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SPECIAL ARTICLE

Why are brown bears protected against atherosclerosis even though their plasma cholesterol levels are twice that of humans?

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Abstract Plasma cholesterol and triglyceride levels are twice as high in hibernating brown bears *(Ursus arctos)* than in healthy humans. Yet, bears display no sign of atherosclerosis development. To explore this apparent paradox, we analyzed lipoproteins from same ten individual bears plasma collected during winter (hibernation; February) and summer (active; June) in the same year. Plasma from fourteen healthy humans were analyzed as comparator. We used standard methods for lipoprotein isolation, composition and functional investigation. The results shows that in brown bears the absence of atherosclerosis despite elevated cholesterol is likely associated with two main athero-protective properties of circulating lipoproteins. First, a significant ten times lower affinity of low-density-lipoprotein (LDL) particles for arterial proteoglycans and secondly, an elevated plasma cholesterol efflux capacity. What does the brown bear data tell us? That elevated total cholesterol and apoB-containing lipoproteins not always associates with atherosclerosis disease. We need to look also at the lipoprotein biochemical features and functionality as they are relevant for arterial pathophysiology. What is the translatability into human of these results? We humans need to control our total and LDL-cholesterol levels. We are not brown bears!

PALABRAS CLAVE
Lipoproteínas;
ApoB;
Aterosclerosis;
Hipercolesterolemia;
Proteoglicanos;
Eflujo de colesterol;
Osos

¿Por qué los osos pardos están protegidos contra la aterosclerosis a pesar de que sus niveles de colesterol plasmático doblan al de los humanos?

Resumen Los niveles plasmáticos de colesterol y triglicéridos son 2 veces más altos en los osos pardos *(Ursus arctos)* durante el periodo de hibernación que en los humanos sanos. Sin embargo, los osos no muestran signos de desarrollo de aterosclerosis. Para explorar esta aparente paradoja, analizamos lipoproteínas del plasmas de 10 osos recolectados durante el invierno (hibernación: febrero) y verano (activo: junio) en el mismo año. El plasma de

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14 humanos sanos se analizó como comparador. Se utilizaron métodos estándar para el aislamiento de lipoproteínas, la composición y la investigación funcional. Los resultados muestran que en los osos pardos la ausencia de aterosclerosis a pesar del colesterol elevado probablemente se asocie con 2 propiedades ateroprotectoras principales de las lipoproteínas circulantes. En primer lugar, una afinidad significativamente, 10 veces menor, de las partículas de lipoproteínas de baja densidad (LDL) por los proteoglicanos arteriales y, en segundo lugar, una capacidad elevada de eflujo de colesterol en plasma comparado con humanos. ¿Qué nos dicen los datos del oso pardo? Ese colesterol total elevado y las lipoproteínas que contienen ApoB no siempre se asocian con la enfermedad de aterosclerosis. Necesitamos observar también las características bioquímicas y la funcionalidad de las lipoproteínas, ya que son relevantes para la fisiopatología arterial. ¿Cuál es la traducibilidad al humano de estos resultados? Los humanos necesitamos controlar nuestros niveles de colesterol total y LDL. ¡No somos osos pardos!

How did we conceive this research idea?

In previous studies of Nordic brown bears we observed that these animals did not develop atherosclerosis,[1] despite high plasma triglyceride and cholesterol levels (Table 1). Interestingly, histopathological examination of the descending coronary artery and aortic arches of 12 free-ranging adult bears revealed no signs of foam cell infiltration, fatty streaks, or advanced atheromatous lesions. Arterial samples from the bears were similar in morphology to muscular arteries found in healthy, non-atherosclerotic humans.[2]

In humans, elevated plasma cholesterol is a major risk factor for atherosclerotic cardiovascular disease.[3] We believe that bears provided an excellent and unique opportunity to investigate this apparent paradox. The bear is an animal that withstands the impact of seasonal environmental extremes. Hibernation in winter means fasting and sleeping versus active living, feeding, and physical activity in summer. We set out to analyse the concentration, composition, and functional characteristics of total lipids and lipoproteins in bears. This was the hypothesis of the paper published in the Journal of Lipid Research.[4]

The results of the study we conducted in collaboration with the laboratory of the Scandinavian Brown Bear Research Project (https://bearproject.info) (Figs. 1 and 2) describe lipoprotein composition and 3 functional properties that appear to modulate early atherogenesis in humans and preclinical mammalian models: the association of LDL with arterial proteoglycans, the ability of plasma to efflux cholesterol from extrahepatic cells (CEC),[5] and cholesterol acyltransferase activity between lipoproteins.[6]

Figure 1 Courtesy of Prof. Ole Frobert, Scandinavian Brown Bear Research Project.

Figure 2 Courtesy of Prof. Ole Frobert, Scandinavian Brown Bear Research Project.

What is the take-home message of this study to the scientific community?

The take-home message is that the apparent paradox described above is due to differences in the structural characteristics of brown bear lipoproteins compared to human lipoproteins. Atherosclerosis is not only caused by high levels of total lipids, cholesterol, and triglycerides, but also by specific structures and biochemical characteristics of the lipoproteins that transport these lipids. Brown bears, although they have winter plasma lipid levels twice as high as humans, possess larger lipoproteins, with significantly less binding capacity to arterial extracellular proteoglycans, a property mechanistically related to atherogenesis in humans and other mammals.[7] Human ApoB-100-containing lipoproteins from healthy subjects show a tenfold higher

Table 1 Mean values of total lipid concentrations and lipoprotein functionality in Nordic Brown bears in winter and in summer compared with humans.

Markers measured in plasma	Brown bear in summer	Brown bear in winter	Humans
Cholesterol (mmol/l)	6.5	12	5
Triglycerides (mmol/l)	2	5	2
Phospholipids (mmol/l)	4.5	6	13
Binding capacity to arterial proteoglycans (μmol/mmol-LDL cholesterol)	.5	10	1.5
CEC receptor SR-B1 (%)	15	12	2.5
CEC receptor ABCA1 (%)	4.5	12	2.5
LCAT (range nmol/mL/h)	4.3—18.2	3.4—6.4	6.3—32

Source: Pedrelli et al.[4]
CEC: capacity of plasma to efflux cholesterol from extrahepatic cells; LCAT: cholesterol acyltransferase activity.

affinity for arterial extracellular matrix proteoglycans than those from brown bears. We believe that our results provide for the first time a mechanistic explanation that may help explain the observed resistance to early atherogenesis in free-living brown bears, despite their high circulating levels of cholesterol and triglycerides, especially during hibernation. Pathological analyses of the arteries of bears that died of natural causes (ageing) show no atherosclerosis in their arteries.[2] In humans, already at an early age pathological studies have shown a high prevalence of atherosclerosis in coronary arteries.[6] Atherosclerosis is the leading cause of cardiovascular disease, the number one cause of death and hospitalisation worldwide according to recent (2020) statistics from the World Health Organisation.[9]

What was the ëureka momentẅhile working on this project?

This was when we observed that, independent of lipoprotein levels and changes in lipid composition during winter (elevated) and summer (decreased), ApoB-100-containing lipoproteins in brown bears showed significantly lower affinity to arterial extracellular matrix proteoglycans compared to healthy humans. We observed that these results provide for the first time a mechanistic explanation that may contribute to the observed resistance to early atherogenesis in free-living brown bears, despite their high circulating levels of cholesterol and triglycerides, especially during hibernation.

Can we make a general analogy to relate this study in bears to humans?

The main factor controlling cholesterol deposition in arteries, the first stage of atherogenesis, appears to be the presence of a specific amino acid sequence of ApoB-100 that binds to proteoglycans. This sequence contains positively charged lysine and arginine residues that allow binding to negatively charged proteoglycans in the arterial wall.[7] In bears, apolipoprotein B-100 contains fewer positively charged amino acids. This contributes to its lower affinity for proteoglycans in the arterial extracellular matrix.[10]

Weight change is another metabolic phenomenon that was associated with unexpected effects. Despite significant weight loss during the hibernation period, when not eating, brown bears have the highest cholesterol and triglyceride levels. This means that fasting and weight loss in brown bears does not lead to a reduction in lipids, which was the opposite of what we expected.

What is the message of this research study for the general public?

The hypothesis of our research study was that the binding of cholesterol-rich lipoproteins containing ApoB-100 is the initial cause of atherosclerosis. The results of this brown bear study support the hypothesis that binding of apolipoprotein B-containing lipoproteins (i.e., mainly low-density lipoproteins) to the arterial wall appears to be more relevant to the disease than high plasma cholesterol levels.[5,8]

What was the biggest obstacle we faced in this project?

One obstacle was that we did not know whether we could apply conventional human lipoprotein analysis techniques to bear lipoproteins. Therefore, we decided to use basic techniques and compare whether they were applicable to brown bear lipoproteins, comparing the size, load, and lipid composition of the lipoproteins. Another limitation was the lack of reagents for the analysis of protein and gene expression in tissues. Plasma lipoproteins were isolated using two different methods normally used for human lipoprotein fractionation. Firstly, size exclusion chromatography, and secondly, sequential density ultracentrifugation in deuterium oxide-sucrose (heavy water) solutions. Both methods are suitable for small plasma volumes and allow rapid analysis of lipoprotein composition and functions, i.e., electrophoretic mobility, lipid content, and other functional characteristics in vitro.

What is your advice to people to take care of their heart health?

Keep cholesterol and triglycerides low, plus physical activity - we are not brown bears!

What are the future directions of this research study?

To verify the validity of our results, we need to compare the structure and composition of arterial wall tissue between bears and humans, as well as their extracellular matrix proteoglycans, and investigate differences and/or similarities.

Conflict of interests

The authors have no conflict of interests to declare.

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