Adverse analytical findings in european anti-doping laboratories

Pedro Manonelles Marqueta[1], Ana García-Felipe[2], Emilio Rubio Calvo[2], Javier Álvarez Medina[1], José Naranjo Orellana[3], Juan Carlos Conte Solano[2], Ana Larma Vela[4], Luis Giménez Salillas[1]

[1]*Departamento de Fisiatría. Universidad de Zaragoza. Zaragoza.*
[2]*Departamento de Microbiología, Medicina Preventiva y Salud Pública. Unidad de Bioestadística. Universidad de Zaragoza. Zaragoza.*
[3]*Departamento de Deporte e Informática. Universidad Pablo de Olavide. Sevilla.*
[4]*Asociación Aragonesa de Medicina del Deporte. Zaragoza.*

Recibido: 08.07.2013
Aceptado: 18.07.2013

Summary

Objective: To study the prohibited substances detected in all the European laboratories according to their number and regional distribution and their possible correlation with the results of European athletes.
Methods: The statistics from the 20 European laboratories accredited by the World Anti-Doping Agency have been studied for the years 2003-2008 and for the 11 groups of substances.
To assess relationships between the 20 laboratories and the substances, we used a multivariate statistical analysis, a method used for the study and prediction of occupational accidents. An analysis of independence (chi-square) and Haberman Corrected Categorized Residuals were performed establishing the dependence between some laboratories and substances. Minkowski distances of power 3 were calculated to establish groups with similar profiles.
Results: The adverse analytical findings in the European laboratories show a very heterogeneus distribution and we found major differences in the substances detected in different laboratories.
Despite the limitations of the study, the main findings were that the detection of anabolic agents and related substances was associated to laboratories from Central and Eastern Europe (Austria, Prague, Cologne, Poland, Russia and Turkey) and the detection of hormones was associated to Mediterranean laboratories (Rome, Barcelona and Madrid).
Conclusions: There is a close relationship between the results of the strength specialties (weightlifting and throwing) athletes and presence of anabolic steroids and related substances in the countries of Middle and East Europe, as well as between the results from the Mediterranean area laboratories and the use of hormones in cycling.
Having more data, such as substances detected by sports, would allow more precise analysis of doping and taking into account the geographical distribution could help to implement more specific strategies to combat doping.

Palabras clave:
Anabolic steroids. Doping. Drug control. Epidemiology. Medical statistician.

Hallazgos analíticos adversos en laboratorios antidopaje europeos

Resumen

Objetivo: Estudiar las sustancias prohibidas detectadas en todos los laboratorios europeos según su distribución regional y numérica, así como su posible correlacion con los resultados de los deportistas europeos.
Métodos: Se han estudiado las estadísticas de los 20 laboratorios europeos acreditados por la Agencia Mundial Anti-Dopaje en los años 2003-2008 para los 11 grupos de sustancias.
Para valorar la relación entre los 20 laboratorios y las sustancias se ha usado un análisis estadístico multivariable, método usado en el estudio y predicción de accidentes laborales. Se realizó un analisis de independencia (Chi2) y de los residuales categorizados corregidos de Haberman para establecer la dependencia entre algunos laboratorios y sustancias. Se calcularon las distancias de Minkowski de potencia 3 para establecer grupos de perfiles similares.
Resultados: Los hallazgos analíticos adversos en los laboratorios europeos muestran una distribucion muy heterogéna y se encuentran importantes diferencias en las sustancias detectadas en los diversos laboratorios.
A pesar de las limitaciones del estudio, los principales hallazgos fueron que la detección de los anabolizantes y sustancias relacionadas se encontró en los laboratorios de la zona europea central y del este de Europa (Austria, Praga, Colonia, Polonia, Rusia y Turquía) y la detección de hormonas se encontró en los laboratorios de paises mediterráneos (Roma, Barcelona y Madrid).
Conclusiones: Hay una intensa relación entre los resultados de los deportistas de especialidades de fuerza (halterofilia y lanzamientos) y la presencia de anabolizantes y sustancias relacionadas, en los paises de la zona central y este de Europa, así como entre los resultados de los laboratorios de la zona mediterránea y el uso de hormans en ciclismo. Tener más datos, como las sustancias detectadas por deporte, permitiría un análisis de dopaje más preciso y tener en cuenta la distribución geográfica podría ayudar a implementer estrategias más específicas para combatir el dopaje.

Key words:
Anabolizantes. Dopaje. Control de dopaje. Epidemiología. Estadística médica.

Correspondencia: Pedro Manonelles Marqueta
E-mail: manonelles@telefonica.net

Introduction

Doping is a big concern for the Sports authorities and governments around the World and, as a result, the World Anti-Doping Agency (WADA) was created to lead the fight against doping through the guidelines of the World Anti-Doping Code[1]. One of the actions is to carry out doping controls through an international network of accredited laboratories with excellent reliability and accuracy of results.

In addition to other actions, such as research, education and close international cooperation[2], the fight against doping requires a clear understanding of aspects such as the different ways to use the prohibited substances and methods or how to circumvent controls. The so called "risk zones" include not only the athletes themselves and the characteristics of sports, but also the geographical areas. The World Anti-Doping Code[1] recommends controls taking into account these characteristics.

Successes in sport seem to be related to the resources allocated to it and the number of practitioners. On the other hand, one might think that the adverse analytical finding (AAF) are related to the results and the number of high-level participants in each sport. So, the prohibited substances detected in a laboratory could be indicators of the kind of sports practiced in a region and they could indicate the number of athletes involved.

Europe has half of the accredited laboratories and a high number of doping controls are performed, but there are few studies relating the AAF with certain sports or the regions where they are practiced[3-12].

The aim of this work was to study the prohibited substances detected in all the European laboratories (number and regional distribution) and their possible correlation with the results of European athletes. These data could help to implement more specific strategies to combat doping.

Material and methods

We have studied the statistics[13,14] from the 20 European laboratories accredited by WADA for the years 2003 to 2008 (Table 1).

The groups "Enhancement of Oxygen Transfer" and "Chemical and Physical Manipulation" are not included in the study because data only are avalaible for 2007.

Table 1. European accredited laboratories. Substances groups and number of adverse analytical finding.

Laboratory	Substances groups											
	Stim	Narc	Cann	Anab	Horm	Beta2	Antio	Mask	Gluco	BetaB	Other	Total
Seibersdorf, Austria	22	0	60	335	12	58	1	38	14	3	0	543
Ghent, Belgium	256	31	358	642	36	218	6	89	174	13	0	1823
Prague, Czech Rep.	34	0	47	435	10	32	7	37	4	4	0	610
Helsinki, Finland	5	0	16	156	6	85	1	10	11	3	0	293
Paris, France	184	15	631	944	57	436	1	153	745	30	10	3206
Cologne, Germany	150	8	111	988	18	143	7	128	120	19	6	1698
Kreischa, Germany	33	8	33	392	7	164	3	33	72	9	1	755
Cambridge, U. Kingdom	11	0	4	19	2	9	0	3	0	0	0	48
London, U. Kingdom	136	4	71	246	11	32	1	21	1	1	1	525
Athens, Greece	41	1	71	240	5	32	2	26	53	2	0	473
Rome, Italy	130	9	154	572	37	178	1	82	101	11	36	1311
Oslo, Norway	36	2	29	267	7	101	3	35	61	3	0	544
Lisbon, Portugal	47	0	93	263	9	43	2	55	99	32	5	648
Moscow, Russia	48	5	47	377	5	7	2	80	2	8	0	581
Barcelona, Spain	24	1	59	266	32	89	10	43	18	3	3	548
Madrid, Spain	69	11	147	503	39	356	1	53	189	6	11	1385
Stockholm, Sweden	28	0	16	447	8	163	2	19	122	0	0	805
Lausanne, Switzerland	39	2	67	422	68	84	7	28	56	5	6	784
Ankara, Turkey	28	0	20	115	1	8	0	34	1	0	0	207
Warsaw, Poland	28	3	32	364	3	4	4	20	6	2	0	466
Total	1349	100	2066	7993	373	2242	61	987	1849	154	79	17253

Stim: Stimulants; Narc: Narcotics; Cann: Cannabinoids; Anab: Anabolic agents; Horm: Peptide hormones; Beta2: Beta-2-Agonists; Antio: Agents with antioestrogenic activity; Mask: Masking Agents / Diuretics; Gluco: Glucocorticosteroids; BetaB: Beta-Blockers; Othe: Others.

Statistics

We used a multivariate statistical análisis, a method used for the study and prediction of occupational accidents[15,16].

To assess relationships between the 20 laboratories and the substances found, we used the information showed in Table 1, with data grouped into a contingency table 20x11.

According to a previous study[17], an analysis of independence (chi square) and Haberman Corrected Categorized Residuals[18,19] were performed establishing the dependence between some laboratories and substances.

With this information, a correspondence analysis[20,21] was performed to show graphically the relationships between the different forms of the two variables. Data are represented by points, whose proximity means greater affinity. Since the analysis was performed with three dimensions, graphs are presented for dimension 1 with 2 and 1 with 3 to get a fuller picture. Other two statistic techniques have been used from the factorial punctuation: cluster analysis, to establish groups with similar profiles[22,23] and Minkowski distances[22] of power 3.

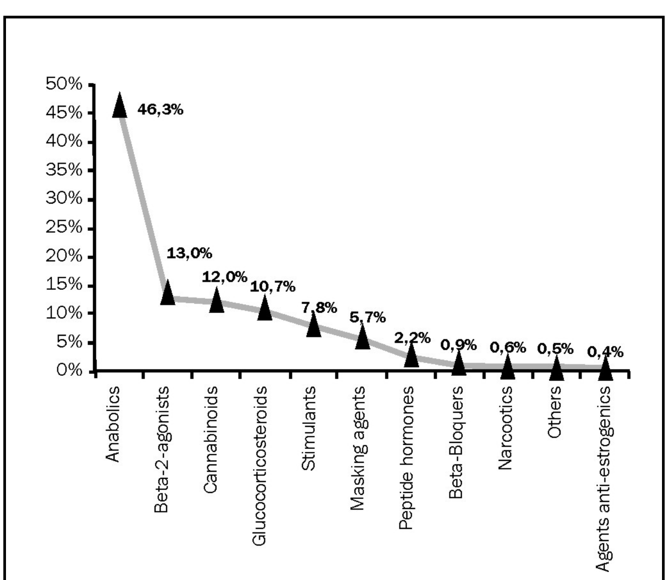
All data were processed with software SPSS 15.0.

Results

A descriptive statistical analysis shows an average pattern of substances detected in laboratories (Figure 1), highlighting anabolic steroids with 46%. However, this pattern varies significantly from one laboratory to another (Figure 1).

The chi square value (3877,995) was significant ($p<0,0001$) as it rejects the hypothesis of independence. So, an association exists between the two variables (laboratories and substances).

Figure 1. Descriptive statistical analysis showing the average pattern of substances detected in laboratories. Anabolic steroids are the most frequent finding (46,3%).



The cumulative proportion of inertia in the three-dimensional model was 0.786.

Figures 2 and 3 show the laboratories and substances in the three dimensions, with the four groups established by the cluster analysis and Table 2 shows the Minkowski distances, which provides support for the analysis of the relationships: the lower value for Minkowski distance, the hugher association.

According to these criteria, an association of anabolic agents can be observed with Austria (0,099), Cologne (0,170), Czech Republic (0,484) and Poland (0,644). A bit more distant were Turkey (0,800) and Russia (0,691). In the adjacent group Barcelona (0,495), Switzerland (0,419) and Norway (0,453) were the closest.

Concerning the antiestrogenic agents, the laboratories with higher associations were Czech Republic (0,436), Poland (0,612) and Austria (0,533), although only one case was detected.

The laboratories more associated to masking agents were Cologne (0,308) Greece (0,252) and Turkey (0,531). In Figure 2 Cambridge seems to be close to this substance; however, with a different projection (Figure 3) it is not, and this is consistent with the Minkowski distance (1,694).

Stimulants were associated to London (0,395), Belgium (0,5) and Cambridge (0,761). In the same group, narcotics were close to Belgium (0,556) and Cambrige (0,678).

In the adjacent group, beta-blockers were associated with Portugal (0,359), glucocorticosteroids with France (0,343) and cannabinoids with Belgium (0,493), although Belgium was included in the previous group.

Figure 2. Three dimensions analysis. This graph shows dimensions 1 with 2. Different laboratories and substances groups are represented by points, whose proximity means greater affinity.

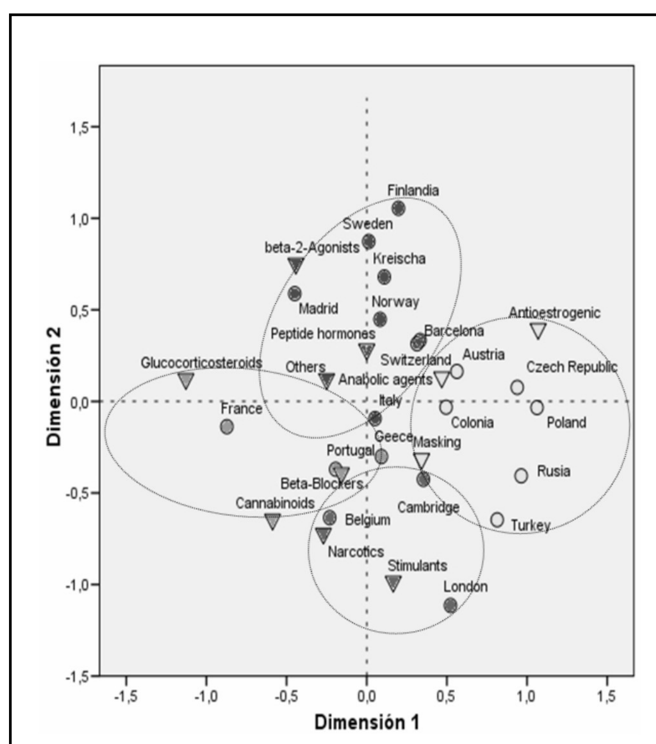
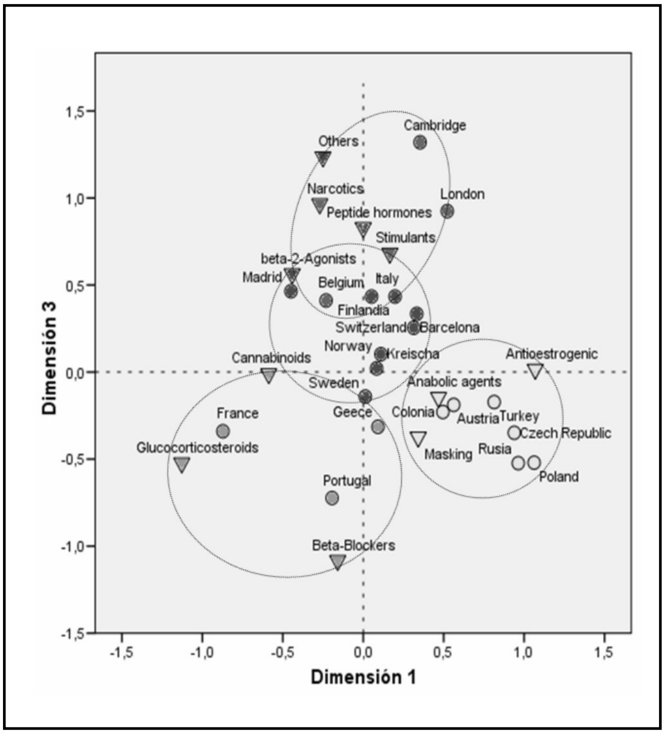


Figure 3. Three dimensions analysis. This graph shows dimensions 1 with 3. Different laboratories and substances groups are represented by points, whose proximity means greater affinity.



In the last group, beta-2-agonists were close to Madrid (0,176) and a bit more distant were Finland (0,662), Kreischa (0,643), Norway (0,693) and Sweden (0,764). In Figure 3 Belgium seems to be associated to this substance but in Figure 2 it is very far, what is consistent with the Minkowski distance (1,390).

Peptide hormones were associated to Italy (0,488), Barcelona (0,541), Madrid (0,551) and Suiitzerland (0,606), while Norway and Kreischa were more distant, as shown in Figure 3.

Finally, substances classified as "Others" only were related to Italy, but not very close (0,821).

Table 3 shows the laboratories with the most detected substances and Table 4 allows for the preferential association of substances with laboratories.

Discussion

Few works analyze the results of doping by geographical locations[3-7] and some of them refer to controls performed in Olympic Games and other international competitions[8-12]. In this case, although the work is done in a geographical zone, the results include different countries.

There are works analyzing some sports[24,25] or the drug declaration made by the athletes themselves during the sample collecting process[26,27] or trough a specific survey[28].

An overall analysis of analytical finding shows an average pattern of detected substances in the accredited laboratories (Figure 1). Theoretically, this pattern should be repeated roughly in all laboratories. However, the profiles observed are very different.

All accredited laboratories follow identical analytical procedures laid down by WADA[29,30], so associations between laboratories and substances can be assessed by Minkowski distances (Table 3).

Stimulants

The laboratories linked to these substances were London (0,395), Gante (0,500) and Cambrigde (0,761).

Stimulants are used for various reasons including medical treatment, weight loss and improvement of physical performance[31-33]. In addition, this group comprises substances as different as meaning ephedrine, amphetamines or cocaine but laboratories only provide data for the entire group and not for each substance.

So, we can not make any hypothesis about the results.

Narcotics

These drugs do not improve athletic performance[33] but they are used as analgesics to continue training, so they are detected mainly in controls out of competition. In any case, it is possible that some athletes are addicted to morphine.

The laboratories more correlated to this Group are Gante (0,556), Cambrigde (0,678) and Rome (0,755). It is important to note that there has been only 100 cases of narcotics over this period.

Cannabinoids

Cannabinoids have shown no ergogenic effect and could even be detrimental to athletic performance[34]. However, they are the third most frequently detected substance with 2066 AAF. Most of the samples show concentrations compatible with consumption in the previous days (late elimination period)[35].

Cannabis can be used for relaxation before competition[35] by the proven effect it has on the reduction of anxiety[36].

Laboratories associated with this substance are Gante (0,493) and París (0,579) with 358 and 631 AAF respectively.

The use of these substances could be associated with social habits[36] and the night lifestyle in Mediterranean countries.

The analysis of Football (Soccer) should support this hypothesis. In the 166 AAF from 20750 controls performed by the International Football Federation (FIFA)[37] there was 85 cannabinoids. 122 of these AAF (73,49%) were made in Europe (UEFA) corresponding to the following countries: France (30), Italy (21), Portugal (21), Belgium (20), Netherlands (8), Norway (10), Turkey (5), Spain (2), Malta (2), Croacia (1), England (1) and Greece (1).

As one might anticipate, these detections were made in the laboratories of Paris (30 from France), Gante (20 from Belgium and 8 from Netherlands), Rome (21 from Italy and 1 from Malta) and Lisbon (21 from Portugal).

This indicates a significant use of cannabis and cocaine in European Football players which is mainly associated with the laboratories with higher correlation (Paris and Gante) and with those whith a higher number of detections (Lisbon and Rome).

Table 2. Minkowski distances between detected sustances and laboratories.

Laboratories	Minkowski distances										
	Stimu	Narco	Cann	Anab	Hormo	Beta2	Antio	Mask	Gluco	BetaB	Other
Seibersdorf, Austria	1,308	1,410	1,272	0,099	1,073	1,180	0,533	0,503	1,694	1,081	1,507
Ghent, Belgium	0,500	0,556	0,493	0,987	0,952	1,390	1,497	0,890	1,253	1,496	0,998
Prague, Czech Rep.	1,403	1,657	1,584	0,484	1,353	1,547	0,436	0,649	2,067	1,221	1,782
Helsinki, Finland	2,040	1,801	1,763	1,000	0,807	0,662	1,007	1,458	1,589	1,873	1,123
Paris, France	1,409	1,383	0,574	1,345	1,328	1,151	1,959	1,216	0,343	0,925	1,610
Cologne, Germany	1,184	1,354	1,148	0,170	1,103	1,218	0,656	0,308	1,627	0,982	1,527
Kreischa, Germany	1,688	1,511	1,387	0,610	0,765	0,643	0,968	1,034	1,322	1,430	1,187
Cambridge, U. Kingdom	0,761	0,678	1,473	1,492	0,803	1,370	1,465	1,694	2,130	2,411	0,727
London, U. Kingdom	0,395	0,823	1,318	1,467	1,422	1,953	1,634	1,394	2,111	2,063	1,335
Athens, Greece	1,095	1,304	0,725	0,517	1,193	1,260	1,094	0,252	1,238	0,778	1,566
Rome, Italy	0,898	0,755	0,803	0,653	0,488	0,901	1,075	0,826	1,362	1,522	0,821
Oslo, Norway	1,479	1,355	1,172	0,453	0,812	0,693	0,986	0,806	1,254	1,250	1,231
Lisbon, Portugal	1,452	1,695	0,764	0,847	1,593	1,528	1,421	0,582	0,980	0,359	1,970
Moscow, Russia	1,349	1,733	1,571	0,691	1,546	1,777	0,876	0,624	2,102	1,166	1,946
Barcelona, Spain	1,328	1,180	1,207	0,495	0,541	0,819	0,757	0,856	1,553	1,496	0,980
Madrid, Spain	1,605	1,334	1,256	1,029	0,551	0,176	1,532	1,221	1,111	1,672	0,829
Stockholm, Sweden	1,911	1,758	1,549	0,795	1,038	0,764	1,090	1,199	1,252	1,421	1,451
Lausanne, Switzerland	1,315	1,186	1,178	0,419	0,606	0,818	0,763	0,792	1,514	1,418	1,043
Ankara, Turkey	0,979	1,401	1,403	0,800	1,330	1,724	1,050	0,531	1,985	1,191	1,645
Warsaw, Poland	1,493	1,814	1,693	0,644	1,545	1,728	0,615	0,736	2,190	1,268	1,973

Stimu: Stimulants; Narc: Narcotics; Cann: Cannabinoids; Anab: Anabolic agents; Horm: Peptide hormones; Beta2: Beta-2-Agonists; Antio: Agents with antioestrogenic activity; Mask: Masking Agents / Diuretics; Gluco: Glucocorticosteroids; BetaB: Beta-Blockers; Othe: Others.

Anabolic agents

These substances are widely used in sport and they constitute the greatest number of AAF in all reports of WADA[13,14].

Possibly, anabolics are the best known substances to improve performance[33,38]. Their main effect is the increase in muscle mass and strength, so they are used in strength and power sports. Given that this effect is dose-dependent, the use of supra-physiological doses is common[39] and, furthermore, several agents are often used in combination along weekly cycles[40,41]. These reasons justify their high detection in sport.

Anabolic agents have been widely used throughout the History: The Soviet weightlifters in the 50s[38,42], American athletes in the preparation of the Mexico Olympic Games in 1968[38,42], American weightlifters[43] or High School Football players[44]. But perhaps, the case with most media coverage was the detection of Stanozolol in the Canadian athlete Ben Johnson in the Olympic Games of Seoul in 1988[45] or the use of tetrahydrogestrinone by several American hight level athletes[45].

The laboratories more associated with these substances were Austria (0,099), Cologne (0,170), Switzerland (0,419), Norway (0,453), Prague (0,484) and Barcelona (0,495). The higher numbers of detections were in Cologne (988), Paris (944), Gante (642), Rome (572), Madrid (503) and Prague (435).

In the spatial analysis showed in Figures 2 and 3, a very close relationship is observed between anabolic, antiestrogenics and mask agents and the laboratories of Austria, Prague, Cologne, Poland, Russia and Turkey.

This association is logical. In fact, anabolics are used in combination with antiestrogenics to stimulate the secretion of testosterone, enhancing the anabolic effect, and to counteract some side effects such as gynecomastia. On the other hand, mask agents are used to hide the presence of anabolics.

In view of this regional association, we studied some sports where strength is critical and could be benefited with the use of anabolics.

Table 5 shows the ten first positions of the European Senior Weightlifing Championships Ranking 2009[46].

Predictably, the laboratories of Austria, Czech Republic, Cologne, Poland, Russia and Turkey have done most of the analysis for the athletes from Eastern Europe, so the analysis of the countries bold-marked in Table 5 should have been carried out in these laboratories.

Table 3. Substances with lower Minkowski distances detected in every laboratory.

Laboratory and country	1st substance (Minkowski distance)	2nd substance (Minkowski distance)	3th substance (Minkowski distance)
Seibersdorf, Austria	Anabolics (0,099)	Masking/Diuretics (0,503)	Antioestrogenics (0,533)
Ghent, Belgium	Cannabinoids (0,493)	Stimulants (0,500)	Narcotics (0,556)
Prague, Czech Republic	Antioestrogenics (0,436)	Anabolics (0,484)	Masking/Diuretics (0,649)
Helsinki, Finland	Beta-2-Agonists (0,662)	Hormones (0,807)	Anabolics (1,000)
Paris, France	Glucocorticosteroids (0,343)	Cannabinoids (0,579)	Beta-2-Agonists (0,925)
Cologne, Germany	Anabolics (0,170)	Masking/Diuretics (0,308)	Antioestrogenics (0,656)
Kreischa, Germany	Anabolics (0,610)	Beta-2-Agonists (0,643)	Hormones (0,765)
Cambridge, United Kingdom	Narcotics (0,678)	Others (0,727)	Stimulants (0,761)
London, United Kingdom	Stimulants (0,395)	Narcotics (0,823)	
Athens, Greece	Masking/Diuretics (0,252)	Anabolics (0,517)	Cannabinoids (0,725)
Rome, Italy	Hormones (0,488)	Anabolics (0,653)	Narcotics (0,755)
Oslo, Norway	Anabolics (0,453)	Beta-2-Agonists (0,693)	Masking/Diuretics (0,806)
Lisbon, Portugal	Beta-Blockers (0,359)	Masking/Diuretics (0,582)	Cannabinoids (0,764)
Moscow, Russia	Masking/Diuretics (0,624)	Anabolics (0,691)	Antioestrogenics (0,876)
Barcelona, Spain	Anabolics (0,495)	Hormones (0,541)	Antioestrogenics (0,757)
Madrid, Spain	Beta-2-Agonists (0,176)	Hormones (0,551)	Others (0,829)
Stockholm, Sweden	Beta-2-Agonists (0,764)	Anabolics (0,795)	
Lausanne, Switzerland	Anabolics (0,419)	Hormones (0,606)	Antioestrogenics (0,763)
Ankara, Turkey	Masking/Diuretics (0,531)	Anabolics (0,800)	Stimulants (0,979)
Warsaw, Poland	Antioestrogenics (0,615)	Anabolics (0,644)	Masking/Diuretics 0,736)

Anabolics: Anabolic agents; Hormones: Peptide hormones; Antioestrogenics: Agents with antioestrogenic activity; Masking/Diuretics: Masking Agents / Diuretics.

From the athletes in the 10 first positions of the ranking, 66 males (82,5%) and 52 females (77,6%) come from the mentioned countries and we could assume thet they have been tested in laboratories highly associated to anabolic agents.

Table 6 shows the 30 first positions in the Top List 2009 Outdoor Senior Men in Athletics for Shot Put, Discus, Hammer and Javelin Throw[47].

If we exclude non-European countries and consider the same laboratories mentioned above, the controls of marked countries in

Table 4. Minkowski distances by laboratories.

	1st Laboratory (Minkowski distance)	2nd Laboratory (Minkowski distance)	3th Laboratory (Minkowski distance)
Stimulants	London (0,395)	Ghent (0,500)	Cambridge (0,761)
Narcotics	Ghent (0,556)	Cambridge (0,678)	Rome (0,755)
Cannabinoids	Ghent (0,493)	Paris (0,574)	Athens (0,725)
Anabolics	Seibersdorf (0,099)	Cologne (0,170)	Lausanne (0,419) Oslo (0,453) Prague (0,484) Barcelona (0,495)
Hormones	Rome (0,488)	Barcelona (0,541)	Madrid (0,551)
Beta-2-Agonists	Madrid (0,176)	Kreischa (0,643)	Helsinki (0,662)
Antioestrogenics	Prague (0,436)	Seibersdorf (0,533)	Warsaw (0,615)
Masking/Diuretics	Athens (0,252)	Cologne (0,308)	Seibersdorf (0,503)
Glucocorticosteroids	Paris (0,343)	Lisbon (0,980)	
Beta-Blockers	Lisbon (0,359)	Athens (0,778)	Paris (0,925)
Others	Cambridge (0,727)	Rome (0,821)	Madrid (0,829)

Table 6 should have been carried out in these laboratories. This is the situation for 13 Shot Put athletes (81,2% of 16 Europeans), 12 Discus throwers (70,5% of 17), 20 Hammer throwers (76,9% of 26) and 15 Javelin throwers (68,1% of 22).

Of course, it is not our intention at all to say that these results are due to doping!

What we want emphasize is that the association observed between anabolic agents and the laboratories of the Eastern European zone is related to the results that athletes from this area obtained in sports where strength is important.

Hormones

Erythropoietin (EPO) is used in endurance Sports to increase the availability of oxygen. The first suspicion about the use of EPO in sport was in the late 1980s when several cyclists died just after the appearance of this substance[48].

EPO has led to several international scandals such as the withdrawal of six Chinese female athletes in Sydney 2000, the "Operation Puerto" in Spain in 2006, with at least 50 professional cyclists involved (23 were disqualified)[45,49] or the operation of the Italian Police in Mantua, in April 2005, with more than 50 spotsmen, coaches and physicians involved.

These data show that Cycling is one of the Sports in which EPO is used more.

If we observe the world ranking of the Union Cycliste Internationale (UCI) on 22 March 2010[50] (Table 7), 33 of the 40 top positions (82,5%) correspond to European cyclists; 10 of them are Italian and 6 are Spanish.

The laboratories with a higher correlation (Minkowski distance) with these substances were Rome (0,488), Barcelona (0,541) and Madrid (0,551) with 37, 32 and 39 AAF respectively.

Although the group "Hormones" is heterogeneous, EPO and similar substances are the most common. So that, it appears to exist again a connection between the use of a substance and the laboratories of a geographical area.

Beta-2-Agonists

The prevalence of asthma and the consumption of bronchodilators in athletes have been widely studied[51-59]. It is known that this prevalence is higher in sportsmen than in general population[51,53,57] and it is higher in endurance sports[52,53]. Even prevalence ranges have been reported in summer (3,7-22,8%) and winter sports (2,8-54,8%)[51].

Table 5. European senior weightlifing championships ranking. 2009.

Position	Men							
	56 Kg	62 Kg	69 Kg	77 Kg	85 kg	94 Kg	105 Kg	>105 Kg
1	BEL	**TUR**	**ARM**	**BLR**	**RUS**	**GER**	**RUS**	**UKR**
2	ITA	**AZE**	FRA	**ALB**	**AZE**	GRE	**UKR**	**GER**
3	**MDA**	CYP	**RUS**	**RUS**	**BLR**	**RUS**	**LTU**	**RUS**
4	**TUR**	**TUR**	**AZE**	**AZE**	FRA	**AZE**	**RUS**	**POL**
5	**MDA**	**MDA**	**ROM**	**POL**	**POL**	**UKR**	**POL**	**HUN**
6	**BUL**	**BUL**	**TUR**	**ROM**	**ALB**	**LTU**	**GEO**	**CZE**
7	**TUR**	**POL**	**TUR**	**UKR**	FRA	**CZE**	**ARM**	**CZE**
8	**ROM**	ESP	**AZE**	**SVK**	**ARM**	NOR	**LTU**	FIN
9	**ROM**	FIN	**UKR**	**ROM**	**ARM**	**ALB**	**AZE**	**SVK**
10	FRA	**CZE**	ESP	FIN	**UKR**	**HUN**	**TUR**	**CRO**
Position	**Women**							
	48 Kg	**53 Kg**	**58 Kg**	**63 Kg**	**69 kg**	**75 Kg**	**> 75 Kg**	
1	**TUR**	**UKR**	**BLR**	**TUR**	**RUS**	**RUS**	**RUS**	
2	**MDA**	**TUR**	**UKR**	NOR	**ARM**	**ARM**	**RUS**	
3	**POL**	**TUR**	**ALB**	**RUS**	**RUS**	ESP	**UKR**	
4	ITA	**POL**	**ROU**	**ARM**	**UKR**	**UKR**	**TUR**	
5	FRA	**BUL**	**UKR**	**POL**	**POL**	**BLR**	**GER**	
6	**BUL**	ESP	FRA	**CZE**	**UKR**	**BUL**	**TUR**	
7	**ROU**	FRA	**BLR**	**POL**	**POL**	**TUR**	GRE	
8		**POL**	GRE	FRA	**UKR**	**BUL**	**POL**	
9		**RUS**	GRE	**BLR**	**GER**	ESP	ITA	
10		**ROU**	FIN	**ISR**	**GER**	SWE	**ROU**	

ALB: Albania; ARM: Armenia; AZE: Azerbaijan; BEL: Belgium; BLR: Belarus; BUL: Bulgaria; CRO: Croatia; CYP: Cyprus; CZE: Czech Republic; ESP: Spain; FIN: Finland; FRA: France; GEO: Georgia; GER: Germany; GRE: Greece; HUN: Hungary; ISR: Israel; ITA: Italy; LTU: Lithuania; MDA: Moldova; NOR: Norway; POL: Poland; ROM: Romania; RUS: Russia; SVK: Slovakia; SWE: Sweden; TUR: Turkey; UKR: Ukraine.

The prevalence of asthma in Cycling is between 30 and 45%[54,55] and the requests for Therapeutic Use Exemption (TUE) show a higher use of Beta-2-Agonists in Cycling[60] and swimming[61]. Between 2003 and 2008 the International Olimpic Committee received 3174 TUE applications, 868 of which (27,3%) were for β2-agonists and 690 (79,5%) were approved[62].

The laboratory more closely associated to this substance was Madrid (0,176) with 356 AAF. París, Gante and Roma had 436, 218 and 178 AAF, but with lower Minkowski distances.

Agents with antioestrogenic activity

These agents are used to stimulate the secretion of gonadotropins and testosterone in males[63,64] and to counteract some side effects such as gynecomastia or the suppression of endogenous testosterone[65].

The laboratories related to these substances were Prague (0,436), Seibersdorf (0,533) and Warsaw (0,615), but there were only 68 detections.

Masking/diuretics

The laboratories more closely associated to these substances Athens (0,252), Cologne (0,308) and Seibersdorf (0,503). They are used to hide or mask other drugs[66].

Glucocorticosteroids

Glucocorticosteroids are widely used in sport[67] to treat soft tissues injuries, asthma or other allergic processes and systemic diseases, but it is not excluded to be used to improve performance in combination with other substances.

Table 6. Athletics. Top List 2009 outdoor senior men.

Position	Shot put	Discus throw	Hammer throw	Javelin throw
1	USA	**EST**	**SLO**	**NOR**
2	**POL**	**LTU**	**HUN**	**LAT**
3	USA	**GER**	**BLR**	**FIN**
4	USA	**POL**	**LAT**	**FIN**
5	**GER**	USA	**RUS**	CUB
6	KSA	**HUN**	**ITA**	**FIN**
7	USA	**ESP**	KUW	**FIN**
8	JAM	**ESP**	**UKR**	**CZE**
9	**BLR**	EGY	**ITA**	**POL**
10	USA	IRI	**BLR**	**RUS**
11	**BLR**	**EST**	**GER**	USA
12	CAN	USA	**POL**	**GER**
13	**RUS**	**NED**	**TJK**	**POL**
14	**LAT**	**RUS**	**CZE**	**GER**
15	**RUS**	USA	**SVK**	**RUS**
16	USA	**SWE**	**FIN**	USA
17	**POR**	IRI	JPN	KOR
18	**SLO**	**EST**	**RUS**	JPN
19	**GER**	AUS	**GER**	USA
20	**SRB**	**GER**	**CRO**	**FIN**
21	AUS	CUB	**BLR**	**LAT**
22	USA	**CZE**	**TUR**	**RUS**
23	**GER**	USA	USA	USA
24	**SRB**	IRI	**FRA**	**EST**
25	CHN	USA	**BLR**	**UKR**
26	CUB	**FIN**	**UKR**	**EST**
27	**ESP**	**AUT**	**GRE**	**CZE**
28	AUS	CUB	EGY	**LAT**
29	**GBR**	**GER**	**FRA**	CHN
30	**CRO**	CUB	**GER**	**FIN**

AUS: Australia; AUT: Austria; BLR: Belarus; CAN: Canada; CHN: People's Republic of China; CRO: Croatia; CUB: Cuba; CZE: Czech Republic; EGY: Egypt; ESP: Spain; EST: Estonia; FIN: Finland; FRA: France; GBR: United Kingdom of Great Britain & Northern Ireland; GER: Germany; GRE: Greece; HUN: Hungary; IRI: Iran; ITA: Italy; JAM: Jamaica; JPN: Japan; KSA: Saudi Arábia; KUW: Kuwait; LAT: Latvia; LTU: Lithuania; NED: The Netherlands; NOR: Norway; POL: Poland; POR: Portugal; RUS: Russia; SLO: Slovenia; SRB: Serbia; SVK: Slovakia; SWE: Sweden; TJK: Tadjikistan; TUR: Turkey; UKR: Ukraine; USA: United Status.

The declared use in UCI in 2005 was higher than 35% and this forced to revise the TUE applications for corticosteroids by its high consumption[60]. Glucocorticosteroids are also widely used in swimmers, perhaps because of the high prevalence of bronchial hyperreactivity[61].

The laboratory with a higher correlation with these substances was Paris (0,343) with 745 AAF. This correlation may be due to this laboratory has reported all tests with these substances, even those with a very low level[68]. Nevertheless, we can not rule out that this finding may be related to other situations such as its administration by local infiltration or the wide use of inhaled corticosteroids by cyclists (a sport of great tradition in France).

Table 7. Union cycliste internationale (UCI). World ranking – 2010 (22 Mar 2010). Men.

Position	Country	Position	Country
1	**ESP**	21	**NOR**
2	**GER**	22	AUS
3	AUS	23	**ESP**
4	**ITA**	24	**ITA**
5	**ESP**	25	**RUS**
6	**ESP**	26	**ITA**
7	**ESP**	27	**BEL**
8	**BEL**	28	**ITA**
9	**ITA**	29	**EST**
10	NZL	30	**SVK**
11	**KAZ**	31	RSA
12	**ITA**	32	**BEL**
13	AUS	33	**ITA**
14	**ITA**	34	**FRA**
15	**CZE**	35	**POR**
16	**ITA**	36	**ITA**
17	AUS	37	**GER**
18	**ESP**	38	**FRA**
19	**NED**	39	**NED**
20	**GER**	40	AUS

Beta-Blockers

These substances are prohibited only in some Sports which require precision or driving. They are used to decrease heart rate and blood pressure, to decrease anxiety and, mainly, to decrease the muscle tremor, which improves performance in precision sports[32,69].

The laboratory more closely associated to these substances was Lisbon (0,359). We can not explain this association according to the results of Portugal in archery, modern pentathlon, motor-sports or any other precision sport.

Others

The heterogeneity of the Group and the few AAF do not allow any analysis.

Study limitations

This study has several limitations because incomplete information is available about sports analyzed by each laboratory. It is possible that one laboratory can analyze samples from another geographic area. In addition, there are groups with different substances and different effects for dopping.

In adition, adverse analytical findings are not separated from the true positive cases in the information provided by WADA.

On the other hand, the existence of a regional preference for the practice of some sports would change detections in the studied areas.

Some factors to be taken with caution are: the low number of findings with antiestrogens, the low number of findings in Cambridge or the low discrimination power of the model for the laboratories in Italy and Switzerland.

Nevertheless, this work may be a starting point to identify risk areas in relation to the consumption of certain substances.

Conclusions

- The AAF in the European laboratories show a very heterogeneus distribution.
- Detection of anabolic agents and related substances are associated to laboratories from Central and Eastern Europe (Austria, Prague, Cologne, Poland, Russia and Turkey).
- Detection of hormones is associated to Mediterranean laboratories (Rome, Barcelona and Madrid).
- Having more data, such as substances detected by sports, would allow more precise analysis of doping.

Competing interests

Authors do not have any competing interests.

References

1. World Anti-Doping Agency. *World Anti-Doping Code.* Montreal: World Anti-Doping Agency, 2009.
2. Mottram DR. Banned drugs in sport. Does the International Olympic Committee (IOC) list need updating? *Sports Med.* 1999;27:1-10.
3. Bahr R, Thornton M. Prevalence of doping in sports: doping control in Norway, 1977-1995. *Clin J Sport Med.* 1998;8:32-7.
4. Epstein S, Eliakim A. Drug testing in elite athletes. The Israeli perspective. *Isr Med Assoc J.* 1999;1:79-82.
5. Alaranta A, Alaranta H, Holmila J, Palmu P, Pietilä K, Helenius I. Self-reported attitudes of elite athletes towards doping: differences between type of sport. *Int J Sports Med.* 2006;27:842-6.
6. Scarpino V, Arrigo A, Benzi G, Garattini S, La Vecchia C, Bernardi LR, Silvestrini G, Tuccimei G. Evaluation of prevalence of "doping" among Italian athletes. *Lancet.* 1990;336:1048-50.
7. Van der Merwe PJ, Kruger HS. Drugs in sport. Results of the past 6 years of dope testing in South Africa. *S Afr Med J.* 1992;82 151-3.
8. Catlin DH, Kammerer AC, Hatton CIC, Sekera MH, Merdlnk JL. Analytical chemistry at the Games of the XXIIIrd Olympiad in Los Angeles, 1984. *Clin Chem.* 1987;33:319-27.
9. Corrigan B, Kazlauskas R. Drug testing at the Sydney Olympics. *Med J Aust.* 2000;173:312-3.
10. Chan SC, Torok-Both GA, Bfflay DM, Przybylski PS, Gradeen CY, Pap KM, Petruzelka J. Drug Analysis at the 1988 Olympic Winter Games in Calgary. *Clin Chem.* 1991;37:1289-96.
11. Park J, Park S, Lho D, Choo HP, Chung B, Yoon C, *et al.* Drug testing at the 10th Asian Games and 24th Seoul Olympic Games. *J Anal Toxicol.* 1990;14:66-72.
12. Segura J, Pascual JA, Ventura R, Ustaran JI, Angel Cuevas A, Gonzalez R. International cooperation in analytical chemistry: Experience of antidoping control at the XI Pan American Games. *Clin Chem.* 1993;39:836-45.
13. Laboratory Statistics WADA-AMA. (Consulted 1/02/2010). Available in: http://www.wada-ama.org/en/Science-Medicine/Anti-Doping-Laboratories/Laboratory-Statistics/Archived-Laboratory-Statistics/.
14. Laboratory-Statistics WADA-AMA 2008. (Consulted 1/02/2010). Available in: http://www.wada-ama.org/Documents/Science_Medicine/WADA_LaboStatistics_2008.pdf.
15. Conte JC, Rubio E, García AI, Domínguez AI. OMEGA: metodología para la previsión de accidentes en poblaciones laborales. Aplicación a microempresas. *Medicina y Seguridad en el trabajo* 2006;LII(203).
16. Conte JC, Rubio E, García AI, Domínguez AI. Método OMEGA: aplicación a pequeñas y grandes empresas. *Medicina y Seguridad en el trabajo* 2006; vol LII, nº 203.
17. Manonelles Marqueta P, García-Felipe A, Rubio Calvo E, Alvarez Medina J, Naranjo Orellana J, Conte Solano JC, *et al.* Una aproximacion a las relaciones entre laboratorios acreditados europeos y hallazgos analíticos adversos. *Arch Med Deporte.* 2011;141:161-272.
18. García Felipe AI, Conte JC, Rubio E, Pérez Prados A. Accidente laboral. ACSOM una nueva orientación para gestión automática del riesgo. *An Sist Sanit Navar.* 2009;32:23-34.
19. García Felipe A, Alvarez JM, Alcalde V, Rubio B, Bascuas B. Monitorización epidemiólogica en vigilancia de la salud, mediante el residual de incidentabilidad estandarizado (RIS). *Mapfre seguridad.* 2004;24:33-42.
20. Benzecri JP. *L'Analyse des Données II: L´Analyse des Correspondances.* Paris: Dunod, 1982
21. Greenacre M, Blasius J. *Correspondence analysis in social science. Recent developments and applications.* San Diego: Academic Press, 1994.
22. Hair J, Anderson R, Tatham R. Black W. *Análisis Multivariante.* 5ª Ed. Lebanon: Prentice Hall. 1999.
23. Conte JC, Cano F, García AI, Molina A, Rubio Calvo E. Interpretación de las relaciones intragrupales de riesgos y de lesiones mediante análisis cluster jerarquizado. *Revista de matemática: Teoría y Aplicaciones.* 2008;15(2):175-86.
24. Dent A. Doping control in professional football. *Br J Sports Med.* 1998;32:96-7.
25. Solberg S. Anabolic steroids and norwegian weightlifters. *Br J Sports Med.* 1982;16:169-71.
26. Corrigan B, Kazlauskas R. Medication use in athletes selected for doping control at the Sydney Olympics (2000). *Clin J Sport Med.* 2003;13:33-40.
27. Tsitsimpikou C, Tsiokanos A, Tsarouhas K, Schamasch P, Fitch KD, Valasiadis D, Jamurtas A. Medication use by athletes at the Athens 2004 Summer Olympic Games. *Clin J Sport Med.* 2009;19:33-8.
28. Tscholl P, Junge A, Dvorak J. The use of medication and nutritional supplements during FIFA World Cups 2002 and 2006. *Br J Sports Med.* 2008; 42:725-30.
29. World Anti-Doping Agency. *International Standard for Laboratories.* Montreal: World Anti-Doping Agency, 2009.
30. World Anti-Doping Agency. *International Standard for Testing.* Montreal: World Anti-Doping Agency, 2009.
31. Bohn AM, Khodaee M, Schwenk TL. Ephedrine and other stimulants as ergogenic aids. *Curr Sports Med Rep.* 2003;2:220-5.
32. Davis E, Loiacono R, Summers RJ. The rush to adrenaline: drugs in sport acting on the b-adrenergic system. *Br J Pharmacol.* 2008;154:584-97.
33. Peters Ch, Schulz T, Michna H, eds. *Biomedical side effects of doping.* Köln: Sport & Buch Strauβ, 2001.
34. Eichner ER. Ergolytic drugs in medicine and sports. *Am J Med.* 1993;94: 205-11.
35. Strano Rossi SS, Abate MG, Braganò MC, Botrè F. Use of stimulants and drugs of abuse in sport: the Italian experience. *Adicciones.* 2009;21:239-42.
36. Campos DR, Yonamine M, de Moraes Moreau RL. Marijuana as doping in sports. *Sports Med*. 2003;33:395-9.
37. Dvorak J, Graf-Baumann T, D'Hooghe M, Kirkendall D, Taennler H, Saugy M. FIFA's approach to doping in football. *Br J Sports Med.* 2006;40(Suppl I): i3-i12.
38. Cowan DA, Kicman AT. Doping in sport: Misuse, analytical tests, and legal aspects. *Clin Chem.* 1997;43:1110-3.
39. Bhasin S, Storer TW, Berman N, Callegari C, Clevenger B, Phillips J, *et al.* The effects of supraphysiological doses of testosterone on muscle size and strength in normal men. *N Engl J Med.* 1996;335:1-7.
40. Perry PJ, Lund BC, Deninger MJ, Kutscher EC, Schneider J. Anabolic steroid use in weightlifters and bodybuilders: an internet survey of drug utilization. *Clin J Sport Med.* 2005;15:326-30.
41. Tricker R, O'Neill MR, Cook D. The incidence of anabolic steroid use among competitive bodybuilders. *J Drug Educ.* 1989;19:313-25.

42. Todd T. Anabolic steroids: the gremlins of sport. *J Sports History*. 1987;14:87-107.
43. Curry LA, Wagman DF. Qualitative description of the prevalence and use of anabolic androgenic steroids by United States powerlifters. *Percept Mot Skills.* 1999;88:224-33.
44. Stilger VG, Yesalis CE. Anabolic-androgenic steroid use among high school football players. *Community Health.* 1999;24:131-45.
45. Baron DA, Martin DM, Magd SA. Doping in sports and its spread to at-risk populations: an international review. *World Psychiatry.* 2007;6:118-23.
46. International Weightlifting Federation (IWF). (Consulted 08/03/2010). Available in: http://www.iwf.net/. 47. International Association of Athletics Federations (IAAF). (Consulted 08/03/2010). Available in: http://www.iaaf.org/statistics/toplists/inout=o/age=n/season=2009/sex=m/list.html. 48. Eichner ER. Blood doping: infusions, erythropoietin and artificial blood. Sports Med. 2007;37:389-91.
49. Barroso O, Mazzoni I, Rabin O. Hormone abuse in sports: the antidoping perspective. *Asian J Androl.* 2008;10:391-402.
50. Union Cycliste Internationale. (Consulted 28/03/2010). Available in: http://www.uci.ch/templates/BUILTIN-NOFRAMES/Template3/layout.asp?MenuId=MjExMw&LangId=1. 51. Haahtela T, Larsson K, Bonini S. Epidemiology of asthma, allergy and bronchial hyperresponsiveness in sports. *Eur Respir Mon*. 2005;33:1-4.
52. Lund T, Pedersen L, Larsson B, Backer V. Prevalence of asthma-like symptoms, asthma and its treatment in elite athletes. *Scand J Med Sci Sports.* 2009;19:174-8.
53. Maiolo C, Fuso L, Todaro A, Anatra F, Boniello V, Basso S, De Lorenzo A, Pistelli R. Prevalence of asthma and atopy in Italian Olympic athletes. *Int J Sports Med.* 2004;25:139-44.
54. Weiler JM, Layton T, Hunt M. Asthma in United States Olympic athletes who participated in the 1996 Summer Games. *J Allergy Clin Immunol.* 1998;102:722-6.
55. Langdeau JB, Turcotte H, Thibault G, Boulet LP. Comparative prevalence of asthma in different groups of athletes: A survey. *Can Respir J.* 2004;11:402-6.
56. Drobnic Martínez F, Casan Clara P. Prevalence of asthma and the use of bronchodilators in the professional athletes in Spain. *Arch Med Dep.* 2002;87:37-42.
57. Nystad W, Harris J, Borgen JS. Asthma and wheezing among Norwegian elite athletes. *Med Sci Sports Exerc.* 2000;32:266-70.
58. Naranjo J, Centeno RA, Carranza MD. The use of beta-2 agonists in sport. Are the present criteria right? *Br J Sports Med.* 2006;40:363-6.
59. Naranjo Orellana J, Carranza Márquez MD. Beta-2 agonists in sport. Are the Anti-Doping rules meeting the needs of asthmatic athletes? *Br J Sports Med.* 2009; accepted 4/nov/2009. Published online December 2, 2009 (doi: 10.1136/bjsm.2008.056903).
60. Thuyne WV, Delbeke FT. Declared use of medication in sports. *Clin J Sport Med*. 2008;18:143-7.
61. Lund TK, Pedersen L, Backer V. The use of anti-asthmatic medication among Danish elite athletes. *Ugeskr Laeger.* 2007;169:4355-9.
62. Fitch KD, Sue-Chu M, Anderson SD, Boulet LP, Hancox RJ, McKenzie DC, *et al.* Asthma and the elite athlete: summary of the International Olympic Committee's consensus conference, Lausanne, Switzerland, January 22-24, 2008. *J Allergy Clin Immunol.* 2008;122:254-560.
63. Stenman UH, Hotakainen K, Alfthan H. Gonadotropins in doping: pharmacological basis and detection of illicit use. *Br J Pharmacol.* 2008;154: 569-83.
64. Handelsman DJ. Clinical review: the rationale for banning human chorionic gonadotropin and estrogen blockers in sport. *J Clin Endocrinol Metab.* 2006;91:1646-53.
65. Strauss RH, Yesalis CE. Anabolic steroids in the athlete. *Annu Rev Med.* 1991;42:449-57.
66. Ventura R, Segura J. Masking and manipulation. *Handb Exp Pharmacol.* 2010;195:327-54.
67. Dvorak J, Feddermann N, Grima K. Glucocorticosteroids in football: use and misuse. *Br J Sports Med.* 2006;40 (suppl I): i48-i54.
68. WADA Technical Document TD2010MRPL. *Minimun required performance levels for detection of prohibited substances.* (Consulted 25/05/2010). Available in: http://www.wada-ama.org/Documents/World_Anti-Doping_Program/WADP-IS-Laboratories/WADA_TD2010MRPLv1.0_Minimum%20Required%20Performance%20Levels_Sept%2001%202010_EN.pdf
69. Kruse P, Ladefoged J, Nielsen U, Paulev PE, Sørensen JP. Beta-Blockade used in precision sports: effect on pistol shooting performance. *J Appl Physiol.* 1986;61:417-20.