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Smoking-induced changes in leptin serum levels and c/EBPalpha-related methylation status of the leptin core promotor during smoking cessation

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ABSTRACT

The damaging effects of smoking: Smokers look 8 to 20 years older than their actual age. Smoking for 10 minutes reduces oxygen delivery to the skin for an hour. Researchers conducted this study to investigate the relationship between smoking and DNA methylation. DNA methylation is a specific type of epigenetic change that alters a number of biological processes. 2,728 people from different racial and ethnic groups participated in the study. The researchers were able to identify 408 methylation markers associated with smoking. Two of these markers were not the same in all participants, and race or ethnicity played a significant role in this difference. The researchers calculated the total nicotine equivalents to determine a measure of smoking. The researchers used data from a database to conduct the first study. Then, biological samples were collected from 1,994 participants to determine each person's smoking dose and the level of DNA methylation across the genome. After examining, the researchers concluded that smoking was associated with DNA methylation at 408 sites. 45 new sites were identified. Of these 408 sites, two sites played a significant role in changing the risk of developing the disease based on race or ethnicity. In the African American participants who smoked, one site in the CYTH1 gene was changed. In the Latino participants who smoked, another site in the MYO1G gene was identified.

Introduction

In smokers, hair turns gray earlier. Smoking increases the male hormone, which increases the likelihood of hair loss [1]. Smoking causes thinning of the upper layer of the skin, a decrease in the thickness of the skin underneath, and damage to hair follicles [2-4]. Studies show that smoking's damaging effects on gene function can lead to chronic inflammation and an increased risk of diseases such as cancer [5], heart disease, and autoimmune disorders. Overall, smoking has a significant impact on gene function in the body [6], leading to mutations and epigenetic changes. Quitting smoking is one of the most effective ways to reduce these risks and improve overall health outcomes. Smoking is a risk factor for many common eye diseases, such as age-related macular degeneration, glaucoma [7], cataracts, and amblyopia. Many of these diseases lead to irreversible vision loss. Smoking reduces blood levels of antioxidants [8-10]. These antioxidants protect retinal cells. Smoking damages the protective layer between the retina and blood vessels, leading to reduced blood supply [11], irritation, and the formation of scar tissue. Smoking exposes the body to free radicals that cause cell damage and deprive the retina and lens of nutrients [12-14]. Carbon monoxide in cigarette smoke reduces oxygen supply [15], resulting in decreased night vision. Smoking produces cyanide, which is toxic to the retina and may lead to a condition called toxic amblyopia [16-18]. Amblyopia (lazy eye) is vision loss without any visible structural changes in the eye [19-21]. In other words, the eye has a normal

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structural appearance, but the person does not have good vision [22].

Age-related macular degeneration is 2 to 3 times more common in smokers and people who live with smokers. Smoking causes teeth to turn yellow in a short period of time [23]. There are toothpastes specifically for smokers on the market that contain more abrasive ingredients than regular toothpastes, but it should be noted that the effect of these toothpastes is temporary and the definitive treatment for this tooth discoloration is to quit smoking. Smokers' teeth are more prone to the formation of tartar and plaque, both of which are the cause of gum disease [24-26].

Gum infections heal more slowly because of the reduced oxygen in the bloodstream of smokers. Infections and gum disease are the most common causes of premature tooth loss. Other effects of smoking on the mouth include bad breath and oral cancer [27]. Many smokers are resistant to local anesthesia and still feel pain despite the injection of dental anesthetic. Irritants in tobacco smoke (tar and cyanide gas) are responsible for the development of chronic bronchitis and emphysema. In the lungs, there are small hairs called cilia that clean the lungs of contaminants. These irritants stop the action of the cilia and cause the lung glands to secrete more mucus.

When the cilia become inactive, the process of clearing the lungs of excess mucus, tar, and other impurities must be done by coughing. Smokers cough more and produce more phlegm than non-smokers. Other respiratory symptoms include shortness of breath, chest tightness, wheezing, chest pain, sinusitis, and frequent colds [28].

Almost all patients with emphysema are smokers. Emphysema is caused by damage to the lungs, which can be due to direct damage from cigarette smoke, induced oxidants, or the production of new inflammatory mediators due to exposure to smoke. Lung cancer is 20 times more common in smokers than in nonsmokers.

The development of lung cancer is directly related to the number of cigarettes smoked per day, the duration of smoking, and the tar and nicotine content of the cigarettes smoked. Heavy smokers are 15 to 25 times more likely to die from lung cancer than nonsmokers, and it takes only 15 to 20 years of smoking for lung cancer to develop, and few patients survive more than 5 years with the disease [29].

Smokers are more likely than nonsmokers to develop cancer of the mouth, throat, and esophagus. In the case of bladder cancer, the concentration of carcinogens in the urine appears to be a factor in causing cancer. Gastric, pancreatic, and cervical cancers occur more frequently in smokers than in non-smokers. Not smoking does not restore the function of a number of human genes to their original and correct state [30-32].

Although they are few in number, they can cause significant complications. In the case of sperm, the effect of quitting smoking will show itself within 3 to 6 months.

Smoking is not the only cause of infertility, and those who smoke in inappropriate physical conditions will suffer serious damage and may become infertile. In mammals, DNA methylation occurs at carbon number 5 of cytosine in the CpG dinucleotide substrate and by DNA methyl transferase enzymes. In the genome of these organisms, CpG-rich regions are seen in many promoters and form CpG islands [33].

Methylation of these islands can suppress gene expression by preventing transcription factors from accessing the promoter or by recruiting proteins that bind to methylated CpGs. Proteins that bind to methylated CpGs, by recruiting complexes containing histone deacetylases, cause DE acetylation of proteins that bind to histone lysine residues and close the chromatin structure, resulting in transcriptional silencing [34].

Roles and functions of DNA methylation

DNA methylation, as an epigenetic system, plays a role in the development of organisms, inactivation of one of the X chromosomes in female mammals, cellular memory, patterning of non-coding RNA expression, gene silencing, and combating cancer. In these methods, DNA is first exposed to restriction enzymes and then amplified by PCR.

This method is usually used to study DNA with a high percentage of methylation. Other methods that are very common today are methods based on chemical modifications with sodium bisulfite and genome sequencing after treatment with bisulfite. The basis of the operation of techniques based on biochemical modifications by bisulfite is that unmethylated cytosines are converted to uracil, but it does not work on methylated cytosines [35].

Therefore, bisulfite creates specific changes in DNA depending on the presence or absence of methyl on cytosine, which can be evaluated by PCR, sequencing and microarray methods. There are other methods that are based on the affinity of antibodies against methylated DNA or proteins that bind to it to determine methylation. In general, there are several main methods in the process of examining methylation:

- ✓ Methods based on cleavage by methylation-sensitive restriction enzymes.
- ✓ Methods based on treatment with sodium bisulfite [36].
- ✓ Antibody-based methods.

Bisulfite-based method

Bisulfite treatment of DNA is one of the most common techniques in this field, in which the DNA is first treated with bisulfite, to convert methylated

cytosines to uracil, which will be replaced by thymine in PCR [35].

Primers are designed to be outside the regions containing CpG and usually generate the target sequence regardless of the methylation site, or two pairs of primers can be designed specifically for methylated and methylated CpG sites, as in the methylation-specific polymerase chain reaction method. Bisulfite sequencing provides a more accurate assessment of the methylation site compared to the PCR method, in which primers are designed for methylated and methylated sequences, and the PCR products are finally used for sequencing. Methods based on PCR amplification of bisulfite-treated DNA can be divided into two groups based on the PCR primers:

- ✓ A group of primers that specifically amplify methylated or methylated templates, e.g. methylation-specific polymerase chain reaction [36].
- ✓ A second group of primers that allow amplification of template DNA regardless of methylation status for post-PCR methylation analysis, including high-resolution melting, enzyme digestion, and sequencing after bisulfite treatment.

Combined restriction enzyme and bisulfite cleavage analysis

This method combines bisulfite treatment, PCR, and restriction enzyme and is used to examine methylation at a specific site. First, DNA is treated with bisulfite and the desired fragment is amplified with primers designed for BSP. The product is treated with a restriction enzyme. Due to the change of methylated cytosines to thymine, the enzyme cleavage sites may have changed and it cannot digest the DNA.

As a result, its electrophoretic band pattern is different from that of the methylated sample. One of the disadvantages of this method is that only cytosines are detected at the enzyme recognition site, but this method is quantitative and easy and can be used for small amounts of initial DNA and paraffin-fixed samples. In the human body, there is almost a structure called the nucleus. The center of the cell is controlled by the nucleus, and inside the nucleus are 23 pairs of chromosomes that are made up of genes [37].

Genes transmit codes to cells on how to behave. When one or more genes in a cell do not work well and a cellular mutation occurs, it causes cancer in the body. Today, techniques such as PGD can identify destructive genes before fertilization. Most cancers develop during a person's life due to genetic mutations. Smoking has a direct impact on the development of various types of cancer in people at different ages.

DNA methylation and telomere length

To examine this enzyme, the amount of phosphorylated TERT can be measured, which in its phosphorylated form leads to the inhibition of telomerase. On the other hand, the two proteins TRF1 and TRF2 bind to telomeric DNA by their carboxyl termini and are essential for the normal function of the telomeric region. Examining the expression of each of these genes can be a good option for examining telomere length. To measure telomere length, a real-time PCR reaction can be used to measure telomerase enzyme activity, using the Telomerase Repeated Amplification Protocol (TRAP) method. In real-time PCR, the number of copies of a telomere-specific primer can be compared using a telomere-specific primer and a single-copy primer [38].

Discussion

A wide range of pollutants, including organic and inorganic pollutants, pesticides, and biological agents, can have harmful effects on living organisms and are present in the environment. These pollutants are endocrine disruptors, carcinogens, mutagens, neurotoxins, etc., and enter the environment from various point and non-point sources.

Recent findings on the role of epigenetic changes induced by toxicity in the progression of degenerative diseases have opened a promising research avenue to explore epigenetic-based therapeutic approaches and prognostic biomarkers. Epigenetic changes have been identified in various diseases, including cancer, autoimmune disorders, pulmonary diseases, as well as cardiovascular, gastrointestinal, and bone diseases. In general, epigenetics is defined as any stable and heritable change in chromatin structure that can lead to changes in gene expression and the appearance of an abnormal phenotype.

Environmental factors are external factors that modulate the aberrant changes in inflammatory pathways. Cigarette smoke, metal ions, bisphenol A, benzene, alcohol and other toxicants with their genotoxic potential induce epigenetic changes. Currently, a number of epigenetic drugs are available on the market and many of them are in preclinical stages and clinical trials. In addition to pharmacological approaches, there is a lot of evidence that dietary compounds have epigenetic protective roles in regulating pathological processes and can be considered as a potential option for epigenetic-based therapy [39].

While previous studies have focused on the role of decreased appetite, orexin, and neuropeptide Y, and increased inflammatory cytokines in muscle wasting in smokers, recent evidence suggests that the skeletal muscle growth inhibitory factor, myostatin, plays a prominent role in reducing protein synthesis and muscle atrophy in smokers. Myostatin plays a key role in regulating skeletal muscle mass, and

mutations in the myostatin gene cause muscle hypertrophy, and its systemic increase leads to muscle atrophy. In the present study, consistent with the findings of some studies, it was observed that serum myostatin levels were higher in smokers than in non-smokers at baseline. Therefore, our results support the idea that smoking may be an inhibitor of muscle protein synthesis, and the higher prevalence of sarcopenia and muscle wasting in smokers is related to higher myostatin levels. On the other hand, studies clearly show that inactivity is associated with sarcopenia and muscle atrophy [28]. Therefore, in the present study, we sought to find out whether resistance training in smokers will lead to improvements in strength and muscle mass or not? Our results showed that in both groups participating in the 8-week resistance training program, adiposity indices, including mass and fat percentage, did not change. Our findings confirm studies that suggest that, at least in non-obese individuals, exercise training alone and without dieting for the short term does not reduce fat mass.

The results also showed that muscle strength and lean mass increased in both groups after resistance training, although the changes observed in the non-smoking group were greater. In fact, the findings of the present study are consistent with other reports and support the positive effects of resistance training on improving muscle mass and function in both smokers and non-smokers. Several mechanisms responsible for the improvement in strength and muscle mass following resistance training have been proposed. Changes in myokine production may play a role in these adaptations [39]. The present study shows for the first time that 8 weeks of resistance training in smokers leads to a decrease in serum myostatin while increasing strength and muscle mass. However, in the non-smoking group, the observed decrease in myostatin following training was greater [40]. This inverse relationship is consistent with the theoretical negative role of myostatin in regulating muscle mass [41]. The results of this study showed that at the end of the aerobic exercise period, maximal oxygen consumption in inactive obese men increased significantly and serum leptin levels, weight, body mass index, body fat percentage, and waist-to-hip ratio decreased significantly [42-44]. Also, a comparison of the two experimental and control groups showed that there were significant differences between their weight, body mass index, body fat percentage, waist-to-hip ratio, and maximal oxygen consumption, although no difference was observed between the two groups regarding serum leptin hormone levels.

Conclusion

Atherosclerosis is the most common cause of coronary heart disease and, consequently, the main cause of ischemic heart disease, and for this reason, the number of myocardial infarction cases increases progressively with age and the presence of other risk factors such as hypertension, smoking, and diabetes. Inflammatory cells and their mediators are also involved in the initiation, progression, and complications of atherosclerotic lesions. Leptin, which is produced by the obesity gene, is a protein hormone with a molecular mass of 16 kDa, which is mainly secreted by adipose tissue and plays a role in regulating body weight. Some researchers have mentioned leptin as an alarming mechanism in regulating body fat content. Another proinflammatory indicator predicting cardiovascular disease is interleukin-6. Serum interleukin-6 levels are an independent risk factor in cardiovascular disease, and there is a strong association between interleukin-6 levels and many risk factors for heart disease. Among the methods of treatment or prevention for cardiovascular diseases is lifestyle adjustment, and it seems that these diseases can be prevented through sports activities and nutrition. Sports activity can be effective in different ways. Sports activity can have various benefits, including reducing visceral fat, reducing pro-inflammatory cytokines such as interleukin-6, and reducing leptin.

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Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

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