

## A New Frontier for Reverse Cholesterol Transport The Impact of Intestinal Microbiota on Reverse Cholesterol Transport

Kazuhiro Nakaya, Shunichi Takiguchi, Katsunori Ikewaki

Numerous epidemiological studies have demonstrated that high-density lipoprotein cholesterol (HDL-C) levels are inversely associated with cardiovascular risk.<sup>1</sup> However, despite intense efforts to develop new pharmacological strategies to increase HDL-C levels, such as with niacin and cholesterol ester transfer protein inhibitors, few robust associations with improved clinical outcomes have been observed.<sup>2</sup> This failure of HDL-raising interventions has been accompanied by a shift toward gaining a more rigorous, basic understanding of HDL as a molecule with multiple functions that can be differentiated from simple measures of HDL-C mass.<sup>3</sup> Reverse cholesterol transport (RCT) is a pivotal pathway involved in the return of excess cholesterol from peripheral tissues to the liver for excretion in the bile and eventually the feces. RCT and cholesterol efflux, the first step and a highly important component of the mechanism of RCT from macrophages in atherosclerotic plaques, are crucial to the antiatherogenicity of HDL. In human studies, it has been shown that the capacity of HDL to promote cholesterol efflux from macrophages *ex vivo* is inversely related to the risk of cardiovascular disease even after adjusting for HDL-C levels.<sup>4,5</sup> Furthermore, although niacin treatment raises HDL levels in statin-treated patients, it does not augment cholesterol efflux, which could explain lack of efficacy of niacin.<sup>6</sup> In view of this, an increasing number of studies have investigated the hypothesis that specific therapies, such as those using reconstituted HDL and various drugs, could increase cholesterol efflux and RCT and thereby improve cardiovascular outcomes.<sup>7-9</sup>

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Our microbiota has been linked to intestinal health, immune function, bioactivation of nutrients and vitamins, and recently, complex disease phenotypes, such as obesity and insulin resistance.<sup>10</sup> Interestingly, recent studies in this regard by Wang et al<sup>11</sup> and Tang et al<sup>12</sup> showed that intestinal microbial processing of dietary choline to trimethylamine, which is further metabolized to trimethylamineoxide by flavin monooxygenases in human and rodent livers, was significantly

correlated with cardiovascular disease. Furthermore, Koeth et al<sup>13</sup> found that trimethylamineoxide suppressed RCT via an intestinal microbiota-dependent mechanism *in vivo*. These findings suggest a new concept that specific combinations of intestinal microbiota and host genetics may provide cardiometabolic regulation.

In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Mistry et al<sup>14</sup> show that complete absence of intestinal microbiota significantly enhances *in vivo* macrophage-to-feces RCT, mainly affecting the fecal bile acid fraction. They show that plasma cholesterol levels and mass fecal neutral sterol excretion are not altered, whereas fecal bile acid excretion is reduced, in the absence of intestinal bacteria. These findings add to those of previous studies showing the importance of the intestinal microbiota to HDL metabolism and development of atherosclerosis. The findings of Mistry et al<sup>14</sup> also suggest possibilities for new approaches to increasing RCT.

A direct approach is to reduce the load of intestinal bacteria, and intriguingly, low-dose antibiotics have been used to reduce trimethylamine production in the human intestine,<sup>15</sup> but it should be noted that antibiotic trials in humans set up to test the hypothesis that certain microorganisms, such as chlamydia, may directly infect the arterial wall have not shown any cardiovascular benefit.<sup>16</sup> However, if bacterial species responsible for decreasing RCT could be identified and eliminated with selective antibiotics, this would be ideal, therapeutically sufficient, and less disruptive to the intestinal microbiota than broad-spectrum antibiotics.

Another approach is to use probiotics, live microorganisms that either inhibit or promote various species in the intestinal microbiota. In a mouse model carrying a humanized microbiota, administration of a certain probiotic reduced trimethylamineoxide production, whereas another probiotic increased it.<sup>17</sup> Also, previous clinical studies have shown that probiotics improved the low-density lipoprotein/HDL ratio, as well as blood pressure, inflammatory mediators, blood glucose levels and body mass index.<sup>18</sup> It would, therefore, be interesting to investigate the effect of probiotics on HDL metabolism and RCT in humans in future studies.

Although the above findings are extremely provocative, cholesterol profiles in the absence of intestinal bacteria observed in the study by Mistry et al<sup>14</sup> and other previous studies have varied. The authors suggest that the phenotypes observed might depend on diet, genetic background, and the composition of the microbiota. In addition, there are large differences between rodents and humans regarding bile acid and cholesterol metabolism.<sup>19</sup> Furthermore, they do not address a possible effect of intestinal microbiota on atherosclerosis. Therefore, further studies are needed to investigate whether the present findings translate into the clinical setting.

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(*Arterioscler Thromb Vasc Biol*. 2017;37:385-386.)

DOI: 10.1161/ATVBAHA.117.309006.)

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*Arterioscler Thromb Vasc Biol* is available at <http://atvb.ahajournals.org>

DOI: 10.1161/ATVBAHA.117.309006

In summary, the study of Mistry et al<sup>14</sup> focuses on the observation that the absence of intestinal microbiota increases RCT, suggesting that the intestinal microbiota might be a new therapeutic target for enhancing RCT, leading to the prevention of atherosclerosis and cardiovascular disease. However, as mentioned earlier, further studies are needed to see whether their findings in mice translate into the clinical setting in humans. In the light of their study, it would be of substantial interest to determine the impact of intestinal microbiota on RCT in patients with atherosclerotic risk. If such an impact were found, it should then be considered whether specific targeting of intestinal microbiota would improve HDL functionality and cardiovascular outcomes in patients with atherosclerotic risk.

### Disclosures

None.

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KEY WORDS: Editorials ■ bacteria ■ cholesterol ■ liver ■ microbiota ■ sterols

# Arteriosclerosis, Thrombosis, and Vascular Biology



JOURNAL OF THE AMERICAN HEART ASSOCIATION

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*Arterioscler Thromb Vasc Biol.* 2017;37:385-386

doi: 10.1161/ATVBAHA.117.309006

*Arteriosclerosis, Thrombosis, and Vascular Biology* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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