**PROJECT REVIEW**

***"Trojan horses in blood doping: can liposomes interact with haemoglobin, mask HBOCs or alter the haematological profile"***

**Dr. Botre, Dr. de la Torre, Dr. Donati, Dr. Mazzarino** (Federazione Medico Sportiva (FMSI)

## Project overview

Blood doping is aimed to illicitly increase the oxygen delivery to tissues to improve sport performance, especially in endurance sports/disciplines. At present, WADA-accredited antidoping laboratories can count on several analytical strategies to detect most if not all the different forms of blood doping: these detection strategies may be either "direct" (aimed to detect the "non endogenous" substances administered to obtain the blood-doping effect), and "indirect (where the longitudinal stability of a panel of hematological and/or biochemical parameters is regularly monitored to spot any significant deviation from the individual physiological ranges).

To the best of our knowledge, the only "masking strategy" for blood doping that has been considered so far is the practice of "blood dilution". This strategy is performed before a doping control test, and it is obtained by intravenous infusions of physiologic solutions (with or without the addition of modified polysaccharides, used also as plasma volume expanders). This practice is aimed both to dilute specific target analytes, making therefore more problematic their detection by the WADA laboratories, as well as to reduce the value of some diagnostic haematological parameters that are considered in the framework of longitudinal monitoring of the athletes.

We postulate that an additional, and in principle very effective, masking strategy, would be the administration of supramolecular structures, and primarily among them phospholipidic liposomes, with the objective to reduce the free concentration of circulating peptides, polypeptides and proteins that are analytical targets of both "direct" and "indirect" methods of detection.

The present project is aimed to verify the effectiveness of this potential masking strategy, and to develop specific, selective, and suitable analytical methods to ensure its detection by the WADA-accredited laboratories.

## Results and Conclusions

The detection, and ideally quantification, of doping agents that still remain "invisible" to the anti-doping laboratories are one of the most urgent challenges for the anti-doping community. To be "invisible", a doping agent needs to satisfy one or more of the following conditions: (i) to be still

unknown; (ii) to be identical to an endogenous substance, (iii) to be present in the biological fluids in a concentration smaller than the limit of detection of the available analytical methods, and/or (iv) to be masked by masking agents that are themselves unknown. This research project has specifically addressed this last point.

In the last few years, novel and potentially more effective masking agents have been considered in sport doping. Among them, drug delivery systems (DDS) seem to be particularly attractive to cheaters. DDS may indeed be used to alter the absorption, release, distribution and excretion profile of prohibited drugs, making their detection by the WADA-accredited laboratories more problematic.

In this study we have considered a particular class of prohibited drugs vehiculated by drug delivery systems, and, specifically liposomial encapsulated hemoglobins (LEHs). Basically, LEHs, tha are also defined "hemosomes", are a new form of hemoglobin-based oxygen carriers (HBOCs) that mimic the oxygen diffusivity of erythrocytes, so that they may be considered "artificial red blood cells" rather than "artificial hemoglobins". Besides their multiple, potential clinical applications, and their extremely promising therapeutic utility, LEHs could be misused as a sport doping practice, since they can increase the oxygen carrying capacity of blood, with the consequent improvement of sport performance, especially in endurance disciplines. Although LEH are not commercially available yet, their preparation is relatively straightforward, and this may increase the interest of those seeking an "invisible" way to take advantage of "blood doping".

The main results of the present research project can be summarized as follows:
I. Liposome-encapsulated haemoglobins (LEHs) are stable enough to be illicitly used as performance-enhancing drugs.
II. LEHs are "challenging" to detect with current methods for the initial testing analysis are applied.
III. Direct detection of LEHs in blood can be achieved by targeting the intact liposome-hemoglobin complex by flow cytofluorimetry with double coloring. The performance of the methods is further improved by using coated microbeads.
IV. Indirect detection of the intake of LEHs and/or of other liposome vehiculated drugs can be performed by HPLC-MS/MS using a aqueous normal phase, by monitoring the entire phospholipid and sphingomyelin profile in urine.

The above results confirm what already reported in a previous research project also funded by the WADA (project code 09D9FB), i.e. that liposomes could represent an effective form of "doping delivery systems", with potential masking effects. Efficient detection of LEHs requires upgrade of routine detection methods to identify their intake either in blood (by flow cytofluorimetry based techniques) and/or in urine (by HPLC-MS/MS based techniques).