

# Spatial Analysis of Gene Expression: Impact of Long-Distance Running on Cellular Pathways

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**Abstract:** *This study conducts a systematic review and computational analysis of over 15 research papers to explore the impact of exhaustive endurance running on genetic and metabolic pathways. Focusing on variations in WBC counts, CK serum metabolites, cortisol levels, and gene expression among ultramarathon and marathon runners, the research identifies stress-induced genetic alterations affecting metabolic and oxidative pathways. The findings highlight temporary and permanent genetic changes, underscoring the need for further research into the complex relationship between running and genetic regulation. This work serves as a foundational reference for future investigations in sports physiology, offering valuable insights for the public, coaches, and healthcare professionals.*

**Keywords:** running; exercise; marathon; ultramarathon; genes; biochemical pathways; health

## 1. Introduction

Although humans have evolved over millions of years, the amount of physical activity required for a man is the same as it was thousands of years ago. However, most people in the current generation have unequal energy consumption to expenditure ratio due to prevalence of physical inactivity (8). This imbalance leads to obesity and susceptibility to several diseases. Physical inactivity is a contributor to the worldwide epidemic of non-communicable diseases, Physical activity is any activity involving bodily movements (8). The World Health Organization (WHO) defines the same as “Physical activity is defined purely physiologically, as all body movement that increases energy use beyond resting levels (8).” The WHO also set out recommendations-recommending at least 150 min of moderate-intensity or 75 min of vigorous-intensity aerobic activity per week, or an equivalent combination of both(8,9,10,11,12,13). A brief overview of the benefits of physical activity (PA) and sports is known to all, many refer to PA as a drug metaphorically for having similar qualities(9,14). Little or no PA won't show results and at the same time an overdose has its consequences. Over the years people have started practicing sports to keep their body and mind healthy, however sports may also lead to certain changes within a cell; Endurance training, running, cycling etc. extensively may lead to certain changes in gene transcriptomics and body metabolism. Several meta-analyses, studies in the past have observed the changes in gene regulation between athletes and control groups before and after a certain workout or race, there were several differences in the gene regulation (some upregulated, some down regulated) between the athletes and controls and among different time periods (15,16). This paper attempts to provide a computational summary of the difference in the regulation of certain genes exclusively in runners when compared to other groups. Running is the action or movement of propelling yourself forward rapidly on foot (17). During running both body and mind work concomitantly improving both physical and mental health. Over the years, the popularity of recreational running has drastically increased, some of the common motives include fat loss, well-being, challenge, fitness/health, and addiction (18); There are people however, who run competitively and

intensively– marathon or ultra-marathon runners, track and field athletes etc... Each of which confers several benefits to us, yet, if overdone, the same benefits may overshoot and become toxic (3,14,15). There are several systems, pathways and genes in the body that keep it running to sustain day to day life. If even a small change occurs to a gene, the entire pathway would be affected. In this paper we take a closer look at certain genes that are affected by running. In many cases these return to baseline level during recovery, but some are permanent regulatory changes (2,4,7,19). In fact, these changes depend on the extent of running and factors like age as well. Therefore, the author has included studies from different cohort groups classified as: recreational runners, who run simply for the pleasure of it; master athletes/veteran runners, who are over 30 years ,competing in running events(track/road running/cross country); elite group, a highly selective group of people with high fitness level(adapted to vigorous training)(13) , often derived by estimating the VO<sub>2</sub>max level as , generally, a higher VO<sub>2</sub>max peak is associated with better fitness levels(6). This paper sets out to give an overview of the internal and external side effects of running across various running cohorts; additionally, it includes non-running athletes as well, to determine commonalities or differences in the gene regulations across all types of exercises. The purpose of this study is to systematically review the genetic and metabolic changes induced by endurance running, identifying both the beneficial and adverse effects on health. This research aims to fill the gaps in current understanding and serve as a catalyst for further investigation into the nuanced relationship between physical activity and genetic expression. The significance lies in providing evidence-based insights that can inform training practices, health recommendations, and future scientific inquiries into sports physiology.

## 2. Literature Background

Running is a popular sport that several people participate in for varied reasons. Research shows that some of the most common motives for recreational running include fat loss, well-being, challenge, fitness/health, and addiction (18); additionally, running is also practiced professionally-

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sprinting, marathon running, or even ultramarathons. Like any other activity, running has its pros and cons. While it is beneficial for cardiovascular health, immunity, longevity, prevention from cancer and other diseases, improving gut health, enhancing mood and cognitive health (3,15,20,21); It may also have a negative impact. For example: risk of premature mortality, CVDs, muscular and skeletal atrophy and depression may be the result of excessive training (13,14,15).

## 2.1 Longevity

One of the benefits of PA is longevity. Over the past few decades, one of the leading causes of mortality has been physical inactivity- cited as the 4th leading death cause factor, globally (16, 22). This can be avoided by any form of regular PA. Running is an excellent method for the same. While any form of PA reduces mortality, running has an inherent benefit making it stand out. A study conducted proves that running results in lower risks of mortality compared to other PA, however, engaging in both running and other forms of PA proves to be even better. The study also suggests that the ratio between running, and longevity is 1:7(9). According to research, running causes a 30-50% reduction in cancer related mortality as well as a 45-75% reduction in CHD mortality thus an overall 30% reduced risk of all-cause mortality compared to non-runners (9, 23). Research shows that there is a U-shaped association between running and lower mortality risks, suggesting that the most benefits of lower mortality are gained by 1 to 2.5 hours a week with a frequency of 2-3 times a week. Overshooting this rate by a large magnitude showed negating results (9, 10, 11,14, 24). Some epidemiological studies suggest that there are possible explanations for elite runners to have lower mortality risks compared to non-runners or inactive participants despite engaging in vigorous exercise above the recommended level, which is contrary to the general hypothesis. However, the dose response relation between physical fitness and mortality and natural selection indicates that only a small group of people can be considered “elite runners (13)”, and in contrast to other results, Long-term vigorous exercise training is associated with increased survival rates of these athletes (13). Cardiac overuse injury (COI) in master athletes may lead to fibrosis and scarring of the myocardium, potentially dangerous rhythms, and accelerated coronary atherosclerosis. COIs might eventually trigger mortality, particularly after age 45 or 50 years, by causing adverse effects in the long run thus negating the positive effects of running (14). To conclude, running has been correlated with lowering the risk of mortality, but only when done in a specific dosage; ideally optimum amount of running confers the advantages and anything more does not confer any additional benefits.(13,14,16,23,24); however, the drug effect differs for people of different ages, medical history, lifestyles and genetic physiological history; for instance, elite runners might have a different range of optimum running as they are acclimated to more vigorous running (13).

## 2.2 Cardiovascular health

Running is an aerobic exercise that has a positive effect on cardiovascular health. Research shows that runners have a 30

% higher cardiorespiratory fitness than non-runners, a linear increase of cardiorespiratory fitness with increasing running time, and a 40% reduced risk of stroke mortality. However, running confers both benefits and risks. Risks being more particular to athletes with a history of CVD(9,23). Running is associated with reduced resting heart rate as well as a higher VO<sub>2</sub>max. This correlation is typically the result of the body adapting to exercise such as increasing blood flow (cardiac and stroke volume increases) and control of sympathetic/parasympathetic systems. The benefits are directly proportional to the duration of training (16, 25). 3 On the contrary, some recent studies suggest that excessive endurance exercise (EEE), such as habitual running, may cause adverse effects on cardiac structure and function (9). Vigorous exercise is specially noted to acutely increase the risk of a cardiac event during and for approximately an hour after exercise. Aski-marathon study highlighting Cardiovascular fitness (CVF) risk, involving 52,755 runners, suggests a high risk of developing arrhythmias in those who completed more races, frequent marathon running, and its training seemed to be correlated with myocardial damage (26). The risk of negative cardiovascular health is more prominent in athletes who have suffered from chronic/cardiac malfunctions in the past and veteran athletes. Extreme vigorous running causes a rise of cardiac calcium score, stiffening and thickening of cardiac muscles in veteran athletes, 62% more plaque, and a 5-fold increase of atrial fibrillation due to scarring in ventricles (14). To conclude, the benefits of running for our CVF varies based on several factors. Yet, there are many controversies in the aforementioned research regarding the cause of heart disease and its type, what the reason behind it is and whether it affects all groups of runners similarly or if it is influenced by other factors. These controversies arise mainly due to their study limitations; for instance, having only a single cohort would lead to contradictory results among studies as each would have a different type of cohort.

## 2.3 Psychological and cognitive health

Running has more than just physical benefits for our body. It has been shown that running activates the cortical areas associated with mood regulation. During running, the prefrontal cortex, a brain region implicated in cognition and mood regulation, is partially involved through the reticular activating system (RAS) of neuron in the brain, as it regulates ascending projections to the prefrontal cortex, improving mental health (21). During running, several muscles are being coordinated, which activate the prefrontal region of the brain. The mechanical impact of each foot-strike during running has been shown to increase blood circulation peripherally and centrally, which may benefit brain activation (21). Furthermore, there are several hypotheses suggesting psychological well-being due to running: (i) The affect-regulation hypothesis posits that exercise has dual effects that increases positive affect and reduces negative affect concomitantly (27), (ii) a thermogenic hypothesis postulates that exercise-induced increase in body temperature triggers a relaxation effect with a reduction in anxiety (20). Research also shows that hormones like dopamine, serotonin, and noradrenergic activity are activated in the central nervous system. Evidence shows the augmentation of  $\beta$ -endorphins, peptides which are released

by the pituitary during any form of stress/exercise that arbitrates euphoria and reduces post-run pain (20). A study on twenty-six young Japanese athletes also reports that even a 10 min single bout of running may trigger inhibitory control (21), a core executive function that allows an individual to limit their unwanted behavior or actions by being able to inhibit natural stimuli like one's attention, behavior, thoughts and emotions to override strong internal predispositions or external lures, thus enhancing self-control (28). Finally, the dosage of running that confers most psychological benefits has been investigated by several research studies in the past. The results suggest rather surprisingly that running at self-desired intensity and pace produced the most psychological benefits like increased calmness and enjoyment and decreased rate of fatigue (20). Another research study involving 30 participants who practiced exercise either regularly (around 5 days/week) or irregularly (1-2 days/week) found that running or practicing PA 4 in a static/dynamic nature setting (a static image or dynamic video of nature was presented) showed greater rates of happiness as compared to self-desired setting such as in a gym while listening to music/watching a movie (29). However, other emotions like reduction in anger, dejection, and anxiety were the same in both settings. This suggests that the environment has a correlation to the benefits obtained by running, but since the settings used for testing this hypothesis were just images/videos, further studies would be required to back up these results. Lastly, psychological benefits of running differ from person to person based on level, past experiences, future expectations, desire and several other self-driven factors; therefore, the placebo hypothesis posits that the people's expectations regarding the psychological benefits of exercise are influential in the emerging results, which seems to be applicable in this case (20). To conclude, running has been shown to improve mood and assist in improving functioning of the prefrontal region of the brain.

#### 2.4 Gene regulation, pathways and running (an overview)

The above-mentioned literature gives a brief insight as to how running can be beneficial for health and how the benefits might be negated if not careful. The basic components of a human body are however genes, and it would be more beneficial to understand how genes are regulating: before exercise(T1), during exercise (T2) and after exercise (T3), and also compare these variations among different groups: marathon runners, ultra-runners, veteran athletes, young athletes, cyclists, recreational runners, elite runners, sprinters, and non-athletes. Furthermore, it is important to understand the effects of running on different genes and its regulating pathways like iron, carbohydrate and oxidation (2,4,6,7). During running several genes come into play, some are up regulated, and some are down regulated; sometimes it's beneficial, while sometimes it's not. Genes also influence the ability to perform and are hereditary. It has long been suggested that genetic background plays an important role in sporting potential, by being responsible for determining the anthropometric, cardiovascular, and muscular characteristics of adaptation to physical training thus making some bodies more suitable for the sport (For example: "The African runners phenomenon"- according to which African athletes have special adaptations to the training making them dominate this sport).In fact, over 200 genetic variants have

been identified to come into play in this phenomenon alone(30). The likelihood of becoming a sprinter (elite runner) has been studied in the past and researchers have observed that promising candidate genes like ACTN3 (sarcomeric protein  $\alpha$ -actinin-3 in skeletal muscle fibers) have a role in influencing the same- a high frequency off the 577RR phenotype and a concomitant low frequency of the 577XX ( $\alpha$ -actinin-3 deficient) phenotype regulate the influence. No Olympic-level sprinter has yet been identified with the 577XX genotype, and in fact, a lower frequency of this genotype prevails among elite sprinters from various countries across the globe. Another candidate gene associated with elite performance is the ACE I/D which regulates blood pressure and the skeletal muscles. The prevalence of the ACE D allele allows higher angiotensin II levels and higher proportion of fast, glycolytic, type 2X muscle fibers which affects sprinting performance. This has been proven by a study conducted on 346 elite pure sprinters from Australia, Brazil, Greece, Jamaica, Italy, Lithuania, Poland, Russia, Spain and US(31). Regardless of being a professional athlete or an amateur, runners in general have several genes that are up and down and regulated during running, thus influencing metabolic pathways. A study conducted on sprinters showed the up regulation of several genes which stimulate a particular metabolic reaction (32).

For example, during sprints, there is an up regulation of the IP3 receptor ITPR3 mRNA and of ITPRIP mRNA, which has been shown to modulate IP3-receptor sensitivity for calcium (32). Similarly, there are other receptor and stimuli which are each associated with genes, the genes regulated may however differ in marathon runners, so each pathway being studied has its own set for example the gene regulating iron metabolism includes (PCBP1, PCBP2, FTL, FTH, and TFRC) (7); while the genes expressions involved in carbohydrates and placebo ingestion are IL-1 $\beta$ , IL-6 (2). One study did a whole blood gene analysis in athletes(cyclists) and non-athletes and found 393 genes that were differentially regulated between the two groups at all times (4). Forty-six were different before exercise (T1), 467 immediately after exercise(T2) and 234 after 24 h recovery(T3). Likewise, these genes are associated with some pathways. Specifically, the researchers found correlations with 'oxidative phosphorylation' as one of the top ranked pathways to be regulated for this dataset, which is not surprising as exercise involves aerobic movements and improves blood circulation, moreover, oxidative phosphorylation is the final stage of cell respiration which is key during any activity, thus highlighting their importance for the performance of an exercise, and maintaining general physical health of the body (4). In summary, the regulation of genes might alter, enhance or abruptly stop a pathway, and all the factors such as longevity, heart diseases, aging, mood are the outcomes of these gene regulated pathways. This paper summarizes the mentioned pathways focusing on how they perform differently in runners when compared to non-runners and athletes.

### 3. Methods and Materials

The author has considered reviewing the results and methods of previous studies about running, genes and pathways to determine the side effects they cause besides being beneficial

to health. The papers that are referenced are from sites like PubMed, Google Scholar, Science Direct, accessed 2023. The key search words used include: “running genes”, “metabolic pathways”, “transcriptomics”, “PBMCs”, “Ultramarathon”, “recreational runners” and more such terms. Further, to dive deeper into the details in the paper reviewed, the references were looked into to identify relevant papers that contribute to the topics discussed here. The results obtained in different papers vary to some extent because the participants in each paper have different levels of fitness, participate in different events and also differ in age, and gender. Demography might also be a factor of influence. For the benefit of understanding the diversity of the side effects in different cohorts, tables have been created to summarize the findings and draw plausible conclusions. Considering diversity as an important factor to highlight modifications of genes and pathways in runners, the author considered papers that do not include runners as participants as well to highlight the differences between runners, other athletes(cyclists), and non-athletes. Table 1 gives the

summary of the subjects that have been considered from several studies reviewed in this paper. The types of studies included are mainly related to genetics– gene regulation– and pathways like metabolic, oxidation, iron; however, studies elucidating cardiovascular diseases, longevity, psychological and cognitive benefits of running have also been selected for giving a complete comprehensive understanding of the benefits and risks of running in humans.

4. Results

To support the argument that running has several side effects: positive and negative, several papers part of this review have been summarized in more detail. The tables below show important factors that were determined by the studies; their role, and the method of analysis they used, cohort and size, and the age of participants. These tables are expected to help in better understanding of the data.

**Table 1:** This table summarizes the subjects and methods of the papers surveyed. Furthermore, it gives the key findings as well. \*Vo2 max was given as either mean value or in some cases the percentage of the vo2max peak at which the participants performed

Participants (*n = number of participants )	Age (years)	Vo2 max* peak (mm/kg/min)	Author	Methods of analysis	Time of sample collections
n=8 7-males, 1- female				composition assessment, muscle biopsy.	T2- after 7-day ultramarathon
Non elite marathoners n=47 28-male,19-female	39.0 ± 7.1	-	Canto et al, 2018	Saliva and blood analysis	21 days (about 3 weeks) before marathon and after marathon participants were asked to report symptoms
Ultra trail runners n= 17 12- male, 5-females	38.2 ± 4.3 (male) 35.6 ± 2.2(female)	-	Maqueda et al, 2017	Microarray, RNA tests for gene analysis	T1- 1 day before the run T2– 2days after the run T1- Prior to running T2– immediately after running
untrained healthy men	20 ± 1	63% peak	Febbraio et al, 2000	RT-PCR test, mRNA and tissue analysis	1 familiarization test followed by experimental procedures after 1 week
n=5 Mount Olympus marathon runners	38.4 ± 8.3	50% max	Tsianos et al, 2009	Genotype and phenotype analysis	Samples collected only once after race
n=438 417- male , 21-female 268-participated in 2007, 170-participated in 2008					
Amateur male marathon runners n=20	29.42 ± 4.51	-	Shi et al, 2020	Serum analysis, metabolite extraction LCMS/MS test	T1- 1 day prior to race T2– 1hour after the race
Healthy active male students n=7	21.3 ± 2.5	51.2 ± 7.1	Bury et al,1995	Plasma interleukin I and interleukin 2 determinations, leukocytosis analysis	After insertion of the catheter. The subjects rested for approximately 1 hour before tests started.
ultra-marathon runners n=14	43.3 ± 6.0(male)	-	Atamaniuk et al, 2008	Cell separation, Hematological procedures, RT PCR tests, RNA tests, DNA isolation	T1- before each race T2– after a 6hr race T3-2 hours after T4-after 2hrs
9-male , 5-female	51.6 ± 7.6(female)	-			
Male experienced marathon runners n=10	19-25	70-80 %	Cooper et al, 2008	Muscle biopsies, biochemical analysis	T1-before race T2– immediately after race

Significance of VO<sub>2</sub> max to endurance exercise

VO<sub>2</sub> max is the volume of oxygen that the body can absorb and use during exercise (Everything to Know About VO<sub>2</sub> Max, 2020). A more experienced athlete would have a

greater VO<sub>2</sub> max compared to the untrained group. Thus, it is an important indicator that reflects the endurance of athletes as it is positively correlated to the athlete's aerobic fitness which determines their performance in any race.

Table 1 gives the mean VO<sub>2</sub> max peak of athletes in various studies indicating clearly that VO<sub>2</sub> max of athletes differ from control cohorts and depend on the exposure to endurance levels. The vo<sub>2</sub> peak test or vo<sub>2</sub> % was considered among most if not all studies as it is an important marker of physiological health and exercise. Interestingly, regular running at 50% of VO<sub>2</sub> peak has positive psychological benefits: activating cortical areas associated with mood regulation (21). Previous studies have also associated the VO<sub>2</sub> values with serum metabolism. (Metabolites: Cis-aconitate, galactonic acid and mesaconic acid). Furthermore, when the % of Vo<sub>2</sub> max consumption increases, the effort and therefore the strain on the body increases, which causes several metabolic changes and gene regulations; for instance, cortisol levels rise with strenuous exercise. Therefore, considering VO<sub>2</sub> levels is essential to this paper as it is an indicator of physical fitness which determines the magnitude of the side effects be it positive and/or negative (Shi et al., 2020).

**Gene regulation and metabolic changes in non runners (veteran) but athletic group**

Exercise is key to health at old age. Athletes in the older age groups indeed show positive correlations with differential regulation of genes coding for proteins regulating important pathways in humans like “oxidative phosphorylation” and “cell adhesion-integrin” which are complementary to a healthy body (Mukherjee et al., 2014).

Firstly, metabolic measurements suggest that athletes have a healthier lipid profile, better cardiometabolic and better aerobic fitness profile which correlates with lesser probability of diseases like obesity, CVD, and respiratory disorders (Mukherjee et al., 2014). Then, several genes are differentially regulated between athletes and controls the top ten functions that these genes regulate according to matcore analysis are: Development Blood vessel morphogenesis (angiogenesis resulting from exercise training contributes to improved capillarization, oxygen exchange and blood flow capacity in skeletal muscle and might contribute to cognitive function.), Cell adhesion Leukocyte chemotaxis, Cytoskeleton Intermediate filaments(reflects the physiological impact of exercise training as skeletal muscle

structure and composition change with advancing age), Chemotaxis, Cytoskeleton Spindle microtubules, Inflammation IL-4 signaling, Cytoskeleton Actin filaments, Proteolysis in cell cycle and apoptosis, Immune response Phagocytosis, and Immune response pathway(Mukherjee et al., 2014)).

Additionally, the same study found three genes differently regulated at all three times of measurement between the athletic and control group. These genes were: SLC5A11, OLFM4 and LPIN1 other genes were mainly different at T2 - just after exercise. These results were consistent within the study. However, a similar study conducted on runners only would be necessary to make any conclusions as these genes are important indicators of several diseases/functions like cancer, fat metabolism, and blood vessel dilation/constriction. Table: 2 displays the key genes in disease indication and the corresponding results (Mukherjee et al., 2014))

Lastly, the results obtained from the NMR spectromacy test and urine analysis suggest that the excretion of several metabolites was different in the post-exercise period compared to pre-exercise; lactate excretion is elevated ~4 fold in athletes and ~7 fold in the control groups 24 hours post-exercise, acetate(formed in a radical-removing reaction of hydrogen peroxide) was excreted to a greater extent in the control group, a finding that is consistent with reduced oxidative stress in well trained individuals (Mukherjee et al., 2014)). Few others were fumarate (higher in athletes), malonate (higher in controls) which have key functions in the process of succinate oxidation and TCA cycle (Kreb’s cycle), and hypoxanthine also greater expression in athletes influences greater ATP turnover required to complete the higher absolute work performed by the athletes(Mukherjee et al., 2014)).The increased lactate and hypoxanthine is consistent in runners as well (Fehrenbach et al., 2000; Shi et al., 2020). TCA cycle also seems to be a commonality as it is associated with energy synthesis which would be a necessity for any form of PA((Fehrenbach et al., 2000; Shi et al., 2020)).

**Table 2:** There are several changes in genes post an exercise, yet only few are significant. The table highlights the key genes that were found in the study and their importance to humans (Mukherjee et al., 2014),44)

Gene	Importance	Regulation
SLC5A11	Sodium dependent glucose (myo-inositol) transporter.	Found higher in the control group
OLFM4	An antiapoptotic factor known to act as a marker for colorectal and gastric cancer,supporting the hypothesis of a potential benefit of exercise training against these tumor types	Decreased in athletes
LPIN1	Role in phospholipid metabolism. Additionally, it contributes to enhancing insulin sensitivity and glucose metabolism in healthy men, and exercise induced mitochondrial *biogenesis in skeletal muscle.	More prominent in athletes.
UTS2	Gene Ontology (GO) annotations suggest a role in signaling receptor binding and hormone activity. In fact this gene encodes a mature peptide that is an active cyclic heptapeptide and acts as a vasoconstrictor, it is expressed only in brain tissue.	Higher in athletes (evident from better vascular health).
HSD11B1	Gene Ontology (GO) annotations suggest a role in oxidoreductase activity and 11-beta-hydroxysteroid dehydrogenase (NADP+) activity. It encodes a microsomal enzyme that catalyzes the conversion of the stress hormone cortisol to the inactive metabolite cortisone and its expression is correlated with rate of obesity.	Lower in athletes (its increase is negatively correlated).
OCLN	This gene encodes an integral membrane protein that is required for cytokine-induced regulation of the tight junction paracellular permeability barrier. OCL Ninthe athletes possess a potential protection against gastrointestinal and other geriatric dysfunctions	Higher in athletes
IGFIR	This receptor binds insulin-like growth factors with a high affinity and has tyrosine kinase activity. It	Lower in controls

	is an important gene in transformation events of tumors in malignant cells where it also acts as an anti-apoptotic agent increasing cell survival.	
INSIG2	These encode endoplasmic reticulum (ER) proteins that block the sterol regulatory element binding proteins (SREBPs) by binding to SREBP cleavage-activating protein (SCAP). Therefore, it plays a role in lipid / cholesterol regulations.	Lower in controls
VASN	Enables transforming growth factor beta binding activity	Lower in controls
DOCK4	Encodes a cytoplasmic protein which functions as a guanine nucleotide exchange factor and is involved in regulation of adherens junctions between cells. It is associated with autism	Lower in controls
NRXN1	It is an efficient neurotransmission and is involved in the formation of synaptic contacts.	Lower in controls
VIPR2-	It is involved in smooth muscle relaxation, exocrine and endocrine secretion, and water and ion flux in lung and intestinal epithelia.	Lower in controls
TRPC1	Encodes proteins that form channel permeable to calcium and other cation in cell membranes	Lower in controls
MYH4-	Functions in enabling double-stranded RNA binding activity. Involved in muscle contraction.	Lower in controls
UQCRB	Encodes a protein that plays an important role in hypoxia-induced angiogenesis through mitochondrial reactive oxygen species-mediated signaling.	Lower in controls

### Regulations and effect on proteins (HSP and Shelterin complex)

Heat shock proteins are a family of several proteins with protective roles in the body, affecting endurance training with increased expression. Since, it plays a protective role in leukocytes against exercise induced stress, prepares the body for adaptation to various environments and regulates cellular homeostasis, indicates selective mechanisms in protein conservation, and activates the immunocompetent cells; the exercise induced increase in these proteins genes may be beneficial side effect of running in athletes (Fehrenbach et al., 2000).

The HSP proteins “represent cell-protective and antioxidant systems that may be induced by reactive oxygen species, cytokines, and hyperthermia” (1). They are activated on cellular stress/injury and oxidative/heat/cytokine stress, and have a special function as a protein due to their participation in the folding and intracellular transport of damaged proteins. Running causes augmentation of these proteins which lasts longer than 24 hours thereby activating immunocompetent cells such as monocytes and granulocytes, capable of producing reactive oxygen species (ROS) under physical stress and oxidative stress, as seen with rise of IL-8, TNF- $\alpha$  levels thereby, increasing the immune response (defense mechanism functioning) in our bodies. The augmentation of these 2 genes is consistent with results from other papers (carbohydrate ingestion) (Fehrenbach et al., 2000; Nieman et al., 2003). Other plausible inducers of HSP may be hyperthermia (rise in body temperature) and metabolic stress ((Fehrenbach et al., 2000),(Febbraio & Koukoulas, 2000).

A study comparing trained vs. untrained persons(amateurs) revealed different HSP mRNA expression after applying a heat shock of 42°C for two hours to their blood samples (Fehrenbach et al., 2000; Nieman et al., 2003)). The trained athletes had a much greater rise in HSP27 and HSP70 as compared to the untrained group. The rise in levels was similarly detected in flow cytometry test and RT/PCR test as well. Thus, substantiating the hypothesis of hyperthermia being an inducer of HSP, and the adaptive response of HSP to training (Fehrenbach et al., 2000; Nieman et al., 2003)).

Table 3 gives detailed roles of these HSP genes in human bodies (Fehrenbach et al., 2000; Thompson et al., 2003).

Another HSP gene that seems to be linked to exercise is HSP72. Studied closely by Febbraio et al, this gene is apparently influenced by increased temperature, ischemia, protein degradation, hypoxia, acidosis, reduced glucose availability, oxyradical formation, and increased intracellular Ca<sup>2+</sup>(Febbraio & Koukoulas, 2000).Five normal men were considered in a study and made to cycle; the levels of muscle temperature (T<sub>m</sub>) and lactate seemed to be elevated within the first 10 minutes of exercise however there was no effect on the HSP72 levels during this time as expected by the author instead, its augmentation was noticed only after ten minutes when T<sub>m</sub> levels did not increase and lactate decreased. This may suggest that the duration of exercise is an influencer of HSP activation. Another study however, saw augmented levels within 4 min, which exacerbated 30 min after recovery and continued to remain high even after 3h of recovery; yet, increase in the gene transcription, surprisingly did not increase protein translation (Vogt et al., 2001). Since, there are limited studies studying this gene and its effects, further studies would be required to make any valid conclusions. Moreover, the studies above used different types of exercise in their experiments, so it is suggested that HSP gene expression during exercise is evaluated in exercise that are concentric or non-weight bearing in nature as well to investigate whether cellular events other than muscle protein degradation or damage can induce a heat shock response or if it is limited to exercises like running (Febbraio & Koukoulas, 2000). One study also suggested that swimming caused no HSP amelioration possibly because it does not generate any heat in the body (Hammond et al., 1982). The HSP32 gene was also seen to be augmented after an ultramarathon and together these Heat Shock genes behave as cell protective genes from apoptosis in mononuclear blood cells (Atamaniuk et al., 2008), thus these are some positive side effects of running, but the exact function of differential regulation of HSP at the transcriptional and translational level in response to exercise and to experimental heat stress, remains to be investigated (Fehrenbach et al., 2000; Nieman et al., 2003)).

**Table 3:** This table compares the HSP 27 and 70 gene/protein for better understanding their roles (Fehrenbach et al., 2000; Nieman et al., 2003) (Fehrenbach et al., 2000; Thompson et al., 2003) (Fehrenbach et al., 2000; Thompson et al., 2003))

	HSP27	HSP70
Expression of mRNA levels in athletes	Down regulated at Basal level compared to untrained group but stimulated on heat shock of 42°C. There was a significantly low positive mono and granulocyte expression of this gene in athletes but could be stimulated by running	Up regulated at basal level compared to untrained group and stimulated expression on receiving heat shock of 42°C There was a significantly low positive mono and granulocyte expression of these gene in athletes but could be stimulated by running
Role	Affects actin dynamics by influencing polymerization and depolymerization during times of stress and may also act as a molecular chaperone	Facilitate the proper folding of labile proteins, protect proteins from unfolding during stress and prevent aggregation of already unfolded proteins.

**Shelterin complex, longevity and running**

The earlier hypothesis of longevity influenced by running can be supported through these intracellular factors such as the telomere length influence in physiological aging (Laye et al., 2012). The shelterin complex is a group of proteins which are key in regulating the length “through allowing access to positive and negative regulators of telomere length in both telomerase-dependent and -independent mechanisms (Laye et al., 2012).” Physiological aging is associated with decreased telomerase activity and telomere length in leukocytes and skeletal muscle. In a study on ultramarathon runners mRNA levels of the shelterin complex in both PBMCs and skeletal muscles were altered although there were no changes in skeletal muscle telomerase activity or telomere length .This suggests that some correlation between physiological interventions and proteins in the shelterin complex exists; Yet, surprisingly, stress post ultramarathon had no direct effect on mean telomere length suggesting that the transcriptional regulation of the shelterin complex in muscle may respond to other factors like pathological insults (disease) rather than physiological stimuli(exercise)(Laye et al., 2012).

The hterc and httert (the telomerase subunit/ telomere catalytic subunit.), which are subunits of telomerase also involved in the alteration of telomere length in general but there was no modification noticed after the marathon event

for these subunits or for the other proteins in the shelterin complex, when compared to pre marathon levels. However, the KU70/80 protein complex expression, protein complex interacting with shelterin complex, was increased pre vs post marathon. This complex is altered both in PBMCs and the skeletal muscles; a potential mechanism hypothesized is the influence of endothelial nitric oxide (eNOS) whose expression is increased by endurance expression (Laye et al., 2012).

On the other hand, mRNA levels of certain genes of the shelterin complex showed modifications in PBMCs. Endurance athletes have increased TRF1 and TRF2 mRNA in circulating PBMCs, prolonged exercise increased this expression. The data in PBMCs suggest that multiple components of the shelterin complex are upregulated at the mRNA level in response to physiological stresses such as exercise (Laye et al., 2012).

While further studies are required for better understanding, telomere length is an intrinsic factor that contributes to the increased or decreased longevity, proving that the benefits of running to longevity is dose related because the dosage directly correlates with expression of the genes. Table: 3 shows the observations of certain genes of shelterin complex and associated protein complex(KU 70/80), and their role in respect to telomere and longevity (Laye et al., 2012).

**Table 3:** Genes in the protein complex that play a role in determining longevity (Laye et al., 2012)

Gene/ Protein	OBSERVATIONINPBMCANDSKELETALMUSCLE	ROLE
TRF1 and 2	Higher in Pbmc lysate. The expression was augmented in cancerous gastric surgery tissues.  Lower expression in isolated endothelial cells of coronary artery disease, internal mammary arterial segments for TRF1 while Statin treatment (used to treat high cholesterol) of cultured endothelial cells, originating from healthy donors saw higher expression in TRF 2.	Increased expression is associated with maintaining telomere length in veteran runners.
Pot-1	The expression of these proteins was increased in PBMCs but not in the skeletal muscles.	Binds single-stranded telomeric DNA and can either act as a positive or negative regulator of telomere length and telomerase activity depending on the experimental conditions, increases in PBMCs.
Ku70	Higher in Pbmc lysate, directly proportional to the amount of physical exercise therefore was higher in older subjects	Helps repair double-stranded DNA breaks in response to DNA damage interacting with the shelter in complex
Ku80	Higher in Pbmc lysate, directly proportional to the amount of physical exercise therefore was higher in older subjects	helps repair double-stranded DNA breaks in response to DNA damage interacting with the shelter in complex

**Serum Iron metabolism relations with running**

Iron, as a component of the prostate group in the hemoglobin, plays an important role in the transport of oxygen through RBCs. The rate of transportation of oxygen elicited in athletes thus making it plausible that iron pathways are

regulated differentially. A study tried to evaluate the changes in serum iron and ferritin concentrations together with the changes in leukocyte mRNA levels of genes encoding proteins involved in iron metabolism (Grzybkowska et al.,

2019). No significant changes in ferritin concentrations were observed, but in serum, concentrations were observed to have changes 3h after running. An interesting protein-serum hepcidin-had increased expression, indicating increased iron synthesis from liver cells due to low blood serum iron. This could mean that running leads to increased iron usage thereby decreasing its levels in blood 3h post run, which may be a negative side effect, until the levels return to their normal values 24 hours later. Furthermore, the specific genes whose mRNA levels were changed are PCBP1,PCBP2, FTH, FTL, and CAT. The PCBP 1,2 are iron chaperones that deliver iron to ferritin (iron storage protein). It is expected that an increase in the expression of these genes might play a protective role against iron toxicity; the CAT gene is associated with an increase in antioxidative capacity, thus its expression indirectly shows the level of oxidative stress in the cell which seems to be increased 3h post run; the FTH, FLT encode heavy and light subunits of ferritin protein respectively (Grzybkowska et al., 2019).

All genes except FTL were downregulated immediately after the marathon, and all genes were elevated 3h post marathon; however, these were temporary changes as they all returned to baseline levels after 24 h. Interestingly, the return to basal values occurred faster in faster runners than in slower runners. Yet, further research would be required to understand the influence of speed. Contrarily, the TFRC gene which encodes a cell surface receptor necessary for cellular iron uptake by the process of receptor-mediated endocytosis remained stable throughout the run and recovery, in fact the levels were significantly lower than the other genes at all times. This may be due to controlled intracellular labile iron proteins (Grzybkowska et al., 2019).

#### ***Changes affecting cytokines in runners***

Cytokines are defined as secretions from certain immune cells that affect other cells. These secretions may be interleukins, growth factors or interferon (signaling proteins) and are essential in regulating metabolic functions in our body (Nieman et al., 2003). Researchers have previously analyzed certain commonalities between running and cytokine levels in skeletal muscle cells such as IL6, IL8, IL10 IL1- ra, IL-1, and IL-2 (Bury et al., 1996; Nieman et al., 2003). A plausible explanation for exercise-induced modification in cytokines could be related to enhanced cytotoxic activity observed after exercising and this could in turn cause muscle degradation((Bury et al., 1996; Nieman et al., 2003)) which makes excessive running a risk to cytokine functioning; Furthermore, past studies have associated the effects of running to the duration and endurance intensity, studies hypothesized that intensity may be a modifier of the running -cytokine effect in runners. Experimenting with different levels of intensity, 45%, 60%, 75%  $VO_{2max}$ , they found a correlation with some cytokines while most were independent of intensity((Bury et al., 1996; Nieman et al., 2003)).

Carbohydrate ingestion is an example of one pathway related to cytokine activity. According to multiple studies, epinephrine is an influencer of cytokine release, and carbohydrate ingestion blunts epinephrine release by increasing blood glucose. IL6 cytokine, the primary focus under the influence of carbohydrates or placebo post run, has been observed to be released by muscle and peritendon in the contracting limb and brain, and cleared by liver after exercise((Nieman et al., 2003)) according to a team from the Copenhagen Muscle Research Center. Additionally, some studies have hypothesized that muscle glycogen availability may also influence key signaling molecules to enhance IL-6 gene transcription within skeletal muscle during altered homeostasis (Febbraio & Pedersen, 2002; Nieman et al., 2003).”

In a study on marathon runners, athletes performed a three-hour treadmill run with placebo and or carbohydrate being supplied periodically every 15-30 minutes ((Nieman et al., 2003)), the increase of cytokine was attenuated, more in the carbohydrate condition compared with placebo condition suggesting the probable role of carbohydrates in decreasing cytokine secretion as a result of running. The pattern of change in plasma glucose and insulin was also significantly different between conditions, with post run levels higher in the carbohydrate condition. These changes were not to be seen in participants who were just sitting (control group) with an exception of TNF- $\alpha$  gene, suggesting that running influences cytokine activity due to damage in cells/tissues ((Nieman et al., 2003)). In many cytokines, although exercise influenced their expression, carbohydrate ingestion had no effect, for example: IL-1 $\beta$  mRNA. This suggests that this proinflammatory cytokine is countered by a rapid anti-inflammatory response that successfully combats its elevation in the plasma. Further studies should investigate if such anti - inflammatory responses could be stimulated for other cytokines as well ((Nieman et al., 2003)). This study proves the role of carbohydrate ingestion in attenuating exercise induced cytokine levels only in the secondary proinflammatory cascade (IL-6, IL-8) but not the primary (IL-1  $\beta$  and TNF-  $\alpha$ ) suggesting that carbohydrate supply reduced the rate of cellular/tissue damage as, it decreases the secretion of cytokines and its secondary proinflammatory cascade(which is supposed to be harmful). It had a slight influence in cortisol and anti-inflammatory indicators (IL-10, IL1-ra) as well but not a significant one. Therefore, there is a positive correlation among cytokines and carbohydrate metabolism. The intensity of exercise being a chief influencer, is however, still controversial; IL-1,IL-2 is seen to be influenced by the level of intensity along with the WBC's like monocytes, leukocytes and lymphocytes((Bury et al., 1996; Nieman et al., 2003)).Table 4 gives a more detailed overview of the roles of cytokine/Blood mononuclear cell (BMC) along with their modifications after running(Bury et al., 1996; Nieman et al., 2003).



**Table 4:** Changes in certain cytokines and BMC

BMC/cytokine	Description	Observations
Monocyte	A type of WBC responsible for engulfing bacteria	Monocyte count was found to increase slightly during exercise but significantly after exercise. Yet, the monocyte count was not clearly influenced by the duration or the intensity of prolonged exercise. However, during recovery, the value returned to resting values in test (45% Vo2) but remained elevated in tests 2 and 3. (60% and 75% VO2).
Leukocytes	WBC involved in immune regulation	The leukocyte counts also seemed to increase during exercise primarily due to neutrophil count rise, it returned to basal value during recovery. Lymphocytosis occurs regardless of the intensity, after the 10th minute
Lymphocytes	WBC fighting antigens, immunity related.	Lymphocytosis occurs regardless of the intensity, after the 10th minute of exercise and this increase has no apparent correlation or association to the type of exercise. Surprisingly, the levels of lymphocytes decrease to below basal levels during recovery suggesting the influence of stress hormones.
Il-1	“IL1, is essentially produced by cells of the monocyte/macrophage series in response to a variety of stimuli ranging from bacterial products to immunological factors. Human IL, exists in two functionally active forms: IL1 $\alpha$ and IL1 $\beta$ ”	There was a rise in Il-1 levels, and it continues to increase even 1 hour after exercise. Intensity modified significantly the magnitude of the mean IL levels. The il-1 plasma level increase is therefore directly correlated to the intensity,
Il-2	“The activity of IL is not confined to T cells. It can act as a growth and differentiation factor for B cells and NK (natural killer lymphocytes) cells and can activate macrophages.”	There is a drop in the Il-2 levels after exercise and during recovery, but it is not associated with magnitude or exercise. However, the mean level was associated with lymphocytes.
IL-8	It is released by macrophages post injury and is involved in neutrophil activation and is released from several cell types in response to inflammation, including monocytes, macrophages, neutrophils, and intestine, kidney, placenta, and bone marrow cells. Therefore, it’s a mediator of inflammatory cell response.	Up- regulated in athletes
TNF-	TNF(Tumor Necrosis Factor) is a Protein Coding gene that is secreted by macrophages and binds to and functions through its receptor: TNFRSF1A/TNFR1 and TNFRSF1B/TNFB. Asthma and malaria are associated with this gene.	Up- regulated

**Carnitine influence on running ability and the powerhouse of cells**

Carbohydrates and fats are sources of energy and therefore it is no surprise that runners, especially marathoners, require a diet high in carbs and essential fats as well to sustain them during running. During long duration running, the mobilization of fatty acid as an energy source is proportionally increased so as to compensate for the relative insufficiency of the reserve of carbohydrates. Unfortunately, the elevated energy metabolism leads to proteolysis which may cause imbalanced protein metabolism due to the deamination of amino acids. It is thus essential to also understand the effects of running on carnitine metabolism - an essential cofactor in the catabolism of fats(Cooper et al., 1986).

Besides fat metabolism, carnitine, along with co-factor acetyl carnitine, can stimulate pyruvate oxidation from isolated human skeletal muscle mitochondria, playing a role in muscle metabolism. Previous studies have found that free carnitine decreases in muscles after running- due to excessive usage of fats but at the same time they also suggest that increased fat utilization by muscles for oxidative purposes may not be the sole cause for reduction in plasma carnitine as - “carnitine as a fat transporter is an essentially cyclic process - carnitine is being returned to the cytosol at a rate dictated by the intramitochondrial usage of acylcarnitine esters concomitantly plasma carnitine content also falls to

accommodate extra acetyl carnitine formation(Cooper et al., 1986).” Superoxide dismutase is an essential enzyme for the neutralization of free radicals, which increase post heavy exercises. This enzyme may be inhibited negatively by hypoxia or other inhibitory metabolites, but this change in superoxidase is uncommon in experienced athletes (Cooper et al., 1986) suggesting that they have developed adaptive mechanisms to prevent reduction of superoxide dismutase production which is beneficial as it is responsible for the free carnitine; yet, this was not observed in a study on-endurance marathon athletes plausibly because of their adaptation to the training(Cooper et al., 1986)). For example: during running an athlete is expected to utilize fats as a muscle fuel to a greater extent than an untrained individual which is brought about by adaptive changes to the complement of the mitochondria (Cooper et al., 1986).

They also suggested the loss of cristae and mitochondrial swelling with excessive exercise which could mean adverse effects on tissue oxidation Furthermore, the glutathione in muscles had some altering following a marathon, either due to alterations of NADPH levels or glutathione peroxidase. One study tried to supply carnitine orally and see if that had any increased performance benefits in athletes during the run. While, there were no performance benefits, it abolished the fall in plasma-free carnitine that was seen at the end of the control run, and stimulated a much greater extent of acetylcarnitine production which is beneficial as it

diminishes exhaustion and boosts recovery by retaining carnitine levels in the body. This therefore indicates that very plausibly, carnitine has a positive effect on athletes. Table 5

summarizes the findings of supply of oral carnitine to marathoners vs no supply (Cooper et al., 1986).

**Table 5:** Carnitine and mitochondrial activity after marathon in a study on athletes.

Factor	description	Without supply of oral carnitine	With supply of oral carnitine
Carnitine status in plasma	Carnitine ( $\beta$ -hydroxy-y-trimethyl aminobutyrate), which is present either in its free form or as an ester which may be formed with long chain fatty acids (e.g., Palmitoyl carnitine), plays an important role in the transport of fatty acids into the mitochondrial matrix for metabolism by the $\beta$ -oxidation pathway.	Fall after exercise	No change differential changes after exercise as compared to without supply
Acetylcarnitine	Are formed from short chain fatty acids and may be involved in metabolic control	Increased after exercise	Increased even more substantially after exercise
Mitochondrial activity	Essential for production of energy. Cellular respiration	No observable changes with exercise or carnitine loading were found	
Glutathione in muscles	An enzyme essential for muscle growth and repair	Whilst exercise did not alter the total (oxidized+reduced) glutathione content of the muscle, there was a large increase in the amount of oxidized form present	

### **What an ultra-marathon/EEE can do to genes and other pathways**

The optimal physiological phenotype of athletes competing in long-duration events probably entails an inherent genetic makeup conferring cardiovascular, pulmonary, and skeletal competence to perform during such events and efficient metabolism of available substrates to sustain the performance throughout the event's duration. Furthermore, "PHYSICAL FITNESS is a very complex phenotype influenced by numerous genetic and environmental factors contributing to the observed interindividual variation not only in the general population but also in trained athletes (Tsianos et al., 2010)". Yet, any form of extreme endurance exercise can result in a lot of genomic changes (Tsianos et al., 2010). An analysis was conducted on 17 participants participating in a UMT of which only 3 finished the race (Maqueda et al., 2017). The authors found surprising results, 5,084 protein coding genes resulted in an overrepresentation of 14% of the human biological pathways from the Kyoto Encyclopedia of Genes and Genomes database and 27 out of the 196 transcriptional regulators (TRs) included in the Open Regulatory Annotation database; 193 Reactome pathways were also found to be overrepresented, Gene Expression, Immune System and Disease (infectious diseases particularly) were top affected superclass (Maqueda et al., 2017). The results highlight the numerous amount of side effects that could be caused due to overexertion, the fact that only 3 athletes completed the race elucidates the difficulty. The observations of this study would not necessarily apply to all runners, but plausibly to any one over exerting themselves during the run, which would vary depending on the fitness level. The authors of this paper summarized these results by grouping related genes/pathways. Table 4 gives a brief overview of their findings.

Apoptosis or programmed cell death is another effect of excessive exhaustive running due to factors like tissue damage or hypoxia caused by cellular damage and genetic pathway modifications (Atamaniuk et al., 2008). Positive

correlations with increased DNA plasma levels after running and cell apoptosis was seen in marathon runners. Cell-free plasma DNA increased to highest levels immediately after the race and remained increased even 2h after the race, it contained detectable cell free apoptotic DNA fragments in this plasma 2h and 6h after the race however these fragments disappeared during recovery, that is 24hrs after running. Initially CK and muscle damage were associated with cell apoptosis, but there are other plausible mechanisms such as differential expression in certain genes of mononuclear blood cells (MNC)- where, the balance between pro- and anti-apoptotic genes is altered, or "apoptotic and necrotic polymorphonuclear neutrophils (PMNs) might also contribute to cell-free plasma DNA since PMNs in the circulation are significantly increased in subjects exposed to physical stress. Therefore, high post-exercise levels of cell-free plasma DNA is likely due to apoptosis or necrosis of a number of cell types and not solely due to muscle cell damage ((Atamaniuk et al., 2008))." Additionally, cell-free plasma DNA may additionally be derived from leukocytes and HSP protein effect therefore, it is most likely to be a combined effect of the above-mentioned possibilities ((Atamaniuk et al., 2008)).

BAX and BCL-2 are the pro/anti apoptotic genes that are increased in expression 24 h after run, while Bax is increased immediately after run and remains increased, BCL-2 first decreases then increases again after reaching baseline level. High levels of anti-apoptotic Bcl-2 in relation to pro-apoptotic Bax promote survival, whereas the reverse ratio promotes cell death ((Atamaniuk et al., 2008)).

Overall, there are several side effects when it comes to ultra-running or marathon running (extra exhaustive forms of running). These may influence the body in several ways. Table 6 summarizes the findings of several previous studies on the effects of genes and pathways on runners following an ultra-marathon/ marathon (Atamaniuk et al., 2008; Cantó et al., 2018; Maqueda et al., 2017; Shi et al., 2020; Tsianos et al., 2010).

**Table 6:** Summary of the results found in exhaustive runners (marathon and ultra-marathon runners), in terms of, the factors that running affects, the findings of the studies, and the genes affected

Factor	Overview	Genes Associated (TOP)
Proteins	They were found to be over-represented by down regulation of related intracellular genes (42).	Skeletal muscles protein degradation: The autophagy-lysosomal and the ubiquitin proteasome pathways (UPP)
Infectious disease	36% are found to be caused by parasitic, viral pathogens associated with URTI (upper respiratory tract infections) and 27% with other infectious diseases. Implying that its genetic mechanisms are triggered by strenuous exercise (42).	1. Pathogenic.-Epstein-Barr virus infection, Herpes simplex infection and Influenza A 2. parasitic-Toxoplasmosis 3. other respiratory infections Legionellosis, M
HIF (HIF- $\alpha$ , EPAS1) signaling pathways and genes	The up regulation of genes related to the increased oxygen delivery, decrease of oxygen consumption and associated with TR (transcriptional regulators) was noted. In human skeletal muscle studies, HIF-1 is responsible for mitochondrial activity and VEGF (vascular endothelial growth factor) regulation EPAS1 gene activates genes response to hypoxia, specifically those involved in erythropoiesis and angiogenesis (42).	1. Oxygen delivery- TIMP-1, HMOX-1 2. Consumption reduction- HK, ALDOA and PFK2 3. TR-HIF-1 $\beta$ aka ARNT
Other key genes related to cancer, mitochondrial activity and endocannabinoid signaling	Several genes from the ETC which were systematically down regulated post run were reported to have a direct effect of ETS1 in cancer cells in its role of mitochondrial stress and dysfunction regulation. The TP53 gene has major importance in athletes as it not only stands as a stress sensor of the cell such as oxidative stress, hypoxia and nutrient deprivation but also has been related to the regulation of mitochondrial respiration and possible exercise-induced mitochondrial biogenesis through interactions with TFAM in the mitochondria (42).	1. NDUFA9, NDUFAB1, CYC1, UQCRCQ and ATP5A1 (Influence of ETS1) (down regulated). 2. TP53
Immune and autoimmune responses	Four down-regulated genes responsible for encoding major histocompatibility complex (MHC) class II proteins along with the up-regulated TNF gene, matched with overrepresented KEGG pathways, related to immune, autoimmune or alloimmune responses (42).	HLA-DPA1, HLA-DPB1, HLA-DMA and HLA-DRA, (down regulated) TNF (upregulated)
Neurodegenerative diseases	Diseases like Parkinson's, Alzheimer's and Huntington's diseases share a certain common gene which are downregulated genes from the electron transfer chain in the mitochondrion. Along with certain other genes having responsibilities in different pathways like retrograde endocannabinoid signaling and morphine addiction. Osteoclast differentiation and AGE-RAGE signaling pathway in diabetic complications pathways (Maqueda et al., 2017).	1. Signal communication- MAPK3, PIK3R5 and PIK3CD. (upregulated)-hub1PRKACA and ADCY4 (cAMP messengers) GNAI2- hub2. 2. GABRD-(encodes for a neurotransmitter GABA receptor) (Up regulated) 3. OSCAR and AGER-upregulated.
CK, urea and cortisol	Serum biochemical indicators.Urea is the product of protein metabolism The elevated levels indicate that studied subjects were in a state of fatigue after the match. CK could indicate liver damage (6)	Elevated post marathon
CRP-C reactive protein	Acute proteins that are sharply increased following infections or injuries Might indicate liver damage along with others such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH), and cardiac or skeletal muscles damage, such as LDH, creatine kinase (CK) (6).	Elevated post marathon
serum $\beta$ -alanine	Serum metabolic change that is negatively correlated with score (indicators of time taken for completion of race. Higher score- faster timing) ((6)).	Elevated post marathon
Theobromine, theophylline	Serum metabolic change Important metabolite in caffeine metabolism Theobromine can dilate blood vessels and increase urine output, whereas theophylline may relax the smooth muscle of bronchus. Positively related to score. May mobilize fatty acid to provide energy and may be associated with euphoria and the popular theory of "Runners high" (6).	Testosterone decreased significantly
Serum testosterone	Reproductive hormones.They are positively correlated with several serum metabolites. Indicator of functional status and exercise load. positively related only to the pre-exercise but not the after-exercise metabolic product pyruvic acid reflect the physical abilities in the resting state (6).	Cortisol response is enhanced in prolonged exercise
Serum Cortisol	Stimulates catabolic processes. Indicator of functional status and exercise load. They are produced usually under stress as under these conditions the body consumes large amounts of energy. These substances subsequently activate the hypothalamus-pituitary gland-adrenal gland axis, intensifying cortisol production (6)	Increased expression in athletes post run
Pyruvic acid	It is an intermediate product of the metabolism of carbohydrates, lipids and amino acids that plays important roles in the pathways	Increased in athletes post run

	which link glycolysis, lactic acid, acetyl-CoA, oxaloacetic acid, malic acid, and various amino acids. Furthermore, it is linked to both TCA cycle and hexose diphosphate pathway, two important biochemical metabolic cyclic pathways. In the resting state, pyruvic acid was positively correlated in the marathon runners, which could reflect the physical condition of the athletes (6).	
Serum glycerol and glyceric acid	Part of the lipolysis pathway and indicate the proportion of energy provision by the fatty acid was increased (6).	Decreased in athletes post run
(Serine, L-valine) serum alanine, phenylalanine and N-acetyl glutamic acid	Important amino acids that indicate deamination (catabolism) of amino acids due to excessive synthesis of energy (6)	Decreased in athletes post run
Glucosamine, and N-acetyl-glucosamine	It is an important nutrient substance that maintains the structure of joints and cartilage. Studies show that the level of glucosamine was reduced in the process of aging, particularly when the function of the joint was degenerated (6)	elevated post run in athletes
L-asparagine and hypoxanthine	Hypoxanthine is a metabolic product of purine metabolism. When the energy status is negatively balanced such as in this marathon, the serum level of hypoxanthine is elevated thus it may be regarded as an important indicator of physical fitness (6)	In participants with LRTI, The lysozyme count increased while in those who did not develop LRTI, the count decreased post run. An increase in salivary lactoferrin, one of the most abundant AMPs, has been described in participants in a 50 km ultramarathon.
Salivary lysozyme and lactoferrin (sAMPs)	They protect the respiratory tract from invading microorganisms and have been linked with an increased infection risk in athletes (3).	Decreased participants who did not develop LRTI
Gro $\alpha$ , Gro $\beta$ and MCP-1 (Chemokines)	These are key components in the attraction of neutrophils to the site of inflammation, have a systemic anti-inflammatory effect, daily moderate exercise suppresses Gro $\alpha$ (3)	Deviated in males due to expression of the TT genome.
BDKRB2(rs1799722)	Encodes a receptor for bradykinin and is implicated in the increase of skeletal muscle glucose uptake during exercise, it has also been associated with endurance performance in previous studies. THE variant rs1799722 (also known as 58C/T), is said to functionally impact the gene, with increased transcription rates for the T allele in luciferase experiments. Additionally, it is associated with hypertension, left ventricular hypertrophy, and baroreflex sensitivity (5)	The high transcription allele was overrepresented in this group of endurance athletes and, even more so, among those who are habitual runners.
ADRB2rs1042713	Encodes the $\beta$ 2-adrenergic receptor that is primarily responsible for increases in bronchodilation, ventricular function, and vasodilation, all of which have direct implications on cardiovascular and pulmonary responses to exercise (5). There was some evidence of lower mean arterial blood pressure at rest, during and after exercise for Arg16 homozygotes and a decreased exercise performance in patients with heart failure with Gly16 and decreased thermogenic responses to $\beta$ 2-adrenergic stimulation with Arg1(5)	Associated with best times of male athletes where running was the preferred sport while for all participants, running was associated with specific events and best time ever. Arg16 allele- more prevalent in endurance athletes
AMPD1rs17602729	Functions in skeletal muscle metabolism (salvage of adenine nucleotides) and is involved in the regulation of muscle glycolysis during vigorous exercise (5).	For the time of the specific event and best time ever among all (both male and female) runners along with ADRB2.
PPAR- $\alpha$ (PPARA) rs4253778	It is an intronic variant associated with heart, liver, glucose homeostasis, lipid metabolism and skeletal muscles (5).	It was investigated in a multisport case-control study, where endurance athletes had a higher frequency of the G allele
PPARD (rs1053049, rs6902123, and rs2267668)	It is involved in fatty acid $\beta$ -oxidation, glucose utilization, mitochondrial biogenesis, angiogenesis, and muscle fiber type. PPAR- $\gamma$ coactivator-1 $\alpha$ (PPARGC1A; rs8192678) regulates genes involved in energy metabolism and is associated with mitochondrial biogenesis and skeletal muscle fiber type conversion. They have also been associated with aerobic fitness and insulin sensitivity (5).	The allelic differences of this gene were more common in endurance athletes in case studies while the minor gene PPARGC1A was more common in unfit athletes.

## 5. Discussion and Conclusions

There is a wide range of observable changes in gene expressions resulting from exercise (running or otherwise). The dosage (duration) of running and its strength (intensity) determine the extent of positive or negative impact acquired, and they are better understood when genes, proteins and other biochemical/metabolic indicators are studied.

Additionally, understanding this would help assist doctors and scientists to prescribe the dosage of running suitable to one's age and fitness level and or how the rate or running can be increased in a way that one does not experience negative effects.

Results from this literature review paper unanimously show the apparent influence of running/ endurance training on the

WBC composition (Atamaniuk et al., 2008; Fehrenbach et al., 2000; Grzybkowska et al., 2019; Nieman et al., 2003). The studies that evaluated WBC compositions found the increase in Leukocyte, monocyte, and granulocyte counts; in one paper the decrease in lymphocytes (lymphopenia) was associated with increased DNA apoptosis (Atamaniuk et al., 2008). In contrary, WBC count in the control group of non-runner veteran athletes, and control groups showed no modifications or a decrease from before to after exercise (Febbraio & Koukoulas, 2000; Mukherjee et al., 2014). Therefore, it may be plausible that WBC count variation is a characteristic effect of running alone. Concomitantly, there was a rise in various serum metabolites such as those involved caffeine metabolism, fat metabolism, protein metabolism, metabolic changes were noted in the control group as well. After running a marathon, hypoxia, and ischemia of muscle cells are also not uncommon, these factors cause rise in hypoxanthine which is a valid metabolic indicator to reflect the level of hemoglobin and metabolic state of the energy in the body. Hemoglobin indicates the physical abilities and performance capacities (Shi et al., 2020). Another surprising reaction post running that is determined by these pathways is "Runners high", a popular term used by athletes which is actually a cause of elevated theobromine and theophylline which are part of the caffeine metabolic pathway (Shi et al., 2020).

There is an interesting correlation between differential regulation of mRNA in faster athletes when compared to slower athletes as suggested by one study ((Atamaniuk et al., 2008; Fehrenbach et al., 2000; Grzybkowska et al., 2019; Nieman et al., 2003)). Since these findings are based on organ damage indicators, they could be the result of a better adaptation to a long-lasting effort in the faster group. Additionally, the age may also have caused such variation as the results indicate that faster runners were significantly younger than slower runners (Jastrzębski et al., 2015). Further studies would be required, however; to suggest if this observation holds true for all types of genes or is limited to serum iron Lower respiratory tract infections (LRTI) is a unique side effect of running which is related to intensity and experience of the runner, more acclimated one is to the energy expenditure during a marathon, lesser chances they have of developing LRTI. Salivary IgA (sIgA) is one of the most widely-studied noninvasive biomarkers of mucosal immunity and LRTI risk (Cantó et al., 2018). Further, there is an inverse relation between "salivary lactoferrin and blood lymphocyte counts before the race, which may be explained by the mobilization of neutrophils to maintain blood homeostasis; at the same time there is also a direct positive correlation between blood leukocyte counts and the content of saliva lysozyme after the marathon. Which makes sense as leukocytes are associated with fighting diseases while lymphocytes make antibodies. Moreover, LRTI runners had a higher percentage of systemic basophils. Therefore, suggesting the benefits of exercise training and post-marathon recovery in the immunological profile and also the risks of developing a LRTI (Cantó et al., 2018).

The systemic alterations may affect the content of saliva lactoferrin and lysozyme in runners, and may be the contributor to the development of LRTI after races.

Chemokines also contribute to regulation of immune response during exercise, the decreased chemokins in non LRTI participants indicates that they had a better inflammatory regulation response that may protect against infection. Although athletic performance has been proved to be modulated by training and reducing inflammation (preventing diseases); factors like dehydration may cause the results to vary (Cantó et al., 2018), so further studies would be required to better prove this relation.

SNPs may also create a huge impact in functioning of pathways although they are changes of only a single base pair (nucleotide). For example, after a marathon, an SNP in the bradykinin B2 receptor (BDKRB2) leading to the TT allele expression in the r variant rs1799722 (also known as 58C/T), may cause effects related to hypertension, left ventricular hypertrophy, and baroreflex sensitivity (Tsianos et al., 2010).

Pyruvic acid, which is the product of cellular respiration, is key to several metabolic pathways such as the TCA cycle, which is an important pathway that provides the energy for long-term exercise. In the resting state, pyruvic acid was positively correlated in the marathon runners, which could reflect the physical condition of the athletes suggesting their adaptations to vigorous training (Shi et al., 2020). Carnitine is key for pyruvic oxidation, one study experimented to notice if there are any benefits in athlete performance by supplying oral carnitine since running reduces carnitine levels. Surprisingly, there were no added benefits to the performance, but it helped in prevention of carnitine level reduction therefore the athletes stayed energized throughout and during recovery (Cooper et al., 1986).

Another interesting correlation is DNA with cellular apoptosis/necrosis (cellular death) which was further associated with lymphopenia in ultramarathon runners but not following a shorter run (Atamaniuk et al., 2008). Therefore, intensity and duration (dosage) of exercise (running) comes into play here as well.

The results suggest that the dosage of running determines the side effects. Moreover, it is different in athletes when compared to recreational runners or other control groups. Longevity, cardiovascular health, muscular strength, gastrointestinal health and so on, could therefore be the benefits and disadvantages of running. If this PA is performed appropriately, it could be the best gift to one's body, but at the same time if the same PA becomes strenuous to the body it could start deteriorating the normal functioning of the systems and become a disadvantage. It is necessary to understand the physical fitness of oneself and not overshoot beyond the body's capacities at once. Athletes would have better physical fitness, and therefore greater tolerance to the stress at the same time a non-experienced runner might have side effects doing the same amount of running.

The study limitations in this paper include, limited participant size in previous studies, making the data not 100% reliable and being unable to capture all the available data on side effects of running and thus having a limited scope. Future studies should expand on these findings and gather more support to support the data. The studies may

consider answering questions like: Is running the only PA which responds this way or is it true for other forms as well? Can veteran athletes perform running without heavy side effects? Is sex a factor to be considered? Can the benefits be cherished without experiencing disadvantages?

In conclusion, this paper proposes that running is a “universal drug” and must be used cautiously like any other drug/medicine. Overdose causes side effects while not using it will not shower its benefits. This drug is also accustomed to physical fitness as determined by indicators like  $VO_{2\max}$  and population genetics as seen in the African runner’s phenomenon. While some may cherish more benefits than others, the disadvantages are under our control and they must not be allowed to accumulate.

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