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Examining Transcriptomic Changes in Non-Elite Marathon Runners Following Endurance Exercise

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Abstract

Objective: This study aimed to analyze the differential gene expression and pathways in non-elite marathon runners before and after completing a marathon, with a focus on understanding the potential deregulation of body systems during prolonged physical activity.

Methods: A cohort of 60 non-elite athletes (42 men and 18 women) participating in the Barcelona Marathon provided blood samples at three distinct time points: baseline before the marathon (START), immediately post-marathon (FINISH), and 24 hours post-marathon (24REST). Differential gene expression, Gene Ontology (GO) term analysis, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed for each group and three comparative analyses: C1 (START vs. FINISH), C2 (FINISH vs. 24REST), and C3 (START vs. 24REST).

Results: Comparative analysis revealed 9534 differential gene expressions, 162 GO terms, and 61 KEGG pathways in START vs. FINISH; 9454 differential gene expressions, 131 GO terms, and 59 KEGG pathways in FINISH vs. 24REST; and 454 differential gene expressions, 14 GO terms, and 8 KEGG pathways in START vs. 24REST. Comparing FINISH with C1 and C2 highlighted significant enrichment in immune system-related terms, mitochondria, inflammatory markers, viral transcription and replication, reactive oxygen species, and lipid metabolism. Furthermore, comparing pre-marathon levels with those 24 hours post-marathon revealed enrichment in GO terms associated with mitochondrial activity, reactive oxygen species, and lipid metabolism.

Conclusion: Strenuous endurance exercise induced deregulation in immune system function, inflammatory markers, and mitochondrial activity, elevating the risk of infection post-marathon and potentially altering oxidation environments and lipid metabolism. Although gene expression did not fully recover 24 hours post-marathon, it approached baseline values more closely than immediately post-exercise.

Keywords: Endurance exercise; Gene expression; RNA-seq; Next-generation sequencing

Introduction

Regular exercise is widely acknowledged for its positive effects on health, including reducing the risk of heart disease [1-4]. However, the impact of highly demanding endurance activities on the body remains ambiguous. Studies examining participants of events like the Tour de France have shown lower rates of cardiovascular events and increased longevity compared to the general population [5]. Conversely, subjecting the body to intense

and prolonged exercise, such as running a marathon, may yield contrasting outcomes [6,7]. Age and training conditions are potential factors influencing these divergent effects [8].

This study seeks to elucidate the physiological changes occurring in the human body following participation in a marathon by analyzing gene expression patterns. Previous investigations focusing on gene expression during ultramarathons have highlighted substantial impacts on the immune and inflammatory systems [9]. Alterations in pathways associated with protein synthesis, immune response modulation, and mechanisms related to infectious diseases were observed. Strenuous exercise has been linked to immune system dysfunction and heightened susceptibility to infection, as well as potential effects on fatty acid metabolism and cardiovascular health.

Consequently, there has been a growing interest in understanding the repercussions of intense physical activity on health, whether at a professional or amateur level, with a specific emphasis on mitigating any adverse effects through genomic analysis. Thus, this study aimed to evaluate the influence of exceptionally strenuous physical exertion on gene expression profiles in blood samples. It involved identifying genes exhibiting significantly altered expression levels post-marathon, exploring their biological implications through pathway and gene ontology analyses, assessing their relevance during the race, and determining their expression recovery levels 24 hours post-exercise.

Materials and Methods

Sample Collection

Blood samples were obtained from 60 nonelite athletes (42 men and 18 women) participating in the 2016 Barcelona Marathon, a 42-kilometer running event. A total of 2.5 mL of whole blood was collected from each participant at three different time points: immediately before the race (START), immediately after completing the race (FINISH), and 24 hours post-marathon (24REST) [10,11].

The participants, aged between 20 and 55 years, provided information on their heights, weights, and weekly training regimen, averaging 7.5 hours per week. An overview of the workflow steps is depicted in Figure 1.

RNA Extraction

Intracellular RNA was isolated from whole blood samples collected from each participant using PAXgene Blood RNA Tubes following the protocol provided by the PAXgene Blood RNA Kit (QIAGEN GmbH, Germany). The extracted total RNA was stored at -80°C until further use. Samples obtained immediately after completing the marathon were collected at a pavilion near the race's finish line, while pre-race (START) and 24 hours post-marathon (24REST) samples were obtained at the Hospital de la Sant Creu i Sant Pau. Subsequently, the samples were sent to Banc de Sang I Teixits (BST), Barcelona's blood and tissue bank, for RNA extraction and sequencing. RNA samples were prepared for sequencing using the Illumina TruSeq sample preparation kit and a Globin Block pack, following the manufacturer's instructions, and sequenced on a HiSeq2000 Sequencing System (Illumina).

Quality Control

Both pre- and post-sequencing quality control checks were performed to ensure sample integrity and sequencing output quality. Samples failing to meet quality standards were excluded from subsequent analyses.

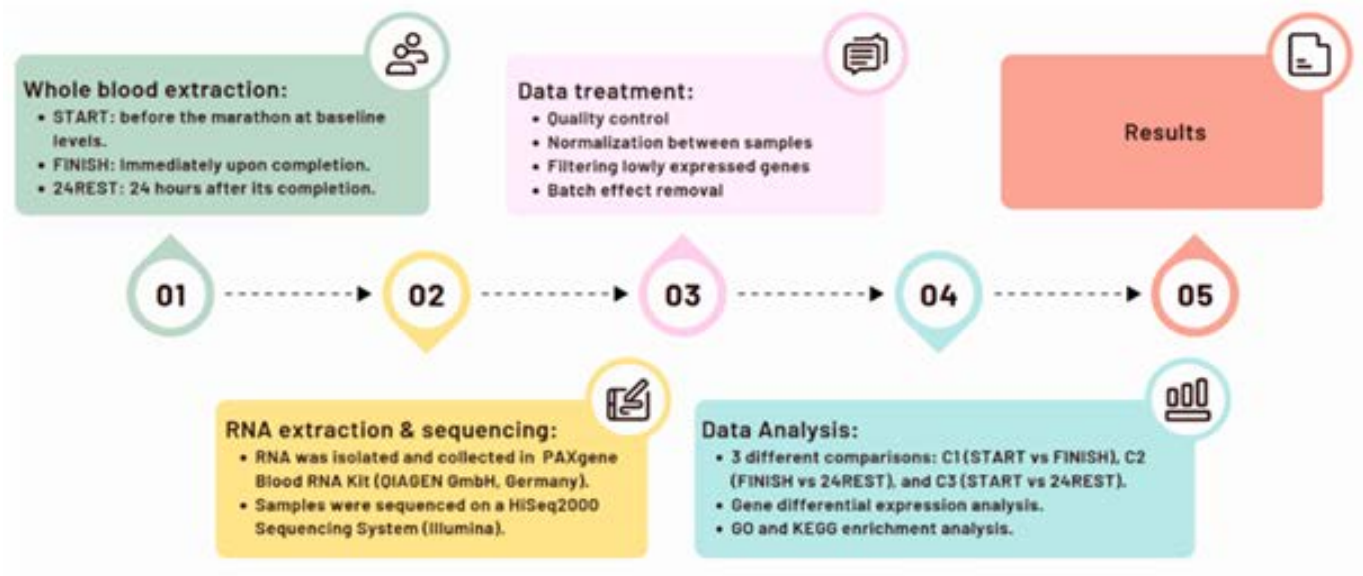

Figure 1: Steps performed in this analysis.

Gene Count and Normalization

Sequenced reads (74-bp paired-end) were aligned to the GRCh38 reference genome using the STAR algorithm for alignment and salmon for gene quantification. Genes were annotated according to Ensembl annotation. Genes with low expression levels were filtered out, and sample normalization was performed using R packages. Normalization factors were calculated to convert raw library sizes into effective library sizes, considering library length variations. Linear regression models of gene expression were fitted using the filtered and normalized count matrix.

Differential Expression Gene (DEG) Analysis

The Limma package was utilized to construct linear models for RNA-Seq data. Differential expression analysis was conducted using moderated t-statistics, moderated F-statistics, and log-odds of differential expression, computed by eBayes. Bonferroni correction was applied to adjust p-values for multiple comparisons.

Gene Ontology Analysis

Gene Ontology (GO) analysis was performed using the GOstats package to identify over- and underrepresented GO terms among significant genes derived from linear models. A conditional hypergeometric test was employed to assess relationships among GO terms, and results were filtered based on size, count, and odds ratio parameters.

KEGG Pathway Analysis

Enrichment analysis of KEGG pathways was conducted using the hypergeometric test with the signature Search package. The enrichKEGG2 function was employed to identify enriched KEGG pathways based on gene identifiers.

Analysis Comparison

All analyses were performed three times to compare gene expression at different time points (C1: START vs. FINISH, C2: FINISH vs. 24REST, C3: START vs. 24REST).

Results

After quality checks and sample filtering, analysis was conducted on 60 participants (42 men and 18 women) out of the initial 78. Subsequently, 180 samples (3 time points for each of the 60 subjects) and 14,235 genes (with Ensembl annotation) were included in the analysis [12,13].

Heatmap Visualization

A heatmap was generated using the gplots package for the top 500 most variable genes (Figure 2), revealing distinct clusters between FINISH (purple) and START/24REST (green and yellow) samples.

Covariate Identification

Principal Component Analysis (PCA) indicated clear separation between START/24REST and FINISH samples, with sex identified

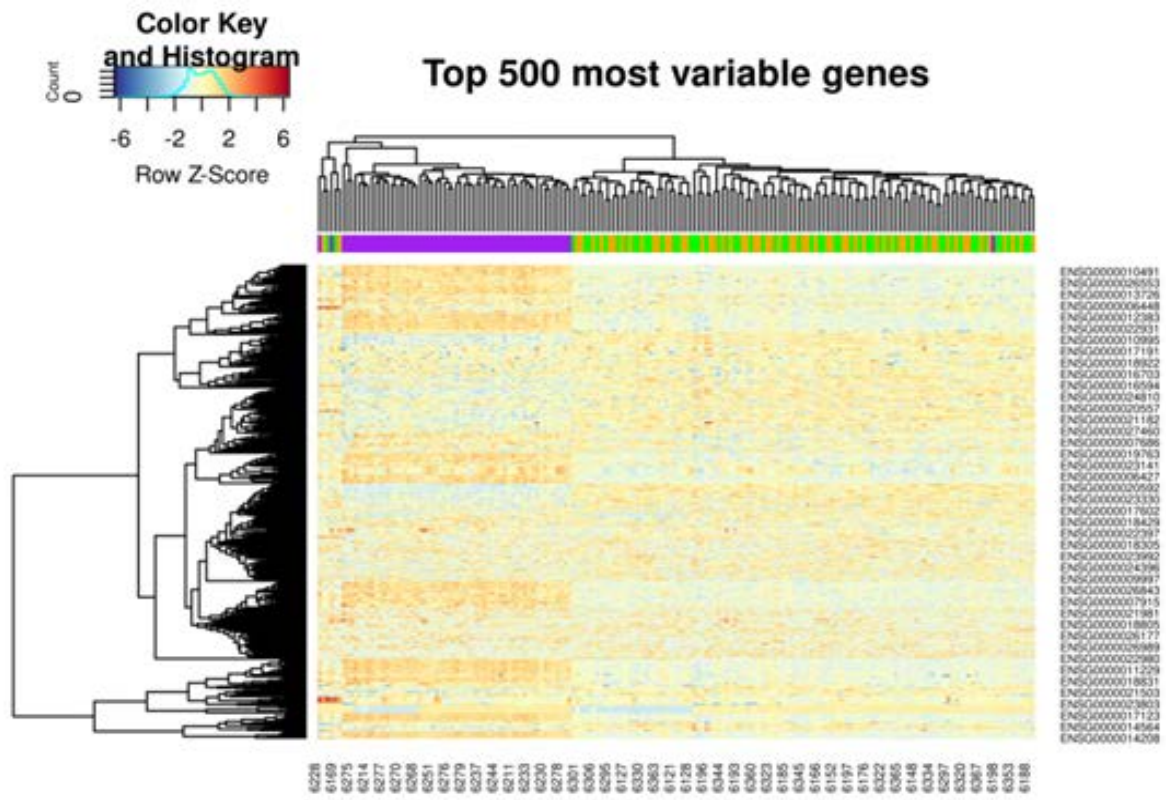

Figure 2: Heatmap: the X-axis indicates all the samples and the Y-axis shows the top 500 most variable genes.

as a parameter of inter-variability. Intra-variability among samples was also observed.

Differential Expression Gene (DEG) Analysis

Differential expression analysis identified 9534 DEGs in C1 (START vs. FINISH), 9454 in C2 (FINISH vs. 24REST), and 454 in C3 (START vs. 24REST). Overexpression in C1 and C2 indicated significantly higher gene expression post-marathon, while C3 showed elevated expression 24 hours post-marathon compared to baseline.

Analysis of DEGs

In C1 (START vs. FINISH), DEGs were associated with immune cell markers, chemokines, interleukins, and inflammatory markers. Notable genes included CD48, CD19, and IL-6.

In C2 (FINISH vs. 24REST), DEGs mirrored those of C1, with notable exceptions such as CLEC10A.

In C3 (START vs. 24REST), fewer DEGs were observed, including genes related to cell progression inhibitors and viral expression.

Gene Ontology (GO) Term Enrichment

GO analysis revealed 162 enriched terms in C1, 131 in C2, and 14 in C3. Enriched terms were related to mitochondrial functions, T and B cell activation, and viral processes.

KEGG Pathway Analysis

Pathway analysis showed enrichment in apoptosis, cellular senescence, mitophagy, and lipid metabolism pathways in C1 and C2. In C2, insulin resistance and VEGF signaling pathways were also enriched.

Overall, the results highlight significant changes in gene expression post-marathon, particularly in immune response, inflammation, and metabolic pathways. These findings provide insights into the physiological effects of strenuous exercise on non-elite marathon runners.

Discussion

This longitudinal study in non-elite athletes aimed to investigate the effects of an endurance event on gene expression and determine if gene levels recovered after 24 hours. The results indicate that strenuous exercise induces an inflammatory response, activates an oxidative environment, and downregulates the immune system [14].

Inflammatory Response

Previous studies have linked endurance exercise to an inflammatory environment, increasing inflammatory mediators and the risk of injury and chronic inflammation. The current study confirmed these findings, with 15 out of 23 inflammatory markers significantly differentially expressed between baseline (START) and FINISH levels.

Immune System Downregulation

The study observed downregulation of the immune system after endurance exercise. Comparisons (C1 and C2) showed downregulated genes associated with B and T cells and other immune components. This downregulation may elevate the risk of infection, consistent with previous research. Terms related to infection and viral replication were upregulated, indicating physiological stress and temporary immunodepression.

Immune System Recovery

Interestingly, 24 hours after exercise (C3), the immune system returned to baseline levels, suggesting a transient effect. Monocyte markers, specific cytokines, and interleukins were overexpressed, indicating acute inflammation. Further research is needed to explore the role of monocytes in exercise-induced oxidative stress.

Oxidative Stress and Mitochondrial Activity

Genes regulating oxidative stress, such as GPX1, GPX3, and SOD2, were downregulated after exercise, suggesting an oxidative environment. DNMT3A, associated with reducing oxidative stress, was overexpressed. Mitochondrial and electron chain-related functions were among the top deregulated genes, reflecting increased energy demands during exercise.

Energy Generation and Recovery

Comparing FINISH to 24REST, some GO terms associated with mitochondrial, electron chain, and ATP metabolism were enriched, indicating incomplete recovery of energy generation capacity 24 hours post-race.

Cardiomyopathy and Atherosclerosis Pathways

Deregulation of KEGG pathways related to diabetic cardiomyopathy and atherosclerosis raises concerns. Previous studies linked long-term marathon running to increased coronary artery calcification and plaque volume.

Conclusion

In summary, completing an endurance exercise like running a marathon significantly alters gene expression levels and disrupts various metabolic pathways and systems. Immunity appears to be temporarily compromised, with T and B lymphocytes downregulated, increasing the risk of infection post-exercise. Additionally, the role of monocytes in lipid metabolism, inflammation, and oxidative stress during strenuous exercise warrants further investigation. Nonelite athletes exhaust considerable energy during such races, leading to incomplete recovery of mitochondrial activity even after 24 hours of rest. Deregulation of pathways associated with atherosclerosis highlights potential health risks post-exercise, necessitating proactive measures.

Insights from this study could inform future training protocols and nutritional strategies tailored to individual gene expression profiles. Moreover, understanding gene expression dynamics may expedite post-exercise recovery through targeted interventions and lifestyle adjustments.

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