that stanozolol and methandienone have significantly lower binding affinities compared with testosterone but all three steroids were potent activators in a cell-based androgen receptor-dependent transactivation assay. Clearly, the degree of physical binding to the androgen receptor, as measured by ligand-binding assays, does not fully explain the biological activity of anabolic steroids. Distinct target gene expression profiles due to androgen receptor activation by structurally different androgens has also been reported (Holterhus *et al.*, 2002), the study including three anabolic steroids nandrolone, oxandrolone and stanozolol, together with what the investigators term three 'virilizing androgens' (testosterone, DHT and methyltrienolone) and two testosterone precursors (DHEA and androstenedione). The model used was three structurally different androgen promoter constructs in co-transfected Chinese hamster ovary cells. All the steroids proved to be potent activators of the androgen receptor, but the anabolic steroids and the testosterone precursors showed characteristic promoter activation profiles distinct from the virilizing androgens. The assumption is that the specific

ligand-induced conformation determines how the hormone receptor complex can specifically interact with coregulators and neighbouring transcription factors and also that the transactivation capability depends on the structure of the response element. Even though many co-activators have been identified as enhancing the ligand-induced transcriptional activity of the androgen receptor, their relative importance with respect to particular cell types and tissues is unclear (Heinlein and Chang, 2002b). The emerging knowledge concerning androgen receptor interaction with its coregulators in different tissues clearly has relevance to understanding how anabolic steroids exert their actions and will give further insight into how favourable anabolic-androgenic dissociation may be achieved.

Most recently, *in vitro* bioassays have been employed to determine that the designer anabolic steroid THG is indeed a potent androgen. Death *et al.* (2004) demonstrated that THG was about one order of magnitude more potent than nandrolone, testosterone and trenbolone in yeast cells expressing human androgen receptors. Friedel *et al.* (2006b) also used a reporter gene assay based in a yeast strain containing transfected androgen receptor constructs and found that THG was about 10 times lower than the EC₅₀ of the reference substance DHT. Jasuja *et al.* (2005) found that THG upregulated androgen receptor expression in mesenchymal multipotent cells by measuring the translocation of the receptor to the nucleus using immunohistochemical and analyses, but this was not significantly different from DHT. The authors make the important point that it is not known whether yeast-based systems express the repertoire of coregulators that is present in mammalian androgen-responsive tissues. Labrie *et al.* (2005) studied the genomic signature of THG and compared it with the effects of DHT on gene expression in mouse tissues by extracting RNA, converting it to cDNA and then transcribing it *in vitro* to produce biotinylated cRNA for analysis. These investigators found that THG and DHT modulated in a similar fashion 671 genes in the mouse levator ani muscle, 95 genes in the gastrocnemius muscle and 939 genes in the prostate.

The use of *in vitro* assays based on androgen receptor expression, as described above, can help to assess whether future designer steroids have anabolic-androgenic activity, and can help to minimize *in vivo* experiments. These approaches can provide useful evidence to government agencies involved in the regulation of drugs to protect public health. Moreover, the employment of such assays should be of particular benefit to sporting authorities to help stifle legal challenges based on the premise that new designer steroids have unproven anabolic activity and thus should not be subject to doping control and the penalties associated with their administration.

Anticatabolic activity

It is accepted that the administration of anabolic steroids to healthy women and children has an anabolic effect, and that with the virilizing effects, there is a gain in muscle mass and strength. However, for many years, it was difficult to prove conclusively that the administration of these steroids had a myotrophic effect in healthy young sportsmen, as discussed

by Ryan (1976) (see the section 'Anabolic steroids as performance enhancers in sport'). Around that period, an interesting but speculative biochemical explanation for this difference in response between the sexes was that due to the exposure to testosterone during puberty in men, there is a downregulation of receptors (decrease in responsiveness of receptors often followed by decrease in numbers) in the skeletal muscle and that the androgen receptor population is then saturated with testosterone in the adult, so that no further response can be induced by pharmacological doses of androgens (Wilson, 1988). It was therefore reasoned that any possible myotrophic effect from administration of anabolic steroids to eugonadal men could be via an anticatabolic mechanism rather than a direct anabolic effect. However, the proposed downregulation of androgen receptors in skeletal muscle because of increased androgen exposure was based on a few animal studies at that time (Dahlberg *et al.*, 1981; Rance and Max, 1984) and conflicting evidence was presented by Michel and Baulieu (1980) and more recently by others, for example, Antonio *et al.* (1999). Indeed, Antonio *et al.* speculate that upregulation may occur with the administration of pharmacological amounts of androgens, converting muscles that normally have a minor, or no response, to muscles with enhanced androgen responsiveness. Androgen receptor regulation in different groups of skeletal muscle in response to physiological and supraphysiological exposure to testosterone is intricate, let alone what may occur following administration of xenobiotic anabolic steroids, and the interested reader is referred to the detailed review by Dr F Kadi in the same issue of this journal.

Indirect evidence of an antiglucocorticoid effect comes from a case report concerning partial androgen insensitivity syndrome (Tincello *et al.*, 1997). A patient with a single amino-acid mutation in the androgen receptor DNA-binding domain (Arg-608 to Lys), which explained his lack of overall response to high-dose androgen treatment at different times in his life, nonetheless, could be induced into a positive nitrogen balance with testosterone administration. An appealing explanation for this finding is that anabolic steroids act as glucocorticoid receptor antagonists. Most binding studies, however, indicate that anabolic steroids have very low binding affinity for the glucocorticoid receptor (Hickson *et al.*, 1990), a notable exception being THG, which binds with high affinity (Friedel *et al.*, 2006b). An alternative hypothesis, therefore, is that anabolic steroids may interfere with glucocorticoid receptor expression at the gene level.

Over the years, it has become apparent that the endocrinology of skeletal muscle is highly complex, and there is a delicate balance between synthesis and breakdown during growth, health, disease and ageing, as considered by Sheffield-Moore and Urban (2004). It is this complexity that makes it challenging to resolve the significance of anabolic steroids as anticatabolic (and anabolic) agents across the spectrum, from the healthy athlete who desires faster recovery from arduous training schedules where cortisol may be somewhat raised (Hervey, 1982) to the patient with severe physical trauma, such as from a burn injury, where there is extreme hypercortisolaemia

and hypoandrogenaemia (Sheffield-Moore and Urban, 2004).

Behavioural mechanisms

The behavioural effects of androgens/anabolic steroids in men and women, including those concerning sexual behaviour, cognitive abilities, aggression and mood, have been reviewed by Lukas (1996), Christiansen (2001, 2004) and Kuhn (2002) and are also discussed in the National Institute on Drug Abuse (NIDA) Research Monographs (Katz and Pope, 1990; Svare, 1990; Yesalis *et al.*, 1990). Androgens are critical to the human male sexual behaviour and they can also enhance female sexual desire and arousal. Testosterone appears to play an important role in cognitive functioning, such as attention and alertness, memory and spatial skills, although based on the conclusions of a limited number of studies. With respect to mood, there are significantly positive correlations of endogenous androgen concentrations with a sense of well-being and joyfulness, and negative correlations with depression and anxiety. Major mood syndromes can arise with anabolic steroid use, including mania or hypomania (mania of a mild type) during exposure and depressive symptoms during steroid withdrawal (Pope and Katz, 1994). Anabolic steroid administration is also associated with increased aggression, especially in high-dose users, but this is not a foregone certainty given that the interaction between androgens and behaviour in men and women is complex. It is an entirely reasonable hypothesis that the athlete may learn to recognize and harness the increase in aggression that can arise with steroid use to help drive their training and increase their competitiveness (Brooks, 1978). Furthermore, male athletes who administer anabolic steroids and then withdraw just before competition in anticipation of a drug test may then experience (in the author's opinion) a lack of motivation and possibly depression, because they will be in a state of androgen deficiency, taking time for testicular steroidogenesis to recover. In an effort to avoid this problem, it is possible that some athletes may switch to using fairly small doses of short half-life formulations of testosterone for replacement purposes in the hope that, at the time of collection of their sample for drug testing, the urinary testosterone/epitestosterone ratio will be below the WADA reporting threshold of 4.

Clark and Henderson (2003) have summarized the literature with respect to the effects of anabolic steroids on the neural circuits that underlie behavioural effects; their review focusing on animal models and steroid exposure that mimic human abuse regimes. Androgen receptors mediate the effects of anabolic steroids in the mammalian brain; the expression of progestogen and oestrogen receptors may also be affected. Non-genomic pathways are important too, the best-characterized example being the allosteric modulation of GABA_A receptor function by anabolic steroids, possibly through a putative binding site for anabolic steroids residing within the transmembrane domain of the receptor. Induction of aggression by anabolic steroids appears to overlap with neural circuits underlying the regulation of aggression by endogenous androgens, these being systems utilizing GABA, serotonin and arginine vasopressin.

Selective androgen receptor modulators

Many anabolic steroids were synthesized and their biological activity characterized (myotrophic-androgenic index, metabolic studies in animal and man) over 40 years ago, at a time when molecular endocrinology was in its infancy. With the knowledge gained in the 1980s and 1990s as to how selective oestrogen receptor modulators, such as tamoxifen and raloxifene, may work at molecular level (Jordan, 1998), perhaps it is not surprising that there is currently an interest in the possibility of modulating the androgen receptor in a similar manner. The development of SARMS, including their

ligand interactions with the androgen receptor, is reviewed by Bhasin *et al.* (2006). Tissue selectivity may be achieved by synthesizing ligands that modulate the expression of the androgen receptor by inducing specific conformational changes that affect its interaction with coregulators. Androgens, steroidal or nonsteroidal, that offer tissue selectivity based on a divergence in intracellular metabolism are also included under the term SARM, such as the steroid MENT, which cannot undergo 5 α -reduction (Kumar *et al.*, 1992) but is almost certainly aromatized to an active oestrogen (Lamorte *et al.*, 1994; de Gooyer *et al.*, 2003). Indeed, the role of 5 α -reductase appears to play a critical part in

Table 2 Non-steroidal AR agonists (Gao and Dalton, 2007a, b)

Chemotype	General chemical structure	Lead compound
Quinolinone analogues		LGD2226
Aryl propionamide analogues		S4
		S1
		C6
Hydantoin analogues		BMS564929
Tetrahydroquinoline analogues		S40503

Abbreviation: AR, androgen receptor.

Table reproduced from the article in *Drug Discovery Today* by Gao, W and Dalton, JT (2007) *Drug Discov Today* 12:241–248, with kind permission from Elsevier (2008).

determining the tissue-specific expression of SARMs (Gao and Dalton, 2007a). Notwithstanding, the clinical applications of steroidal androgens are generally limited by poor tissue selectivity, pharmacokinetics and toxicity, and it is hoped that the amenability to structural modifications of nonsteroidal ligands will overcome these limitations. The current nonsteroidal SARM pharmacophores are analogues of aryl propionamide, bicyclic hydantoin, quinoline and tetrahydroquinoline (Gao and Dalton, 2007b) (Table 2).

Clinically, SARMs may offer unique therapeutic potential to androgen therapy (Negro-Vilar, 1999; Roy *et al.*, 2001; Wolf and Obendorf, 2004) and ultimately those that maintain the anabolic actions of androgens without causing virilization would greatly expand the therapeutic options for women (Gao and Dalton, 2007b). Negro-Vilar (1999) gives a wish list of the desired profile of activity of SARMs, these being tailored to a number of male and female applications. Generally, all include an anabolic effect in muscle and bone, but the androgenic effects are modified to varying degrees from stimulatory, to weak or neutral, depending on the disease state. For example, for the treatment of hypogonadism in elderly men, it is important to minimize induction of growth of the prostate gland to avoid increasing the risk of developing benign prostatic hypertrophy or cancer of the prostate, and, thus, an SARM could be administered with weaker to no activity in this gland. SARMs also could be useful, but not merely confined to, the treatment of osteopaenia, osteoporosis and sarcopaenia in elderly men and postmenopausal women (assuming sufficient anabolic-androgenic dissociation can be achieved for the latter), glucocorticoid-induced osteoporosis, HIV wasting, cancer cachexia and different types of muscular dystrophies.

Clinical applications

The clinical applications of anabolic steroids has been reviewed recently by Basaria *et al.* (2001) and Shahidi (2001). Historically, the usefulness of anabolic steroids in reversing the catabolic state of patients had not proved convincing and, by the end of the 1980s, many anabolic steroids had been withdrawn as licensed products and those remaining were limited for the purpose of hormone replacement therapy and the treatment of specific diseases (see next paragraph). A detailed analysis of the plethora of clinical reports, including uncontrolled trials and case studies, together with consideration of the risks versus benefits of various anabolic steroids for protein-building purposes is beyond this review. What is especially of note, however, is that lately the potential of anabolic steroids as therapeutic agents to increase weight, lean body mass and strength is being currently revisited. Anabolic steroids, such as testosterone esters, and the 17 α -alkylated steroids oxymetholone and oxandrolone, may play a significant role in the treatment of cachexia associated with AIDS, severe burns and renal failure, where nutrition and standard care have been ineffective, as reviewed by Basaria *et al.* (2001). Further, nandrolone decanoate has been demonstrated to be effective in countering sarcopaenia in patients receiving dialysis (Johansen *et al.*, 1999, 2006) and trestolone (MENT) could

be a promising new androgen therapy for sarcopaenia (loss of muscle and strength in senescence). In the older woman, oxandrolone administration stimulates muscle protein anabolism (Sheffield-Moore *et al.*, 2006), but the role of anabolic steroid therapy in women with wasting syndromes very much needs to be evaluated (Basaria *et al.*, 2001). Notwithstanding the above, a number of regulatory and conceptual issues are hindering progress in deciding which clinical conditions may benefit from intervention with anabolic steroids (Bhasin *et al.*, 2006), not least what outcomes should constitute evidence of efficacy in clinical trials. For example, although, theoretically, an increase in lean body mass and weight in HIV-infected individuals suffering from weight loss should lead to improved physical functioning and quality of life, and ultimately to increased survival, this has not been demonstrated (Johns *et al.*, 2005). Carefully designed randomized trials may eventually give the definitive answers as to the clinical usefulness of therapy with anabolic steroids, and whether xenobiotic anabolic steroids offer any advantage over supraphysiological doses of testosterone to men. In designing trials involving women, to help reduce unwanted androgenic effects, the administration of a xenobiotic steroid with a favourable myotrophic-androgenic index should be considered. In the interim, at the very least it seems sensible to consider hormone replacement therapy to men in a catabolic state where there is a significant decrease in circulating testosterone associated with the chronic disease, for example, those with severe burn injuries or HIV-associated wasting.

For hormone replacement therapy, testosterone preparations are used in male hypogonadism and male hormonal contraception (where progestogens are administered to inhibit gonadotropin secretion). Mesterolone is also available for the treatment of male hypogonadism but it is seldom used, if at all. Oxymetholone and stanozolol, which induce the production of a C-1 esterase inhibitor, were used in the prevention and control of attacks of hereditary angio-oedema (except in pregnant women and prepubertal patients due to the risk of virilization) but the latter steroid has been recently withdrawn in the United Kingdom. Anabolic steroids also stimulate erythrocyte synthesis, which can be useful in the treatment of hypoplastic anaemias but their use in wealthy countries is likely to be limited with the relative recent availability of recombinant human erythropoietin and its analogues. In postmenopausal women, the treatment of osteoporosis with anabolic steroids, such as nandrolone decanoate, is not advocated given the success of oestrogen replacement and, more recently, with the introduction of the biphosphonates.

Anabolic steroids as performance enhancers in sport

The action of anabolic steroid in increasing skeletal muscle mass and strength in women is not questioned. Male and female athletes from the German Democratic Republic (GDR), from about 1972 onwards did exceptionally well in international events, being consistently in the top ranking of medal winners. Sporting performance among their female athletes, particularly in strength-dependent events, was

spectacular. Following the reunification of Germany in 1990, ground-breaking documental research was made by the former athlete, Mrs Brigitte Berendonk, and her husband Professor Werner Franke, who had succeeded in acquiring a number of highly classified scientific reports that had not been destroyed. These documents deal with the systematic state-sponsored programme of doping of athletes and included scientific reports, doctoral theses and a handwritten protocol book giving the times and dosage of administration of anabolic steroids to athletes. Several thousand athletes were treated with anabolic steroids every year, including adolescents of each sex. Particular emphasis was placed on the administration of anabolic steroids to women and adolescent girls, despite the virilizing effects, because of the rapid gains in sporting performance. It is important to note that the GDR scientists established (to themselves) that 'androgenic initiation' has permanent effects in girls and women, where increases in strength and performance do not return to pretreatment values after the drug is withdrawn. The current emphasis on out-of-competition drug testing is, therefore, of vital importance to help prevent doping during training followed by a period of drug elimination and then competition.

With respect to men, a most comprehensive review in 1976 of previous results concluded that there was little evidence for supraphysiological doses of testosterone or synthetic anabolic steroids having any appreciable effect on muscle size or strength in healthy men (Ryan, 1976). Even so, many of the studies reviewed had a lack of adequate control and standardization. Despite the debate in the scientific community as to the effectiveness of anabolic steroids as performance enhancers in men, male athletes and bodybuilders continued to use them, knowing from their own experimentation that they were effective. Conclusions from more recent reviews suggested that the administration of anabolic steroids could consistently result in significant increases in strength if male athletes satisfied certain criteria including the timing of doses and dietary factors (Wright, 1980; Haupt and Rovere, 1984; Alen and Hakkinen, 1987; Strauss and Yesalis, 1991). Then in 1996, Bhasin *et al.* (1996) in a very carefully designed study, proved beyond doubt that treatment with testosterone in supraphysiological doses (600 mg i.m. of testosterone enanthate for 10 weeks) increases muscle size and strength, and that with exercise these effects are augmented.

Subsequent work showed that increases in fat-free mass, muscle size, strength and power are highly dose-dependent and correlated with serum testosterone concentrations (Bhasin *et al.*, 2001; Woodhouse *et al.*, 2003) (Note: Strength is the maximum amount of force that can be exerted, for example, the heaviest weight that can be pushed away on a leg press, as opposed to power, which is the product of force and velocity, usually measured in watts, for example, the amount of weight that can be pressed away at speed, often repeatedly. Several track and field events demand explosive power, which depends on athletes first developing a solid strength base). The implications of these subsequent findings need to be emphasized to those concerned with antidoping in sport, in that an approximate doubling of the serum total and free concentrations of testosterone from

the baseline values in eugonadal men over a 20-week period caused significant increases in strength and power (see the results reported by Bhasin *et al.* (2001), regarding the weekly regimen of 300 mg testosterone enanthate). Although the serum testosterone was measured 7 days after previous injection, which reflect the lowest values after administration, such androgen exposure is relatively small in the context of the regimens often written about in connection with bodybuilding. Even with respect to athletes, this dose is small compared with the amounts that some athletes may have been administering around 30 years ago, as Wright (1980) comments during that period that 'it is not uncommon for the dose level in national calibre athletes to exceed 1 mg/kg of body weight per day with a rather large number of individuals using two to four times that quantity.' Over a decade later, it is worth noting that Rogol and Yesalis (1992) remark that 'Endurance and sprint athletes use doses closer to those used medically for replacement levels', and the connotation is, therefore, that such athletes by that time recognized that modest drug-induced gains in strength and power may be all that is required to secure an advantage in these type of sports events. The use of smaller doses of anabolic steroids, particularly those formulated for daily administration (such as transdermal applications of testosterone as opposed to i.m. administered testosterone enanthate) would allow rapid elimination of a steroid in anticipation of a drug test. Out-of-competition testing should counter this strategy. Regardless of the above, it should be stressed that due to anabolic steroid administration being covert in athletics for obvious reasons, very little recent information has come to light regarding the doses of anabolic steroids used by elite athletes who choose to cheat.

Adverse effects

The undesirable effects arising from anabolic steroid administration (Table 3) have been extensively reviewed (Haupt and Rovere, 1984; Di Pasquale, 1990; Graham and Kennedy, 1990; Landry and Primos, 1990; Shahidi, 2001; Kicman and Gower, 2003b; James and Kicman, 2004). Typically, anabolic steroids are taken in cycles of about 6–12 weeks (the 'on period') followed by a variable period off the drugs, from 4 weeks to several months (the 'off period') in an attempt to reduce the likelihood of undesirable effects but some bodybuilders will take them almost continuously.

For clinical purposes, the administration of these drugs can be of therapeutic benefit and reasonably safe, with the physician making objective decisions based on the benefit/risk ratio in relation to a patient's condition. By contrast, for the purposes of enhancing performance in sport or for cosmetic purposes, usually because it is a clandestine activity, the athletes and bodybuilders are making subjective decisions regarding the effect these steroids are having on their health. Many probably have an attitude of personal invulnerability because they regard themselves as smart steroid users (Perry *et al.*, 1990), their knowledge being based on reconnaissance of the considerable amount of popular literature (also in electronic form) written by steroid 'gurus', consultation of colleagues who are steroid users in the gym

Table 3 Adverse effects from anabolic steroid administration

Target	Adverse effect	Comment
Bone	Premature closure of the epiphysis in children	Stunting of linear growth
Breast	Atrophy in women Gynaecomastia and enlarged nipples in men	Gynaecomastia can be pronounced and painful; corrective surgery may be necessary. As some anabolics are known to be resistant to aromatization, other mechanisms need to be considered, such altered hepatic function causing an imbalance between androgens and estrogens
Cardiovascular	Increases risk of thrombotic events such as myocardial infarction or stroke (raised LDL, lowered HDL and apolipoprotein-1, raised haematocrit (due to polycythaemia) and lowered plasma fibrinogen Cardiac damage (left ventricular hypertrophy, fibrosis and heart failure) Sudden cardiac death	The link between long-term anabolic steroid use and cardiovascular events remains to be clearly established but the evidence gathered is fairly compelling. This effect is probably underreported Heart disease may be potentiated by concomitant use of growth hormone or insulin (also misused for anabolic purposes)
CNS	Increased libido in men and women, which may be difficult to control Hypomania (less severe form of mania) Heightened irritability Increased aggression and hostility Destructive Impulses Self-destructive impulses Withdrawal symptoms can include severe depression	Psychological effects are unpredictable. Anabolic steroids are implicated in cases of violent behaviour ('roid rage') including, manslaughter and murder Polypharmacy can increase the risk of violent criminality (Klotz <i>et al.</i> , 2007)
Hair	Hirsutism in women (conversion of vellus to terminal hair and male-body pattern of growth) Acceleration of baldness in men; male-pattern baldness in women.	Hirsutism is at very best only partially reversible on cessation of administration.
Liver	Impaired function Hepatic cholestasis (bile canal obstruction) causing jaundice Peliosis hepatitis (blood-filled sacs in the liver) Liver tumours (increased risk)	Hepatotoxicity is associated with 17 α -alkylated steroids (orally active) For the liver function test, it is important to include GGT and CK, as ALT and AST can be raised naturally due to exercise
Reproductive	Suppression of gonadal steroidogenesis Amenorrhoea Clitoral hypertrophy Testicular atrophy Disproportionate growth of the inner prostate Masculanization of female foetus	Recovery of fertility can take from months up to approximately 1 year after cessation of administration, depending on the extent of abuse Growth of the clitoris is irreversible
Skin	Cystic acne	This can be very severe on the chest, back and face
Vocal chords	Lengthened in women	Irreversible deepening of the voice can result in considerable distress
Other	Serious infection associated with injected drugs Toxicity from unlicensed products?	Needle-exchange programmes are helping to address this problem Extent is unknown

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; GGT, γ -glutamyl transpeptidase; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

and their own personal experiences from experimentation. Furthermore, it may be perceived that athletes who fail a test show no obvious signs of ill-health, such as blatant gynaecomastia, severe steroid acne or hirsutism, and this may imply to others that the adverse effects of anabolic steroid use are exaggerated. These athletes could be exercising moderation in the doses they were administering, which should help to keep adverse effects to a minimum (Millar, 1994). Of note, however, is that many of the adverse effects can be difficult to recognize without a thorough medical examination (and patient-doctor confidentiality would have to be maintained) and other damaging effects are insidious where the athletes themselves will be unaware, such as the potential harmful changes to the cardiovascular system. Even so, it is important not to overstate the medical risks associated with anabolic steroid use (Hoffman and Ratamess, 2006) but to emphasize that the hazards to health are dependent on the sex, the dose, the duration of

administration, whether hepatotoxic 17 α -alkylated steroids are being administered and the susceptibility of the individuals themselves to androgen exposure (likely to be dependent on genetic factors, age and lifestyle). The axiom, particularly among bodybuilders who can use excessively large amounts of steroids, that the 'more you take, the more you grow' should be accompanied with 'the more you may damage your health'. It is difficult to gauge the prevalence of severe adverse effects of what is an underground activity, and, moreover, it would be unethical to mimic the large dose regimens in controlled studies over prolonged periods of time to evaluate the risks to health. Notwithstanding, from the records of the doping programme in the former German Democratic Republic, nowhere did the GDR doctors record a damaging effect that was not described in the 'western' literature. These effects included the irreversible effects of virilization (masculanizing effects) in women and female adolescents, and life-threatening liver damage associated

with 17 α -alkylated steroids (oral-turinabol was commonly administered), which sadly led to the death of the hammer thrower, Mr Detlef Gertsenberg, following postoperative complications.

Conflict of interest

The author states no conflict of interest.

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Metabolism and receptor binding of nandrolone and testosterone under in vitro and in vivo conditions

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Abstract. The metabolism and receptor binding of nandrolone (N) and testosterone (T) were studied under in vitro and in vivo conditions. The results of both in vitro incubation studies with $^3\text{H-N}$ and $^3\text{H-T}$ in tissue homogenates from rats and in vivo infusion studies with $^3\text{H-N}$ and $^3\text{H-T}$ in conscious rats show the importance of the enzymes 5α -reductase and $3\alpha/\beta$ -hydroxysteroid-oxidoreductases in the prostate and the importance of the enzyme 17β -hydroxysteroid dehydrogenase in the kidney for the effects of N and T on these tissues. Following infusion of a combined dose of $^3\text{H-N}$ and $^3\text{H-T}$ there is a preferential retention at the receptor of 5α -dihydrotestosterone (DHT) over 5α -dihydronandrolone (DHN), N and T ($\text{DHT} \gg \text{DHN} > \text{N} > \text{T}$) in the prostate because T is a better substrate than N for 5α -reductase and because DHT binds more strongly to the androgen receptor than DHN, N and T. In the kidney 5α -reductase is not important; there is a preferential retention of N in T (DHN and DHT were only present in small amounts) because N is less susceptible than T for metabolic inactivation by the enzyme 17β -hydroxysteroid dehydrogenase and N binds strongly to the androgen receptor. Both in vitro and in vivo studies show that N and T were relatively stable in spleen, thymus and muscular tissue (only shown in vivo) and, as a result, the same amount of N and T was bound to the receptor in these tissues in the in vivo infusion experiment.

In vitro binding studies with the androgen receptor in intact human cells show that 5α -reduction increases the affinity of T and decreases the affinity of N and of the 17α -ethyl derivative of N (3-keto-ethylestrenol).

The results of the present studies explain the relatively strong effect of N, or derivatives of N, compared to that of T on tissues devoid of 5α -reductase activity (e.g. muscular tissue) and they suggest that in particular there may be a strong effect of N on tissues which in addition

have a high 17β -hydroxysteroid dehydrogenase activity (e.g. kidney).

Androgen-sensitive target tissues differ in their responsiveness to testosterone probably because of differences in the tissue content of androgen receptors and the tissue content of enzymes controlling testosterone metabolism (Krieg & Voigt 1977; Deslypere et al. 1980). The major enzymes controlling testosterone metabolism are shown in Fig. 1. It has also been shown that these target tissues may differ in their sensitivity to the effects of nandrolone (N) and testosterone (T) as shown in the Hersherberger test (Overbeek 1966). Characteristic for a high responsiveness to T is a high tissue content of 5α -reductase activity and a relatively low 3α -reductase activity as found in the prostate and other sex accessory glands (Krieg & Voigt 1977), whereas tissues with a low 5α -reductase activity such as muscular tissues and thymus are more responsive to N (Tòth 1981).

An explanation for this dissociation in activity between N and T was offered recently (Bergink et al. 1981; Tòth & Zakàr 1982); 5α -reduction of T increases its affinity whereas 5α -reduction of N decreases its affinity. It has also been suggested that, in comparison to T, N is less easily converted to its 17-keto metabolite by the inactivating enzyme 17β -hydroxy dehydrogenase in the kidney (Bergink et al. 1985) and this effect may account for a 'renotrophic' effect of N (Buchwald et al. 1977).

In the present study we analysed the metabolism

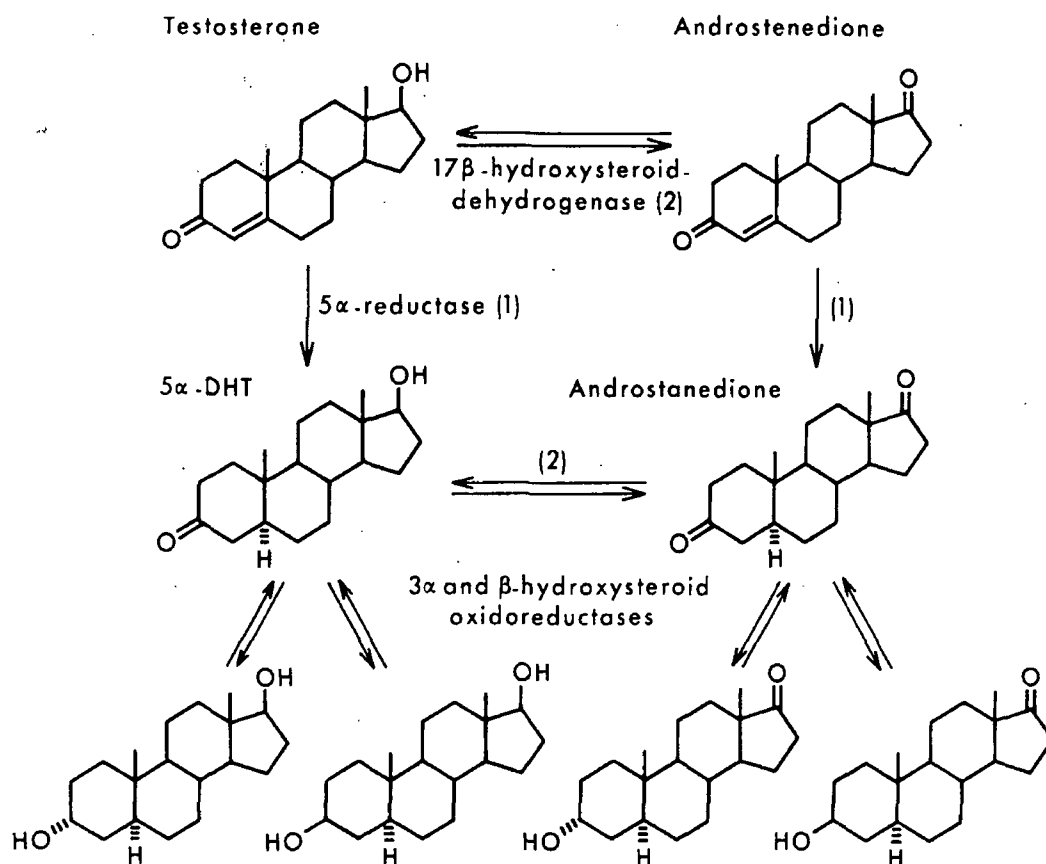


Fig. 1.
Metabolic pathways of testosterone.

and receptor binding properties of N and T in various tissues of the rat under in vitro conditions (only metabolism) and in vivo conditions (metabolism and receptor binding) following a combined infusion of a physiologic dose of ^3H -T and the same dose of ^3H -N in castrated male rats. The receptor binding properties of N, T and their metabolites were also analysed using the androgen receptor in intact human cells.

Materials and Methods

The details of the materials, code names, infusion study, chromatographic procedures and the procedure for receptor binding studies with androgen receptors in intact human cells have been previously described (Bergink et al. 1985).

A) In vitro metabolism studies: tissues were homogenized (1 ml of buffer per 25 mg of tissue) in Tris-HCl buffer (0.25 mol/l Tris-HCl, pH 7.4) and the homogenates were centrifuged for 30 min at 6000 N/kg. The supernatants were diluted 1:1 (v/v) with a solution of cofactors in Tris-HCl buffer (0.25 mol/l Tris-HCl, 1 mmol/l NADPH, 1 mmol/l

NADP, pH 7.4) and incubated for 30 min at 37°C with ^3H -N or ^3H -T at a final concentration of 0.250 $\mu\text{mol/l}$. Following incubation one ml of water was added and the steroids were extracted 3 times with 7 ml ethyl acetate. After evaporation to dryness under nitrogen, the pellets were dissolved in 200 μl acetone for further separation by thin layer chromatography on silica gel.

The steroid solutions (25 μl) were spotted on thin layer sheets and underwent two perpendicular ascents on chloroform-ethyl acetate (4:1 v/v) or ether-hexane (3:1 v/v) in case of ^3H -T and its metabolites or ^3H -N and its metabolites respectively. These chromatographic systems separate T, DHT, androstenedione, androstanedione and androstanediols ($3\alpha + 3\beta$) or N, DHN, estrenedione, estranedione and estrenediols, respectively. Zones corresponding to each steroid were scraped off and the radioactivity was counted.

B) In vivo infusion study: conscious male Wistar rats were infused 24 h after castration via the vena jugularis with a mixture of 5.2 nmoles of ^3H -N and 5.2 nmoles of ^3H -T in 1.2 ml of rat serum in saline (1:1) at a constant rate of 10 $\mu\text{l/min}$ for 2 h. Based on previous studies (Bartsch et al. 1983) this substitution dose was considered physiological. The non-

specific accumulation of $^3\text{H-N}$ and $^3\text{H-T}$ was analysed in a similar experiment in a rat pretreated with 0.25 mg nandrolone propionate and 0.25 mg testosterone propionate 16 h and 1 h before the start of the infusion experiment. After termination of the infusion, the tissues were removed and placed in ice-cold buffer (A) (10 mmol/l sodium molybdate and 0.25 mol/l sucrose, pH 7.4). Tissue homogenates were prepared by homogenization (5 ml of buffer per g of tissue) in buffer A.

The protamine sulphate pellets containing receptor-bound radioactivity were prepared as follows: the homogenates were centrifuged at 1100 000 N/kg for 20 min at 4°C and the supernatant was mixed (1:1 v/v) with a protamine sulphate solution in Tris buffer (2.2 mg/ml protamine sulphate, 10 mmol/l Tris-HCl, pH 7.4). After 10 min at 0°C the solution was centrifuged (10 000 N/kg) and the pellets were extracted three times with 3 ml of ethyl acetate containing 1 µg each of unlabelled T, N, DHT and DHN. Following evaporation to dryness under nitrogen, the pellets were dissolved in 200 µl ethyl acetate for further separation by reversed phase HPLC as previously described (Bergink et al. 1985).

C) Estimation of binding affinities for the androgen receptor in intact human MCF-7 cells. The details of this procedure have been previously described (Bergink et al. 1983). In brief: aliquots (100 µl) of the suspension of MCF-7 cells (10^7 cells/ml) were added to the wells of the microtitration plates and incubated for 45 min at 37°C with $^3\text{H-5}\alpha\text{-DHT}$ either alone or with increasing concentrations of unlabelled competitor. The subsequent freeze-thaw/high salt extraction procedures followed by a dextran-coated charcoal method in low salt were described by Bergink et al. (1983).

Results

Metabolism of nandrolone and testosterone under in vitro conditions

The homogenates from prostate, kidney, spleen and thymus were incubated with either $^3\text{H-N}$ or $^3\text{H-T}$ at a concentration of 0.250 µmol/l (excess substrate) in the presence of excess cofactors (NADPH and NADP) for the converting enzymes 5α -reductase, $3\alpha/\beta$ -hydroxysteroid-oxidoreductases and 17β -hydroxysteroid dehydrogenase. Under these conditions, the conversion of N and T was

Table 1.

Formation of metabolites of N and T under in vitro conditions.

Substrate	Metabolites found (nmol/l)	Prostate	Kidney
$^3\text{H-Testosterone}$			
	5 α -DHT	19	<1
	5 α -Androstanediols	19	<1
	5 α -Androstenedione	<1	2
	Androstenedione	7	63
	Other metabolites	<1	<1
$^3\text{H-Nandrolone}$			
	5 α -DHN	10	<1
	5 α -Estranediols	6	<1
	Estrenedione	3	39
	Other metabolites		

Tissue homogenates were incubated with either 0.250 µmol/l $^3\text{H-T}$ or 0.250 µmol/l $^3\text{H-N}$ for 30 min at 37°C in Tris-buffer containing NADPH and NADP. In thymus and spleen there was no conversion of $^3\text{H-N}$ and $^3\text{H-T}$.

estimated under zero-order reaction kinetics. No conversion of N and T was found in the homogenates from thymus and spleen (Table 1). Substantial amounts of $^3\text{H-N}$ and $^3\text{H-T}$ were converted to 5α -reduced metabolites in the prostate; N was less good a substrate than T for the enzyme 5α -reductase. In the kidney there was a substantial formation of 17-keto-metabolites of N and T, instead of 5α -reduced metabolites. Under these conditions N was as a substrate for the enzyme 17β -hydroxysteroid dehydrogenase less suitable than T.

Metabolism of nandrolone and testosterone during a 2 h infusion of a combination of $^3\text{H-N}$ and $^3\text{H-T}$ in vivo

The radioactivity associated with 5α -reduced and 17-keto-metabolites of N and T in the homogenates of prostate, kidney, thymus, spleen and heart muscle is shown in Table 2. N and T were found to be relatively stable in thymus, spleen, and heart muscle since only small amounts of 5α -reduced and 17-keto-metabolites were detectable. A considerable amount of T and a smaller amount of N were converted to androstenedione and estrenedione respectively in the kidney whereas the 5α -pathway was clearly far more dominant in the prostate. As was found under in vitro conditions,

Table 2.

Concentration of radioactive compounds in the total homogenates from various tissues.

Metabolites found* (fmol/g tissue)	Prostate	Kidney	Thymus	Spleen	Heart muscle
5 α -DHT	13019	151	76	59	—
T	534	1163	2120	955	1888
Androstenedione	350	3210	425	910	180
5 α -DHN	7603	120	—	—	—
N	2216	2413	2572	1379	1753
Estronedione	150	800	210	150	175
Unidentified (mostly polar) metabolites	4840	10449	2080	2525	2278

Compounds were estimated after a continuous infusion for 2 h of 5.2 nmoles of ^3H -N and 5.2 nmoles of ^3H -T in conscious rats 24 h after castration.

* Quantification and detection of androstane diols and estrane diols was not possible since these compounds were not completely extracted with ethyl acetate and not completely separated from other metabolites by HPLC.

the results obtained *in vivo* also show that N was not as good a substrate for the enzymes 5 α -reductase and 17 β -hydroxysteroid dehydrogenase as T.

Receptor binding under in vivo and in vitro conditions at 37°C

A combined dose of tritiated N and T was administered in a continuous infusion for two h to castrated male rats and the concentration of N, T and their metabolites (based on radioactivity measurements) was estimated in the protamine sulphate pellets containing receptor-bound steroids. Highest levels of receptor-bound steroids were found in the prostate (DHT $\gg$ DHN $>$ N $>$ T), lower levels were found in the kidney (N $>$ T $\gg$ DHT $>$ DHN), followed by even lower levels in thymus (N = T), spleen and heart muscle (N = T) as shown in Table 3 and Fig. 2 (results of spleen not shown in Fig. 2).

For the detection of non-specifically bound material an infusion experiment was also performed in an animal pretreated with excess unlabelled nandrolone propionate and testosterone propionate. The levels of radioactive compounds found in the protamine sulphate pellets were less than 10% of those found in the animal treated with

a combined dose of tritiated N and T only (Fig. 2). *In vitro* receptor binding studies were performed with androgen receptors in intact human MCF-7 cells using ^3H -5 α -dihydrotestosterone as radio-

Table 3.

Retention of ^3H -N, ^3H -T, ^3H -DHN and ^3H -DHT in the protamine sulphate pellet (containing receptor-bound steroids).

Tissue	Receptor-bound activity (fmol/g tissue)			
	N	T	DHN	DHT
Prostate	152	40	338	638
Kidney	83	26	6	5
Thymus	35	26	—	—
Spleen	13	10	—	—
Heart muscle	12	12	—	—

Tritiated N, T, DHN and DHT were separated by HPLC (as shown in Fig. 2). Protamine sulphate pellets were obtained after a continuous infusion for 2 h of 5.2 nmoles of ^3H -N and 5.2 nmoles of ^3H -T in conscious rats 24 h after castration.

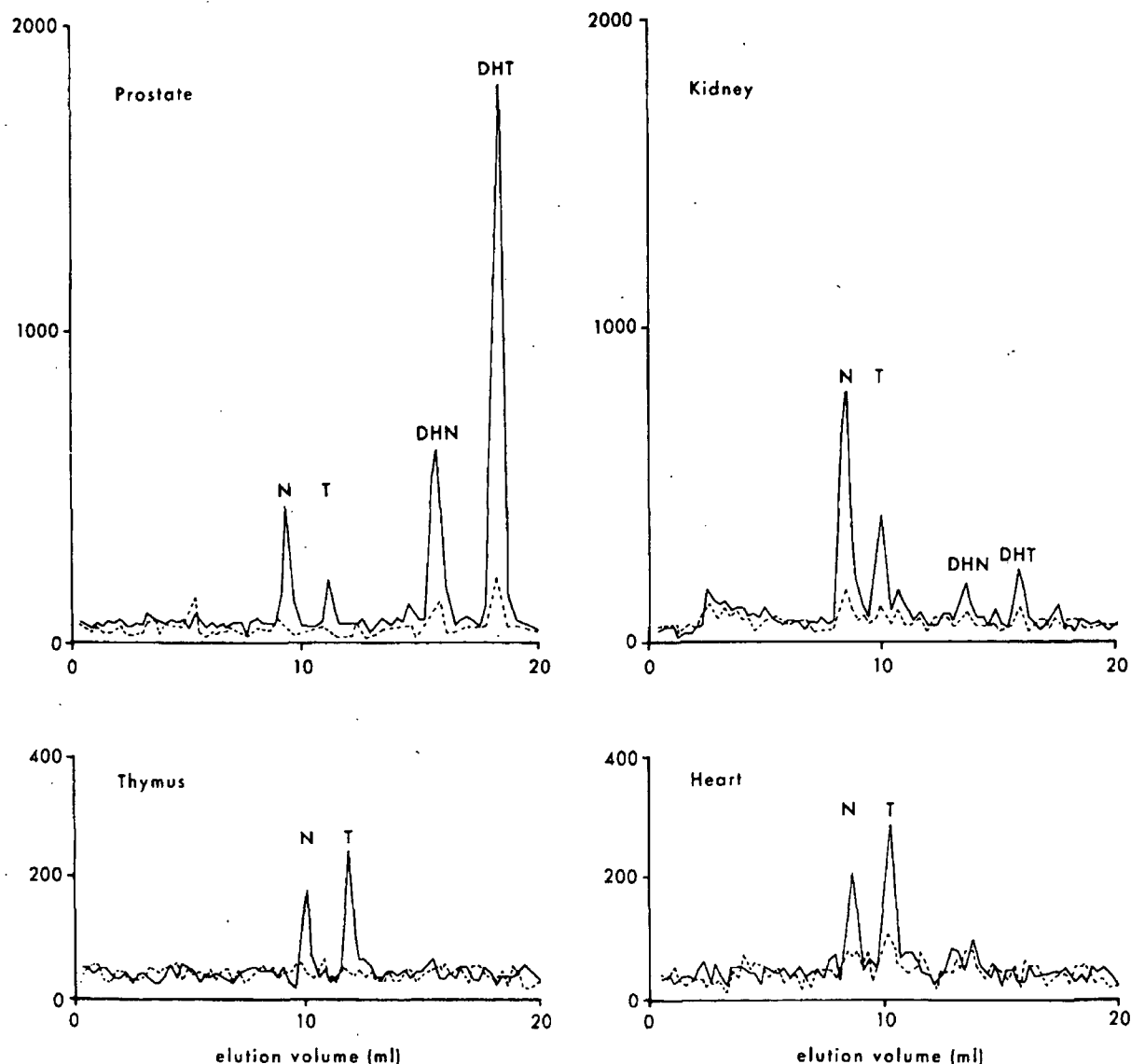


Fig. 2.

Separation by reversed phase HPLC of radioactive compounds present in 100 μ l ethyl acetate extract. Following a 2 h infusion of a combined dose of $^3\text{H-N}$ and $^3\text{H-T}$ the protamine sulphate pellets (which contain receptor-bound radioactivity) from the prostate (A), kidney (B), thymus (C) and heart muscle (D) were extracted with ethyl acetate. After separation fractions of 250 μ l were collected and the radioactivity of each fraction was measured. Straight lines: results obtained from a castrated rat infused with $^3\text{H-N}$ and $^3\text{H-T}$. Dotted line: result from a rat injected with nandrolone propionate and testosterone propionate 16 h and 1 h before the start of the infusion (see Materials and Methods).

active ligand and N, T, DHT, DHN, androstenedione and estrenedione as competitors (Table 4). The ranking in affinity for the human androgen receptor in intact cells at 37°C was as follows: $\text{DHT} = \text{N} > \text{DHN} = \text{T}$. Androstenedione and estrenedione were found to have very low affinity for the androgen receptor. Androgen receptor binding was also analysed for the 17α -ethyl derivative of N, ethylestrenol, its 3-keto-metabolite and the 5α -reduced form of the 3-keto-metabolite. The ranking in affinity at 37°C was: $3\text{-keto-ethylestrenol} > 3\text{-keto-}5\alpha\text{-dihydro-ethylestrenol} \gg \text{ethylestrenol}$.

Discussion

The receptor binding of N and T was studied under in vivo condition ($^3\text{H-N}$ and $^3\text{H-T}$ were simultaneously administered in a continuous infusion) and estimated in the protamine sulphate pellets obtained from various tissues.

The following evidence strongly suggest that the radioactivity recovered from the protamine sulphate pellets is indeed receptor-bound:

A) The levels of radioactive compounds found in the pellets from animals treated with excess unlabelled nandrolone propionate and testoster-

Table 4.
Relative affinities of T, N and ethylestrenol and their metabolites for the androgen receptor in intact human MCF-7 cells at 37°C.

Compound	Relative affinity (5α-DHT = 100%)	
Testosterone	38 ± 6	(6)*
5α-Dihydrotestosterone	100	
Androstenedione	2 ± 0.4	(3)
Nandrolone	92 ± 16	(7)
5α-Dihydronandrolone	50 ± 16	(5)
Estrenedione	2 ± 0.3	(3)
Ethylstrenol**	2 ± 0.5	(3)
3-keto-ethylstrenol	22 ± 5	(3)
3-keto-5α-dihydro-ethylstrenol	14 ± 4	(3)

* Mean ± SEM (number of experiments).
** Ethylestrenol = 17α)-19-norpregn-4-en-17-ol.

one propionate were less than 10% of those found in animals treated with tritiated N and T only (Fig. 2).

B) Only N and T and, depending on the 5α-reductase content of the tissue, also DHN and DHT, but no other metabolites of N and T (which in some cases were abundantly present in the homogenates but do not bind to the receptor) were found in the protamine sulphate pellets. Our present results show that there are specific binding sites for N and T (or DHN and DHT) in the prostate, kidney, thymus, spleen, and heart muscle, suggesting that these tissues are sensitive to the effects of N and T. It is known for a long time that prostate and kidney are androgen target tissues (Selye 1939). More recently it was found that this is also the case for thymus (Roubiniani et al. 1978) and heart muscle (McGill et al. 1980).

The results from our in vitro and in vivo studies also show the importance of both activating and inactivating enzymes as tissue-specific modulators of the biological effects of N and T. In the prostate T is converted for more than 90% to DHT which seems to be a prerequisite for its effect on the prostate (Brooks et al. 1981).

N was found to be not as good a substrate as T for the enzyme 5α-reductase as was also found by Krieg & Voigt (1977). Moreover, the results of present receptor binding studies using human

androgen receptors and the results of previous studies with rat androgen receptors (Liao et al. 1973; Tòth & Zakàr 1982; Bergink et al. 1981) show that 5α-reduction of N to DHN results in metabolic inactivation. Also 5α-reduction of a 17α-ethyl derivative of N, namely 3-keto-ethylstrenol, resulted in inactivation of receptor binding. 5α-Reduced androgens are not always more potent than their corresponding Δ4-compounds.

These results explain why N is much less androgenic than T with respect to its effect on the prostate (Overbeek 1966) and probably also on other tissues with a high 5α-reductase content (e.g. hair follicle cells and pituitary; Lotz 1980). In the kidney, 5α-reductase activity does not play an important role since considerably more T than DHT was bound to the receptor. The results of our present metabolic studies show that the enzyme 17β-hydroxysteroid dehydrogenase plays an important role in the kidney. T is converted to androstenedione which does not bind to the receptor, whereas N is also inactivated to its 17-keto-metabolite estrenedione but to a much lesser extent. In the kidney therefore more N than T was bound to the receptor in the in vivo infusion study.

In thymus, spleen and muscular tissues N and T are relatively metabolically stable; in the in vivo study similar amounts of N (which binds strongly to the androgen receptor) and T were bound to the receptor in these tissues. These results explain why N has a relatively strong effect on muscular tissue as found in the Hersherberger test and probably also on other androgen target tissues devoid of 5α-reductase activity.

In summary, we found the following differences between N and T in metabolism and receptor binding: (1) N is a less suitable substrate than T for the enzyme 5α-reductase. (2) 5α-Reduction of T increases its affinity whereas 5α-reduction of N reduces its affinity. (3) N is less good as substrate than T for the enzyme 17β-hydroxysteroid dehydrogenase which is present in the kidney. These results explain the differences in the biological effects of N and T, e.g. the so called difference in 'androgenic' and 'anabolic' activities.

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Discussion

The discussion following Bergink's paper started with more information with regard to the 5 α -reduction of other anabolic steroids. In the same way as found with nandrolone, derivatives of 19-nortestosterone (nandrolone), especially the alkylated ones, are in general not activated by 5 α -reduction.

The correlation between receptor binding and biological effects of hormones and the competition between nandrolone and testosterone with respect to receptor binding in target organs were questioned. Generally there is a fairly good correlation between receptor binding and biological effects of hormones; when receptor binding data in man are compared with clinical data, there is in most cases a better correlation than that obtained when animal pharmacological data are compared with chemical data.

Furthermore, since the half-life of the receptor complex at 37°C is expected to be less than an hour there is a rapid exchange of receptors, so following nandrolone administration there is a direct equilibrium between nandrolone and testosterone. The potency of testosterone relative to that of nandrolone in the muscles was also questioned. When comparing the results of receptor binding studies with nandrolone and testosterone in the musculus levator ani (MLA) with the effects of the same compounds on the MLA under biological conditions there is a difference. Under biological conditions both compounds stimulate to the same extend, while from receptor binding studies it appears that nandrolone is about twice as potent as testosterone.

CLAIMS

~~Amended claims~~

1. A pharmaceutical composition comprising an anabolic steroid and a vitamin D compound in a carrier liquid suitable for parenteral administration.
2. A pharmaceutical composition according to claim 1, wherein the anabolic steroid is selected from the group consisting of oxymetholone, oxandrolone, nandrolone, and esters thereof, and preferably is nandrolone decanoate.
3. A pharmaceutical composition according to claim 1 or 2, wherein the vitamin D compound is selected from the group consisting of ergocalciferol, cholecalciferol, calcidiol, calcitriol, doxercalciferol, and calcipotriene.
4. A pharmaceutical composition according to any one of the claim 1 to 3, comprising 5 to 600 mg of nandrolone decanoate, and 17.5 µg to 15 mg of cholecalciferol.
5. A pharmaceutical composition according to any one of the claims 1 to 4, wherein the carrier liquid is selected from the group consisting of arachis oil, cottonseed oil, and sesame oil.
6. A pharmaceutical composition according to any one of the claims 1 to 5, for use in the prevention of frailty, particularly as a recovery booster in persons that are in state beyond sarcopenia without having frailty. comprising
✓
7. An injection device for intramuscular administration of a liquid, ~~wherein~~ ^{which} the liquid is a composition according to any one of the claims 1 to 5. a
8. A composition comprising an anabolic steroid and a vitamin D compound for use in the treatment of frailty in an elderly patient, particularly having an age of 60 or older, comprising the concomitant parenteral administration of an effective dose of a combination of an anabolic steroid and a vitamin D compound. for use
✓
9. A composition according to claim 8, wherein the administration is done at least monthly, and preferably weekly.

* for use

10. A composition according to claim 8 or 9, wherein the administration is done for a period of at least 3 months, preferably 6 months.
11. A composition according to any one of the claims 8 to 10, wherein the anabolic steroid is selected from the group consisting of oxymetholone, oxandrolone, nandrolone, and esters thereof, and preferably is nandrolone decanoate.
12. A composition according to any one of the claims 8 to 11, wherein the vitamin D compound is selected from the group consisting of ergocalciferol, cholecalciferol, calcidiol, calcitriol, doxercalciferol, and calcipotriene.
- 10 13. A composition according to any one of the claims 8 to 12, wherein the administration is conducted via parenteral injection of a single formulation comprising both the anabolic steroid and the vitamin D compound, preferably the combination of nandrolone decanoate and cholecalciferol.
14. A composition according to claim 13, wherein the single formulation
- 15 comprises a dosage of from 5 to 600 mg of nandrolone decanoate, and 17.5 µg to 15 mg of cholecalciferol.
15. A composition according to any one of the claims 8 to 14, as a recovery booster in frail elderly people, particularly after hospitalization or surgery.

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