ELSEVIER

Epigenetic adaptation to regular exercise in humans

Charlotte Ling and Tina Rönn

Q1

Department of Clinical Sciences, Epigenetics and Diabetes Unit, Lund University Diabetes Centre, CRC, Scania University Hospital, 205 02 Malmö, Sweden

Regular exercise has numerous health benefits, for example, it reduces the risk of cardiovascular disease and cancer. It has also been shown that the risk of type 2 diabetes can be halved in high-risk groups through nonpharmacological lifestyle interventions involving exercise and diet. Nevertheless, the number of people living a sedentary life is dramatically increasing worldwide. Researchers have searched for molecular mechanisms explaining the health benefits of regular exercise for decades and it is well established that exercise alters the gene expression pattern in multiple tissues. However, until recently it was unknown that regular exercise can modify the genome-wide DNA methylation pattern in humans. This review will focus on recent progress in the field of regular exercise and epigenetics.

Introduction

During human evolution, physical fitness has been a key advantage for survival. However, in today's society, many people are physically inactive and taking the car to work and the elevator to your office is a common scenario. In the meantime, the number of individuals with metabolic diseases such as type 2 diabetes, cardiovascular disease and obesity is increasing at an alarming rate [1]. Notably, although it is well established that regular exercise can prevent the risk of metabolic diseases and improve its course in patients with type 2 diabetes and obesity, many people still live a sedentary lifestyle [1–3]. Changing this trend will most probably improve public health. It is therefore essential to understand, describe and communicate better how long-term exercise alters molecular mechanisms and subsequently improves a person's wellbeing.

Q2

Additionally, research needs to be performed in multiple key tissues that respond to exercise. Indeed, a large body of research has already shown that exercise alters the expression of genes that affect glucose and lipid metabolism as well as mitochondrial function [4–13]. Exercise also changes muscle expression of transcription factors, myogenic regulatory factors and myokines [8,14,15]. These exercise-induced changes in gene expression can contribute to increased fitness, decreased blood pressure and an improved whole-body glucose homeostasis [13,16–18]. This has been demonstrated by manipulating the expression of key genes that are regulated by exercise, resulting in rodents with an altered response to exercise [19–22]. Yet more mechanisms and additional tissues are likely to be involved in the whole-body response to long-term exercise. Interestingly, inherited factors such as single nucleotide polymorphisms (SNPs) could affect a person's response to exercise and thereby its health benefits [23,24]. Recent data further suggest that environmental factors can modify our genes through epigenetic modifications [25–33], which could also affect a person's response to exercise. Epigenetics has been defined as heritable changes in gene function that occur without a change in the nucleotide sequence [26]. Environmental stimuli can alter the epigenome. However, once epigenetic modifications are present they can be stable and inherited [34,35]. Epigenetic modifications include DNA methylation, histone modification and non-coding RNA. In the present review, the impact of regular long-term exercise on DNA methylation in humans will be discussed.

DNA methylation

Until recently there has been limited knowledge about whether exercise induces epigenetic modifications in humans. DNA methylation is the most extensively studied epigenetic marker and is involved in a variety of biological processes. In differentiated mammalian cells, DNA methylation mainly occurs on cytosines in CpG dinucleotides. Although S-adenosyl methionine is a co-substrate involved in methyl group transfers, DNA methyl transferases (DNMTs) including DNMT1, DNMT3a and DNMT3b are responsible

Corresponding author: Ling, C. (Charlotte.Ling@med.lu.se)

1359-6446/06/$ - see front matter © 2014 Published by Elsevier Ltd. http://dx.doi.org/10.1016/j.drudis.2014.03.006

for adding methyl groups to the mammalian genome. Increased DNA methylation has been associated with decreased transcriptional activity because it can repress the binding of transcription factors to promoter regions and attract transcriptional co-repressors and histone deacetyltransferases (HDACs) [26]. This results in a dense chromatin structure and inactive genes. Furthermore, new methods have allowed the analysis of DNA methylation in different genomic contexts, showing for example that DNA methylation within gene bodies has a positive correlation with active gene transcription and/or can affect alternative splicing events [36,37]. It is probably that the function of DNA methylation varies with genomic context and external stimuli, and hence has a complex relationship with gene transcription.

Epigenetics and regular exercise

We recently performed an exercise intervention study in middle-aged sedentary but healthy men [38,39]. The intervention consisted of 6 months aerobic exercise for one hour approximately twice a week. Clinical and metabolic characteristics were analyzed and skeletal muscle and adipose tissue biopsies were taken before and after the exercise intervention. Although this intervention resulted in decreased waist, blood pressure and pulse, it increased VO_{2max} and high-density lipoprotein (HDL) levels. However, the intervention had no significant effects on glucose tolerance, folate or homocysteine [38].

To examine if regular aerobic exercise induces epigenetic modifications, we analyzed the genome-wide DNA methylation pattern in skeletal muscle and adipose tissue from the men included in the intervention. The degree of DNA methylation of a large number of genes changed in response to exercise in both tissues (Fig. 1). After correction for multiple testing using false discovery rate analysis, we found altered DNA methylation in (or near) 2817 and 7663 genes in skeletal muscle and adipose tissue, respectively. Q3 Interestingly, whereas approximately three-quarters of the identified genes showed decreased DNA methylation in skeletal muscle [38], the majority of the genes showed increased DNA methylation in adipose tissue in response to exercise [39], suggesting that epigenetics can have different effects in the two tissues.

A similar pattern was seen for gene expression, where the majority of genes with overlapping changes in DNA methylation and gene expression showed increased expression in skeletal muscle and decreased expression in adipose tissue. It is possible that there is a crosstalk between these tissues, regulated by epigenetic mechanisms. This hypothesis is supported by the fact that the adiponectin receptor shows differential DNA methylation and mRNA expression in skeletal muscle in response to exercise [38]. This could affect muscle sensitivity to adiponectin, a hormone secreted from adipocytes. Additionally, skeletal muscle and adipose tissue showed exercise-induced changes in DNA methylation of candidate genes for type 2 diabetes. Epigenetic modifications of these candidate genes might affect how respective risk of SNP increases susceptibility for diabetes (Fig. 1). This knowledge might encourage people at a genetic risk for diabetes to exercise more frequently. Whereas our muscle and fat papers found an overlap between differential DNA methylation and gene expression, they also demonstrate that elevated DNA methylation has direct negative effects on the transcriptional activity, by the use of luciferase assays.

The genes studied using the luciferase assay include *MEF2A*, a Q4 transcription factor that regulates glucose transporter type 4 (Glut4) expression in human muscle in response exercise [40], *NDUFC2*, which encodes a protein included in complex one of the respiratory chain, *RUNX1*, a transcription factor known to be regulated by exercise [23], and *THADA*, a candidate gene for type 2 diabetes [41]. In agreement with previous studies showing improved mitochondrial function in response to exercise, we also found increased mitochondrial density in skeletal muscle after the exercise intervention. In addition, we found that exercise decreased DNA methylation of *PPARGC1A*, which encodes the transcriptional co-activator PGC 1α, known to regulate the expression of mitochondrial OXPHOS genes in skeletal muscle. In adipocytes, we used the luciferase assay to show that increased methylation of the *RALBP1* promoter results in decreased transcriptional activity. This gene has previously been shown to be involved in the pathogenesis of metabolic syndrome [42] and to affect insulin-stimulated Glut4 trafficking [43]. This experiment was done to mimic the situation in adipose tissue after exercise, where *RALBP1* DNA methylation was increased and mRNA expression was decreased. To mimic the situation in human adipose tissue, we also silenced the expression of two genes in cultured adipocytes (*HDAC4* and *NCOR2*, which showed increased DNA

Q5 Q6

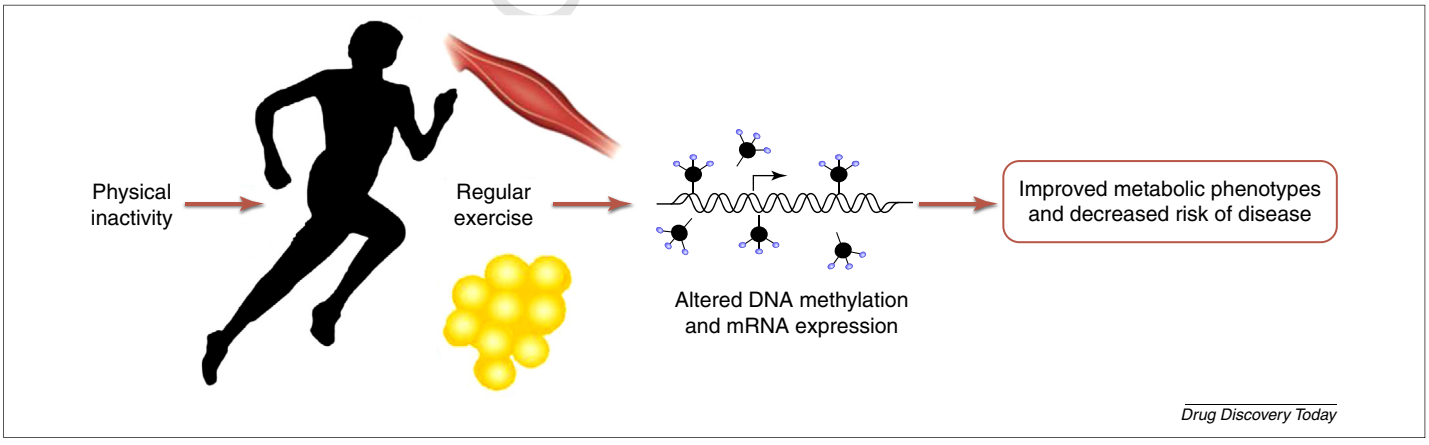

FIGURE 1

Regular exercise induces genome-wide epigenetic modifications in human skeletal muscle and adipose tissue, linked to altered mRNA expression. This could potentially affect metabolic phenotypes and risk of disease.