European Heart Journal Supplements (2019) **21** (Supplement B), B76–B79
The Heart of the Matter
doi:10.1093/eurheartj/suz030

The complex interplay between fitness, genetics, lifestyle, and inflammation in the pathogenesis of coronary atherosclerosis: lessons from the Amazon rainforest

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KEYWORDS
Atherosclerosis;
Inflammation;
Environment;
Genetics

Far from only being a modern disease, atherosclerosis has also been reported also in ancient civilizations, as shown in some studies conducted on Mummies from different latitudes. Conventional cardiovascular (CV) risk factors can explain more than 90% of the attributable risk of coronary artery disease (CAD). In this regard, Tsimane Aborigenous of Amazon rainforest, conducting a subsistence lifestyle with low prevalence of CV risk factors, present the lowest reported prevalence of CAD in the world, despite an elevated inflammatory burden. Experimental and clinical studies have supported the theory that other factors, like genetics and inflammation, are involved in atherosclerosis. Indeed, a large clinical randomized study (CANTOS trial) tested the anti-inflammatory properties of canakinumab, and provided the first evidence to support the 'inflammation hypothesis'. Another field of research, based on Mendelian randomization studies, supports the appealing hypothesis that correcting CV risk factors earlier in life, may prevent or delay the progression of CAD. All these data prove that atherosclerosis is the expression of a complex, dynamic, and continuous interaction between environment and genetics that begins at conception and continues through adulthood.

Introduction

Throughout human history life expectancy has progressively increased. Infection and famine, reported for millennia as the primary causes of death in the world, still represent the main cause of death in developing countries. On the contrary, in recent centuries, cardiovascular (CV) death has become the main mortality cause in industrialized Countries. Conventional CV risk factors (cholesterol, smoking, arterial hypertension, and diabetes) can explain more than 90% of the attributable risk of coronary artery disease (CAD).[1] For this reason, international health organizations have been trying to design and implement different intervention strategies. Primary prevention with meticulous risk factor control leads to a significant, consistent, and dose-related reduction in major adverse cardiac events. Nevertheless, other factors, such as genetic predisposition or inflammatory state, that are only partially modifiable, seem to be relevant determinants of CV risk.

Messages from Amazon rainforest

Despite a process of wild industrialization, there are still small populations around the world with a preindustrial lifestyle. The Tsimane of Bolivia are one of these groups, living in Amazon rainforest, maintaining a subsistence lifestyle of hunting, fishing, gathering, and farming. The Tsimane Health and Life History Project team (THLHP) has

been working with this population to understand the correlation between preindustrial lifestyle and low prevalence of CAD. A recent THLHP study, published on Lancet, evaluated prevalence of coronary atherosclerosis in the Tsimane, measured by coronary artery calcium (CAC) scoring with non-contrast computed tomography (CT).[2] Of 705 Tsimane indigenous considered (mean age: 57 years, male: 349, 50%), about 85% had no CAC, and only 3% had CAC scores higher than 100, suggestive of significant atherosclerotic disease. For individuals older than 75 years, 65% were free from atherosclerosis, and only 8% had CAC scores of 100 or more, with a five-fold lower prevalence than industrialized population ($P \leq 0.0001$). The prevalence of CV risk factors were low in all age groups. However, high-sensitivity C reactive protein (hs-CRP) was elevated (>3 mg/L) in about half the population (360, 51%), without differences across age groups. These data prove that Tsimane aborigines are the population with the lowest reported levels of CAD in the world. This seems to be related to subsistence lifestyle, intense physical activity, low prevalence of CV risk factors, a diet with low processed carbohydrates, and saturated fats. However, the Tsimane aborigines have a high inflammatory burden, related to infections and environmental exposure. The results of Kaplan *et al.*[2] partly contrast other studies according to which inflammation plays a central role in atherosclerosis. It is however possible, that inflammation, in presence of lifelong low LDL levels, might not potentiate atherosclerotic disease. From the Amazon rainforest, a number of lessons can be learnt:

(1) Some populations with very low LDL levels from birth have a very low prevalence of CAD.
(2) Age is an intrinsic and unmodifiable risk factor related to atherosclerosis, although with a smaller order of magnitude.
(3) A healthy lifestyle is crucial for CV protection, even in the presence of a high inflammatory burden.
(4) Inflammation is able to potentiate atherogenetic effects of CV risk factors, but it is not able to determine atherosclerotic disease alone.

This study does not consider the possibility of a favourable genetic predisposition, and the hypothesis that environment and healthy lifestyle are only the tip of the iceberg of CV disease protection remains.

Messages from the past

The low prevalence of atherosclerosis described in a Bolivian population should not get our hopes up regarding the existence of a Paradise lost in the past where CV disease was absent. Far from being only a modern disease, atherosclerosis has been reported in ancient civilizations. To support this hypothesis, the Horus group first studied 52 ancient Egyptian adult mummies (mean estimated age: 38 years) by performing whole body multislice CT to identify arterial calcific deposits in arterial bed as equivalent of atherosclerosis.[3] Among 44 mummies with identifiable CV structures, 20 (45%) had arterial calcifications, especially in the aortic wall, but also in coronary, iliac, femoral, carotid, and peripheral arteries, with no significant gender differences (55% male, 45% female). Mummies with atherosclerotic calcification were generally older, and multivessel involvement ($\geq$3 beds) was significantly more frequent in patients with $\geq$40 years.

The prevalence of CV risk factors in ancient Egypt is very difficult, nay impossible to evaluate: hypertension and diabetes mellitus cannot be estimated, and tobacco smoke was not present. Furthermore, mummification in ancient Egypt was reserved for elite members of society, Royals, and clergy. Based on the interpretation of hieroglyphs and representations, the authors suggest that a sedentary lifestyle and a lipid rich diet, especially amongst the clergy, played a central role in atherogenesis. However, we have to be careful to not extend these hypotheses to the entire population of ancient Egypt since these data belong to a small elite of society.

The Horus Team also conducted a similar, larger study, considering 137 mummies from different Ages through four millennia and from four different parts of the world (ancient Egypt, ancient Peru, Ancestral Puebloans of southwest America, and the Unangans of the modern-day Aleutian Islands), with a mean age of about 36·years.[4] Once again, probable or definite atherosclerotic calcifications were found in a high number of mummies (47, 34%) using total body CT, especially in the aorta, without statistically significant differences among the four populations (38% in the Egyptians, 25% in the Peruvians, 40% in the Ancestral Puebloans, and 60% in the Unangans; P = NS). Atherosclerosis extension and severity were directly related to age, while there was not significant correlation to sex or historical period. Regarding atherosclerotic risk factors, there were differences in diet, climate and environment amongst these four populations. Tobacco was unavailable in these civilizations, but the authors speculated that inhalation of fire smoke could have had a part in the pathogenesis of atherosclerosis. However, chronic infections were common in all civilities, and it is likely that inflammation played a crucial role in the development of CAD. Again, the past can give us some interesting messages:

(1) Atherosclerosis is not exclusively a disease of modern society, but it affected different ancient civilizations around the world. It seems to be a loyal companion of humankind throughout history, and not a characteristic of any specific diet or lifestyle.
(2) A linear correlation exists between age and atherosclerotic disease. Older age mummies often present vascular calcification and more frequently show multivessel involvement.

Inflammation

Several lines of evidence (experimental models and population studies) support the notion that inflammation is involved in all stages of atherosclerosis. Different statin trials (REVERSAL, PROVE-IT and JUPITER) over the last decade have taught us that a reduction in hs-CRP levels—a marker of subclinical inflammation—is associated with improved CV outcomes, independently of modification of traditional risk factors. However, due to the duplicity of statin

drug action, affecting both LDL cholesterol and inflammatory pathways, it is not possible to state that therapy targeting inflammation *per se* can reduce CV events.

The first successful large scale clinical trial that addressed the 'inflammation hypothesis' was the CANTOS trial (Canakinumab Anti-Inflammatory Thrombosis Outcome Study).[5] This randomized, double-blind trial enrolled 10 061 patients with previous myocardial infarction (MI) and a hs-CRP level $\geq$2 mg/L despite the use of aggressive secondary prevention strategies. Patients randomly received placebo or one of three doses of canakinumab, a fully human monoclonal antibody targeting interleukin-1β, with an anti-inflammatory effect, that was previously approved for clinical use in rheumatologic disorders. The primary endpoint included non-fatal MI, non-fatal stroke, or CV death. In the canakinumab group, there was a significantly reduction of hs-CRP levels from baseline compared to the placebo group, without reduction of LDL cholesterol levels but only the 150 mg dose resulted in a significant reduction (15%) of the primary endpoint. These open the way to an era of 'precision medicine', where specific therapeutic strategies are tailored according to the single patient's needs, based on simple biomarkers. Currently, the standard of care for secondary prevention in survivors of acute coronary syndromes includes high-dose statins (plus ezetimibe when needed), anti-platelet drugs, beta-blockers, and myocardial revascularization. Despite optimal medical therapy, many patients continue to experience life-threatening CV events—due to what is known as 'residual risk'—partly due to the insufficient LDL-C lowering ('residual cholesterol risk'), and partly due to an inflammatory component ('residual inflammatory risk').[6] Nowadays we can face residual risk by lowering LDL-C further with the use of proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors—as shown in FOURIER trial[7]—in patients with suboptimal cholesterol control and, in near future possibly further reduce the residual inflammatory risk by inhibiting inflammation downstream.

Genetics and environment

Cardiovascular diseases are the expression of a complex and dynamic interaction between environment and genetics that begins at conception and continues through adulthood. Several environmental variables, such as tobacco exposure, diet, psychosocial stressors, alcohol consumption, and physical inactivity, increase CV risk; however, quantifying the impact of each one, with any precision, is difficult. On the other hand, genetic background influences the atherogenic process: with the exception of monogenic familial hypercholesterolaemias, atherosclerosis is generally a polygenic disease. Genome-wide association studies have recently identified more than 60 genomic regions where single nucleotide polymorphisms affect inherited CV risk. At any particular point in life, each genotype can lead to different phenotypes, according to the particular individual's environmental niche: a patient with a low-risk genotype might develop disease because of adverse environmental exposures. On the other hand, an individual with a high-risk genotype might remain healthy thanks to beneficial exogenous elements. During lifetime, environmental factors might modify CV risk through epigenetic or transcriptional mechanisms that regulate gene expression, or by directly affecting metabolic pathways. The study by Allam *et al.*[3] on the Tsimane does not consider the genetic substratum. However, it is likely that not only a healthy lifestyle but also a protective genetic background, perpetuated generation by generation thanks to the Tsimane's isolation, can explain the extreme low prevalence of atherosclerosis in these aborigines.

Mendelian randomization and lifetime exposure to cardiovascular risk factors

Investigators have recently developed a novel epidemiological study design that can be used to investigate potential risk factors for specific outcomes: Mendelian randomization (MR). These studies were designed to demonstrate whether an outcome of interest (e.g. CV events) is more common in individuals with a risk allele (e.g. LDLR genetic variant) than in those without the allele. Because the risk allele is strictly associated with a risk factor (intermediate variable) of interest (e.g. LDL cholesterol blood levels), it can be shown that the presence of the risk factor is likely a causal factor for the outcome of interest. Anyway, thanks to the experience from families carrying LDL receptor mutations and from the multiple epidemiological and interventional studies, the causal role for LDL in promoting CV diseases had been well-established long before MR studies were conducted. However, an advantage of the MR approach is that it can provide information on the impact of a lifetime modulation of a biomarker, unlike clinical trials that often follow their patients only for a few years.

In this respect, MR studies showed that variants in the LDL receptor gene, increasing LDL cholesterol level from childhood, result in stronger effects on CV risk than predicted by epidemiological or clinical studies. On the contrary, individuals carrying a rare PCSK9 allele, which lowers LDL levels below population average, have a significantly lower incidence of MI. Ference *et al.*[8] have recently estimated the effect of long-term exposure to lower LDL-C on the risk of CV disease, mediated by nine polymorphisms in six different genes known to be associated with lower LDL-C. They found that prolonged exposure to lower LDL-C, beginning early in life, is associated with a substantially greater reduction in the risk of CV disease than that observed in clinical trials involving statin therapies given over shorter periods of time and later in life. All this evidence demonstrates that both the magnitude and lengths of exposure to low LDL-C have an important effect on the risk of CV disease, confirming not only the rule that the lower the better but also the earlier the better. In fact, it is known that coronary atherosclerosis is a chronic and progressive disease that begins early in life and develops slowly over several decades before becoming clinically manifest.

Conclusions

The interesting findings from the Tsimane study need to be integrated with the current knowledge of traditional CV

risk factors in western countries, the findings from MR studies and from intervention trials focusing on lipid lowering therapies and anti-inflammatory drugs.

The genetic profile of this population was not investigated, so it remains unknown whether the low prevalence of atherosclerosis is a consequence of the peculiar lifestyle or of genetic background. Despite the fact that the hypothesis of a protective genetic heritage is attractive, a widespread protection against atherosclerosis in the Tsimanes probably depends on their healthy lifestyle. Prevention strategies should therefore be encouraged and started from a young age with educational strategies promoting a healthy lifestyle.

Conflict of interest: none declared.

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