# Gene x Environment interactions and their impact on the stress response system as studied in space-flight analogs

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## Introduction

Environmental and social cues activate the stress response system and stimulate a series of physiological adaptation processes, with an overload leading to decreased well-being and illnesses.

We investigate how gravitational unloading, isolation and confinement impinge on the stress response system of healthy individuals. The impact of these parameters is important to identify the health effects astronauts can experience. Results obtained from this research has also direct relevance for a better understanding of the health consequences of social isolation, sedentary lifestyle, aging and osteoporosis.

## Objectives

The aim of our studies is to capture the molecular responses and adaptations to the challenging environmental conditions of unloading, isolation and confinement.

Transcriptomics analyses are performed in whole blood. It is envisioned that blood cells sentinel the health and well-being status.

We also investigate the saliva transcriptome and hypothesize that this matrix is also responsive to the environmental conditions, and reflect some of the changes observed in blood. If so, saliva transcriptome might have a potential as an alternative non-invasive and convenient matrix for health monitoring.

## Materials and methods

Here we will report the results of healthy individuals that have been participating in an aerobic bike test (30 minutes exercise at 60% of their maximal heart rate), and individuals that were exposed to hypergravity instead of artificial gravity (30 minutes, 2× gravity force in supine position). Blood samples (3 mL) and saliva samples (approximately 1 mL) were collected just before and just after the experiment using Ambion's Tempus tubes and DNA Genotek's Oragene®•RNA to preserve intact RNA.

RNA was extracted using Tempus Spin kit ( blood) and QIAgen RNeasy Micro kit (saliva). RNA yields from blood were checked using NanoDrop. Globin mRNA was depleted from total RNA preparations using the Ambion Globinclear kit. The integrity of the remaining globin-depleted RNA was determined with Nanodrop and Bioanalyzer. RNA extracts from saliva were measured with Nanodrop.

Next, RNA samples were processed for dual-color microarray analysis using Agilent's workflow for 4× 44K whole genome microarrays. An aliquot of 200 ng RNA and 50 ng RNA was used for blood and saliva, respectively. Yield and specific activity of the single-stranded, labeled cRNA (both blood and saliva) were determined using NanoDrop.

Following overnight hybridization, microarray slides were scanned with an Agilent high-resolution scanner and data was extracted using Feature Extraction software. Statistical analysis was performed in GeneSpring 11.

| Measurement | Saliva | Blood |
|---|---|---|
| Sample volume (mL) | 1 | 3 |
| Concentration (ng/µL) | 20-2,000 | 60-80 |
| RNA quality ($A_{260}/A_{230}$ or $A_{280}$) | ≈2 | ≈2 |
| RNA integrity number | >6.5 | >9 |
| Dye incorporation (pmol/µL) | >8 | >8 |
| Yield (µg) | >2 | >2 |

***Table 1**: Characteristics of the blood and saliva samples and sample processing for microarray analysis. Measurements of the globin-depleted RNA for blood are reported.*

## Results

Statistical analysis revealed that both an acute bout of exercise and hypergravity induced differential gene expression. 182 genes ($p<0.05$; absolute fold change>2) were changed after a bike test (Figure 1). 129 genes ($p<0.15$; absolute fold change>1.5) were changed after hypergravity (Figure 2). The statistical power in case of hypergravity is lower, but this can be attributed to the fact that at present only 5 individuals were tested (9 in the case of the bike test).

Analysis of the saliva transcriptome revealed that 132 genes and 34 genes were significantly altered in expression after the aerobic exercise and hypergravity protocol, respectively.

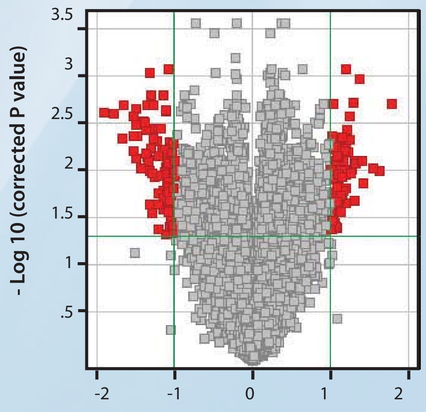


***Figure 1**:*

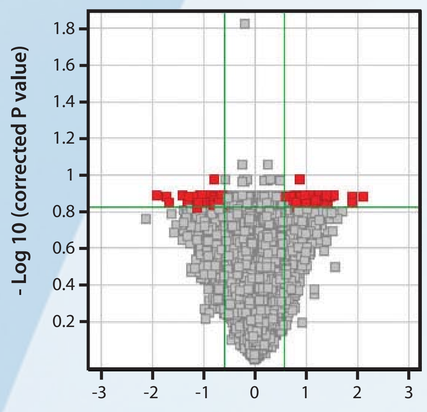


***Figure 2**:*

The XY plots show that the directionality of the differential expression was the same in blood and saliva for both the exercise (Figure 3) and hypergravity (Figure 4) protocol.

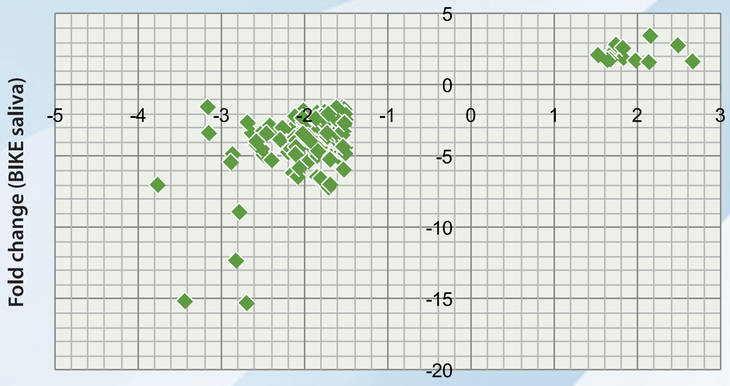


**Figure 3:** *132 mapped genes that are differentially expressed in saliva after a bike test.*

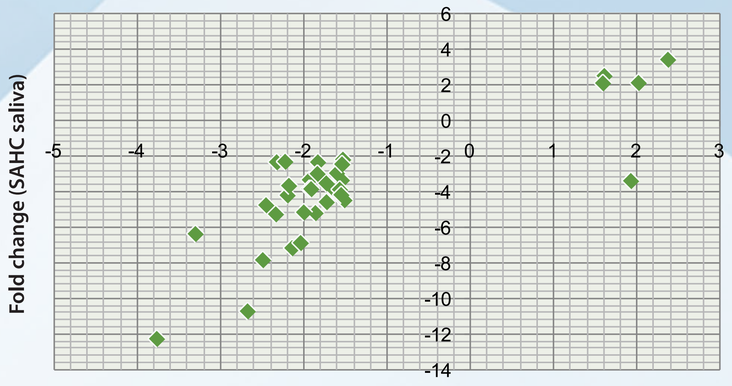


**Figure 4:** *34 mapped genes that are differentially expressed in saliva after a hypergravity test.*

We have identified several differentially expressed genes in saliva that have relevant metabolic, cardiovascular and neurological functions related to the positive health effects of exercise.

- pyruvate kinase (PKLR)
- neurotensin receptor 2 (NTSR2)
- interleukin 2 (IL2)
- adrenergic, alpha-2C-, receptor (ADRA2C)
- neuropilin 2 (NRP2)
- neuritin 1 (NRN1)
- phospholipase A2, group X (PLA2G10)

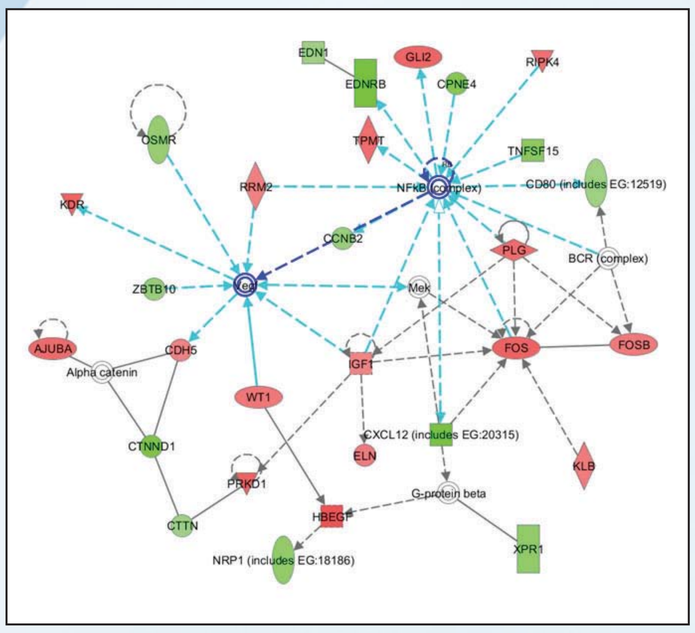


***Figure 5**: Shows a biological interpretation of the differentially expressed genes after the bike test. Using Ingenuity Pathway Analysis we have identified a biological network related to the cardiobascular system. Important gene products in this network are NFkB, VEGF and IGF1.*

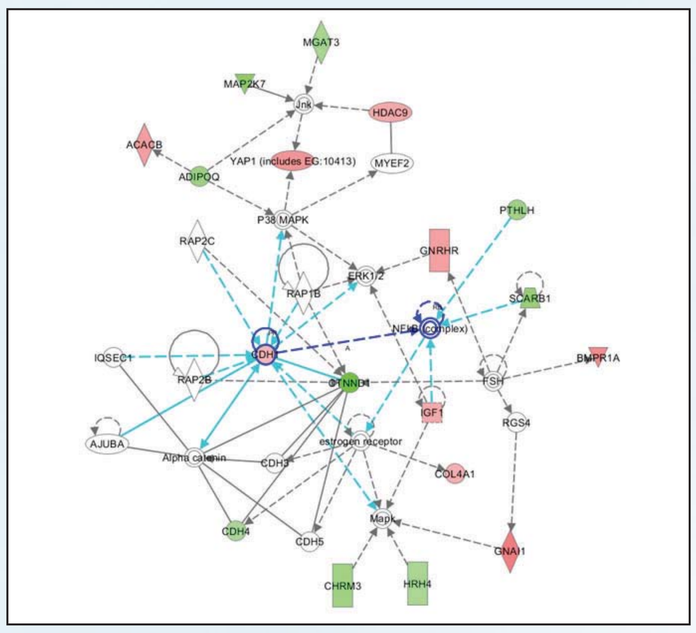


***Figure 6**: Shows a biological interpretation of the differentially expressed genes after the hypergravity test. Using Ingenuity Pathway Analysis we have identified a biological network related to the metabolic system. Important gene products in this network are NFkB, CDH1, CTNNB1 and IGF1.*

## Discussion

We have shown that a short bout of exercise induced differential gene expression in blood. These genes can be associated to vascular biological networks.

For the first time ever, we have analyzed the gene expression response in blood after a hypergravity protocol.

Both exercise and hypergravity induced insulin growth factor 1 (IGF1). IGF-1 is a key anabolic hormone. An increase in Insulin-Like Growth Factor-1 (IGF-1) has been found to improve brain health and cognitive ability.

Whole genome expression analysis of saliva appeared to be feasible and practical. Moreover, saliva transcriptome was responsive to our treatments. We have identified a couple of genes that have relevant physiological functions. Further work will be focused on extending our study population and validation of the differential gene expression using real-time PCR.

We believe that saliva transcriptome is convenient and useful for studying response in our intervention studies. More results are being collected in the context of our hypergravity, parabolic flight and isolation studies.


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