"*Application of transcriptional and proteomic profiling to the detection of recombinant human Growth Hormone (rhGH)*"

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Project Summary

This project seeks to build on the very important findings of a project funded previously by the Australian Government's Anti-Doping Research Program (ADRP), into the potential application of transcriptional profiles derived from peripheral blood mononuclear cells (PBMCs -lymphocytes & monocytes) for the detection of rhGH. That project identified sets of genes that were consistently either "up" or "down" regulated in mRNA expression, changes that were evident at least 21 days after the final administration of rhGH. The 21 day time period is an extremely important finding given that the current detection "window" with the WADA-approved test for rhGH is only 36 hours (Holt & Sonksen, 2008). The findings of the previous ADRP-funded study clearly provide the foundation for the development of a new, more sensitive test for the detection rhGH, a test with a vastly improved detection "window'.

We anticipate that the proposed project will result in the development of a new, more sensitive test for rhGH based on alterations in gene expression assessed at either the transcriptional and/or proteomic level. A cell's transcriptional "profile" is obtained by measurement of (potentially, genome-wide) genespecific mRNA levels via use of oligonucleotide microarray technology. Subsequent quantitative RT-PCR analyses allow verification of alterations in the expression of candidate genes. A cells "translational"/proteomic profile can be assessed via the techniques of SDS-PAGE and western blotting. These technologies will allow the identification of gene "subsets" whose activity at a specific time-point, is either up-regulated, down-regulated or remains unchanged following a specific intervention. The set of genes responding transcriptionally or translationally to a specific intervention and the temporal extent of such changes potentially provide a set of unique indicators that are characteristic of that intervention.

Results and Conclusions

This project has been carried out with the support of The World Anti-Doping Agency. The investigation aimed at pinpointing a set of differentially expressed mRNA genes associated specifically to the uptake of recombinant growth hormone.

Particular attentions of this project focused on the determination of exercise related induce gene expression and to compare whether such expression profile shared similarities with rhGH related expression profile. In other

words, this project aimed at finding the unique and specific rhGH related gene expression signature eliminating possible additional unrelated expression variables that different types of exercise induce.

Three types of exercise regimen (endurance, resistance and anaerobic exercises) were analysed in untrained participants and temporal PBMC extracted cell gene expression profiling using microarray technology was determined for each type of exercise. In addition, mRNA expression profiling was performed on trained individuals carefully sub-grouped in the same types of exercise mentioned previously.

Our analysis used a series of state of the art bio-informatics tools aimed at reducing background noise, batch effects between microarrays, false positives and any other un-necessary variables to aim at narrowing the candidate genes at the highest significant relevance to our study and conclusions.

The main results obtained in this document are reporting the PBMC's temporal expression profiling from:

I. participants administered with rhGH;
II. untrained participants undertaking three types of exercise. In addition, non-temporal expression profiling at rest was evaluated on trained participants grouped in the same types of exercise (endurance, resistance and anaerobic).

The comparison of all the different profiles has shown that:

I. 47 genes are specific to rhGH and unrelated with any of the three exercise related gene expression in untrained individuals.
II. 2 genes specific to rhGH are seen to be similar to both the Trained and Untrained expression profiles. These two genes consisted of the interferon gamma and the tubulin folding cofactor C.

Of note, this two candidate genes were observed specifically in the resistance groups. Finally, these two genes showed fold gene expression levels that are, and across all time points, lower in rhGH related PBMCs than the fold expression values of the resistance groups.

The common limitations of this present study are:
- Batch effect related to the analysis of the rhGH related study that required the action of the use of a bio-informatic batch effect removal tool.
-Possible dosage of the rhGH that was administered to reflect a more pronounced expression profile.