Restraint Stress Restrains Cholesterol in the Intestine

Arnold von Eckardstein

Epidemiological studies have observed that psychosocial stress, for example, by unemployment, low socioeconomic status, or work stress, increases the risk of cardiovascular events. The pathophysiological basis of this association is poorly understood. Restraint stress, which is an experimental model for psychosocial stress, is characterized by the activation of the sympathetic nervous system involving the fast release of epinephrine and norepinephrine and the hypothalamic-pituitary-adrenal axis, resulting in a slow but sustained increase in circulating glucocorticoids. Both neuroendocrine axes elicit multiple and complex responses, aiming to protect the threatened organism. As yet, there is little and mixed epidemiological evidence about which of these pathways is relevant for the pathogenesis of cardiovascular diseases. Diurnal and other intradividual variation of cortisol and catecholamine plasma concentrations make the design of meaningful observational studies difficult. More recently, measurements of cortisol in saliva or urine found positive associations between cortisol and risk of incident coronary events. Elevated cortisol levels in plasma or saliva have also been associated with increased blood pressure, diabetes mellitus, hyperglycemia, hypertriglyceridemia, and central adiposity. However, these associations have been questioned by recent population studies that did not find any significant association of cortisol awakening response, evening cortisol, cortisol decline across the waking day, total cortisol output, and cortisol after dexamethasone suppression with metabolic syndrome or its individual components.

In this issue of Circulation Research, Silvennoinen et al. investigated the effects of stress and corticosterone, the stress hormone of rodents, on cholesterol homeostasis in mice. They focused on reverse cholesterol transport (RCT) that describes the transport of excess cholesterol from peripheral cells to the liver for excretion into the bile, either directly or indirectly after conversion into bile acids, or to steroidogenic organs for hormone production (Figure). Since the original description of the RCT model by Glomset and Wright, which has been very much refined since then, high-density lipoproteins (HDLs) have been considered as the pivotal mediator of RCT: The majority of HDL particles originate from lipid-free or poorly lipidated apolipoprotein (apo) A-I that is secreted by hepatocytes and the intestinal mucosa or dissociate from lipolyzed chylomicrons and very-low-density lipoproteins as well as from interconverting mature HDL particles. The interaction of apoA-I with the ATP-binding cassette transporter A1 (ABCA1) leads to efflux of phospholipids and unesterified cholesterol from many cells, including hepatocytes, enterocytes, and macrophages, and thereby to the formation of discoidal HDL particles. These HDL precursors become lipid-rich, spherical, and larger through the esterification of cholesterol by the enzyme lecithin:cholesterol acyltransferase, the acquisition of additional phospholipids and unesterified cholesterol from both cells and apoB-containing lipoproteins, the association of additional (apo) proteins, and the fusion with other HDL particles. Lipids of mature HDL are removed from the circulation by at least 3 pathways. The indirect pathway is mediated by the cholesteryl ester transfer protein that exchanges cholesteryl esters of HDL with triglycerides of apoB-containing lipoproteins. Mice, however, do not express cholesteryl ester transfer protein, so this pathway is not operative in the model used by Silvennoinen et al. Two direct pathways are mediated by HDL receptors, one well-characterized involving the selective uptake of lipids into the liver and steroidogenic organs via scavenger receptor-BI (SR-BI) that is also crucial for the supply of steroidogenic organs, including adrenals, with cholesterol. The other less characterized holoparticle uptake is stimulated by the interaction of apoA-I with ectopic F0F1-ATPase and subsequent activation of the P2Y13 receptor.

From the liver cholesterol is excreted into the bile, either directly by the ABCG5/G8 dimer or after conversion into bile acids through ABCB11. In addition to this HDL-mediated hepatobiliary delivery of cholesterol to the intestine, there is increasing evidence for a direct and HDL-independent transintestinal cholesterol excretion at least in rodents. From the intestinal lumen, large proportions of both cholesterol and bile acids are reabsorbed by Niemann-Pick type C1-like protein type 1 (NPC1L1, the target of the cholesterol-lowering drug ezitimibe) and the apical sodium-dependent bile acid transporter, respectively. The ABCG5/ABCG8 dimer...
expressed by enterocytes resecretes some of the absorbed cholesterol into the intestinal lumen. The remainder is either incorporated into chylomicrons or effluxed by ABCA1 to apoA-I in the lymph or secreted as part of chylomicrons (CM). The intestinal bile acids are taken up into the enterocytes by the apical sodium-dependent bile acid transporter (ASBT). For reasons of clarity, the trafficking of bile acids by enterocytes is not shown but symbolized by an asterisk (for details see text).18

B. The stress hormone corticosterone enhances macrophage reverse cholesterol transport most prominently by inhibiting NPC1L1 through induction of peroxisome proliferating agent receptor α (PPARα), which in turn downregulates NPC1L1 expression. Upregulation of ABCA1 in macrophages and hepatocytes by PPARα is prevented by direct counterregulatory effects of corticosterone on ABCA1 expression. Selective cholesteryl ester uptake into the liver is also enhanced possibly because the corticotropic hormone adrenocorticotropic hormone (ACTH) induces SR-BI not only in adrenals, but also in the liver. oxLDL indicates oxidized low-density lipoprotein.

Figure. Reverse transport of macrophage-derived cholesterol in nonstressed (A) and stressed mice (B). A. ATP-binding cassette transporter A1 (ABCA1) is required to generate high-density lipoprotein (HDL) by the liver and to allow efficient cholesterol efflux from macrophages. Scavenger receptor B1 (SR-B1) mediates selective uptake of cholesterol from HDL into the liver, from where cholesterol is secreted into the bile, either directly by ABCG5 and ABCG8 or by the bile salt export pump ABCB11 after conversion into bile acids through the cytochrome P450 enzyme 7α (CYP7A). The Niemann-Pick type C1–like protein type 1 (NPC1L1) mediates the absorption of biliary (and dietary) cholesterol into the enterocytes of the ileum. From the enterocytes, cholesterol is either resecreted into the intestinal lumen by ABCG5/ABCG8 or effluxed by ABCA1 to apoA-I in the lymph or secreted as part of chylomicrons (CM). The intestinal bile acids are taken up into the enterocytes by the apical sodium-dependent bile acid transporter (ASBT). For reasons of clarity, the trafficking of bile acids by enterocytes is not shown but symbolized by an asterisk (for details see text).18

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knockout of ABCA1) or with the concentration and composition of HDL in plasma (eg, by knockout or overexpression of apoA-I) led to the expected alterations in RCT. This model has been repeatedly exploited to explore the effect of genetic or pharmacological interventions on RCT. In the study by Silvennoinen et al, both restraint stress and corticosterone enhanced RCT. Interestingly, however, neither cholesterol efflux from macrophages, nor cholesterol delivery to the liver, nor biliary secretion from the liver was altered significantly or strongly enough to explain the observed increase in fecal sterol excretion. Rather the reabsorption of cholesterol from the intestinal lumen by NPC1L1 into the enterocytes was strongly reduced, thereby interrupting the enterohepatic circulation of cholesterol and enhancing fecal sterol excretion. By using gavage experiments, the authors also provide indirect evidence that via suppression of NPC1L1 psychosocial stress will also suppress the resorption of dietary cholesterol. Also of note, HDL cholesterol levels dropped upon stress without compromising RCT. The authors explain this by the slightly enhanced hepatic cholesterol uptake into the liver, possibly as a result of scavenger receptor BI activation by the corticotropic hormone that is induced by stress and known to upregulate SR-BI in the adrenals.

The authors could reproduce the RCT-accelerating effects of stress by treating the mice with corticosterone, and they could abrogate the effects of stress by pharmacological inhibition of corticosterone synthesis. These observations strongly indicate that corticosterone is the humoral mediator of stress effects on RCT. Interestingly, however, corticosterone did not suppress NPC1L1 directly but indirectly via upregulating the expression of the peroxisome proliferating agent receptor α (PPARα). PPARα was already known to downregulate the transcription of NPC1L1. In PPARα knockout mice, neither stress nor corticosterone enhanced macrophage RCT, clearly demonstrating the necessity of PPARα for mediating the stress response on RCT. Interestingly, Silvennoinen et al ruled out the contribution of PPARδ, which was previously found to downregulate NPC1L1 and to enhance transintestinal cholesterol excretion.

PPARα is a transcriptional regulator of many target genes involved not only in lipid metabolism, but also in inflammation. Silvennoinen et al found stress- and corticosterone-induced upregulation of PPARα not only in the intestine, but also in the liver and macrophages. Because PPARα is known to induce ABCA1 via upregulation of the liver-X-receptor α, ABCA1 expression and cholesterol efflux should be increased. The authors explain the lack of these changes by direct counterregulatory effects of corticosterone on ABCA1 expression. Because of this complexity, it will be important to test whether stress also alters the expression of other PPARα target genes involved in lipid metabolism (eg, lipoprotein lipase) and inflammation. With respect to the latter, it is interesting to note that prolonged or severe stress activates immune cells to produce inflammatory mediators, including cytokines, free radicals, and prostaglandins. The parallel induction of PPARα by stress may hence mediate counterregulatory effects aimed at the protection of the organism.

Based on the presented results, it will be interesting to study the effects of stress on cholesterol metabolism in humans. Specifically, the effect on NPC1L1 can be tested by the measurement of plant sterols in plasma, which are considered as surrogate markers of cholesterol resorption. Typically, inhibition of cholesterol resorption leads to counterregulatory increase in hepatic cholesterol synthesis. In mice, Silvennoinen et al only observed a very moderate increase in cholesterol synthesis, so it will also be important to verify this in humans, for example, by measuring the surrogate marker lathosterol. Finally, it will be interesting to see the effects of fenofibrate and other PPARα agonists on stress response both in cholesterol metabolism and in inflammation.

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References

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