Is stress response a new link between adipose tissue and atherogenesis? The role of HSPs/HSF1

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The role of lipids in atherogenesis is well established; sub-endothelial deposition of oxidised low density lipoprotein (ox-LDL) initiates atherosclerotic plaque formation.[1] Circulating cholesterol is regulated by its dietary intake and endogenous synthesis on one hand, and its elimination from the circulation via LDL-receptors/excretion in the bile on the other hand.[2] Various lipid-lowering therapies targeting these regulatory mechanisms are used in patients with dyslipidaemias, significant impacting cardiovascular risk. However, the residual risk after applying existing lipid-lowering strategies highlights the complexity of lipid metabolism and the necessity for broader understanding of the links between lipids and the cardiovascular system.

Over the last decade, it became clear that adipose tissue is an active endocrine organ producing various adipocytokines, the secretory profile of which is dysregulated in cardiovascular and metabolic disease. Adipocyte differentiation, a process whereby small, pre-adipocytes with little lipid content transform into large, differentiated lipid-rich adipocytes, is altered in cardiometabolic disease[3]. Obesity-related diseases are characterised by adipose inflammatory infiltration, which prevents pre-adipocyte differentiation, affecting lipid and energy consumption/expenditure and modifying the secretory profile of the tissue.[3] The literature on the roles of adipocyte differentiation status in metabolic and cardiovascular disease is, however, contradictory. Indeed, obesity-related diseases are associated with large, lipid-rich adipocytes[4]. Conversely, recent evidence suggests that insulin resistance may occur with small adipocytes, whereas adipocyte hypertrophy may be associated with preserved insulin sensitivity[5]. Thus, the consequences of adipocyte differentiation status may depend on the underlying disease status. Furthermore, obesity is associated with stressful stimuli (such as abnormal protein modifications and mitochondrial/oxidative/endoplasmic reticulum stress) disturbing adipose tissue homeostasis and enhancing metabolic imbalance.[6] The response of

adipose tissue to cellular stressors may thus be a crucial disease mechanism and a potential therapeutic target.

Heat shock proteins (HSPs) comprise a conserved family of molecular chaperones induced by diverse stress stimuli, allowing for cellular adaptation in response to stress.[7] HSPs are implicated in atherosclerosis, but the underlying mechanisms remain controversial. HSPs are upregulated in the lesions, but also in the plasma of patients with atherosclerosis.[7] HSPs also display immunomodulating properties and may induce autoimmunity[7], whereas Hsp25 facilitates proliferation of vascular smooth muscle cells. Heat shock factor 1 (HSF1) is an HSP trans-activator with pleiotropic effects on vascular tissue, ranging from anticoagulant/vasorelaxant properties to autoimmune/proinflammatory reactions and oxidative stress response modification.[8, 9] HSPs also mediate metabolism and adipose tissue biology, thus providing a link between diseases such as diabetes mellitus and atherosclerosis that share common metabolic traits. More specifically, HSPs display depot-specific adipose tissue upregulation upon *in vivo* heat treatment; this suggests that HSPs may regulate depot-specific adipose tissue responses in health and disease.[10] Additionally, HSF1 activates mitochondrial and thermogenic gene programs to increase energy consumption, and also reduces lipid content and expansion of adipose tissue.[11] Therefore, HSF1 may be a reasonable target in obesity-related diseases However, the integral roles of HSPs in obesity and atherosclerosis are unclear. Are HSPs (like HSF1) ubiquitously protective molecules being upregulated as local defence mechanisms, or do they also carry pro-atherogenic effects?

In this issue of CVR, K. Krishnamurthy *et al*[12] explored the impact of HSF1 inhibition on atherosclerotic lesion size, circulating lipid/adipokine profile and adipose tissue biology. Using a $HSF1^{-/-}/LDLr^{-/-}$ double knock-out (KO) mouse model, they demonstrated that western diet induced lesser atherosclerotic disease burden in the presence of HSF1 inhibition, whereas double KO mice exhibited reduced circulating LDL cholesterol, triglycerides and adiponectin,

and increased leptin levels; ablation of HSF1 resulted in reduced expression of Hsp25 in aortic tissue. Visceral adipose tissue from double KO mice was characterised by reduced macrophage infiltration and differentiation status, indicated by small size and lipid content as well as impaired PPARγ2 expression and AMPK activity. Trying to unravel the underlying mechanisms, the authors documented reduced hepatic steatosis, enlargement of gallbladder and bile ducts, and transcriptional upregulation of the CYP7A1/MDR1/Pgp axis (which enhance bile production and cholesterol clearance) in double KO mice. This study[12] proposes for the first time that HSF1 may be a reasonable target in atherosclerosis, since its inhibition improves lipidaemic profile via increased cholesterol clearance as well as, possibly, metabolic effects on adipose tissue, and reduces atherosclerotic lesion burden.

The Krishnamurthy *et al*[12] study provides new insights into the role of HSF1 in atherosclerosis, but it also introduces new questions regarding the effects of HSF1 inhibition on adipose tissue, both local (e.g. perivascular) and distal (e.g. visceral) to the cardiovascular system. PPARγ is required for adipocyte differentiation and there is vast literature about its beneficial effects on the lipid-buffering capacity of adipose tissue, as well as on insulin resistance status and cardiovascular disease. Adiponectin, an anti-atherogenic adipokine (through favourable metabolic, insulin-sensitizing and direct vascular antioxidant/anti-inflammatory effects[13, 14]) is also under the direct control of PPARγ.[15] Therefore, the consequences of PPARγ and adiponectin downregulation accompanying HSF1 knock-down would be conceptually detrimental for the metabolic and cardiovascular status, which is in apparent contrast with the study findings.[12] Additionally, the reported negative effects of HSF1 inhibition on adipocyte differentiation are controversial, since the functional significance of adipocyte differentiation and size are, as explained, miscellaneous. Therefore, a long-term and detailed characterisation of the global metabolic effects of HSF1 inhibition is required for better understanding of its roles. Adipose tissue biology is a crucial link between atherosclerosis and

metabolism, and such questions should be mechanistically investigated before the findings of the present study are translated in humans. An overview of the study findings as well as the remaining open questions, are presented in figure 1 (Fig. 1).

Despite these unresolved issues, this study by Krishnamurthy *et al*[12] comprises a significant step forward for the better understanding of the roles of HSF1, and HSPs in general, in the integrated stress responses in atherogenesis, while also proposing a novel therapeutic target with a great translational potential in humans.

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Figure 1 legend: HSF1 ablation reduces vascular expression of HSP25, whereas it also activates the CYP7A1/MDR/PgP axis in the liver. The latter leads to increased duodenal cholesterol excretion through the bile, thereby lowering circulating LDL cholesterol, an important mediator of atherogenesis upon its oxidation by reactive oxygen species (ROS). Inhibition of HSF1 is also associated with reduced lipid accumulation and adipocyte differentiation in visceral adipose tissue (AT), as well as with decreased expression of PPARγ and adiponectin, an adipokine with anti-inflammatory and anti-oxidant properties. The immediate phenotype resulting from HSF1 ablation in mice is athero-protective; however, the specific biological effects of HSF1 ablation on different AT depots (e.g. perivascular (PVAT) versus remote to the vascular wall depots) and its global metabolic effects regarding obesity and insulin resistance status remain unanswered.

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