Does Endothelium Buffer Fat?

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Endothelial cells (ECs) are the metabolic gatekeepers of the body. All tissues require nutrients for growth and function, and those nutrients must pass the endothelial layer. In some tissues like the liver, the endothelium is discontinuous and marked by “fenestrae” (from the Latin for “window”), dedicated transcellular openings that allow free movement of nutrients. But in most tissues such as the heart, muscle, and brain, endothelia lack fenestrae, and nutrients must traverse the endothelial wall directly. The list of nutrients that must do so is long and includes sugars, amino acids, and fats. Of those, the latter have received the least attention, although organs like the heart consume copious fatty acids for the generation of ATP.

Fatty acids travel the blood stream largely in 2 forms: esterified as triglycerides in lipoprotein particles, including chylomicrons and VLDL (very-low–density lipoprotein), or unesterified and noncovalently bound to albumin. Esterified fatty acids are liberated by lipoprotein lipase, which, importantly, is located on the luminal side of the endothelium. Therefore, all fatty acids must traverse the endothelial layer before reaching underlying parenchyma. Recent work has demonstrated that paracrine factors, both proteins and metabolites, secreted by parenchymal cells can promote adjacent endothelium to transport fats. For example, VEGFB (vascular endothelial growth factor B), a VEGF family member, is secreted from fat-consuming oxidative skeletal and heart muscles to promote transendothelial fat transport, thereby coordinating myocyte metabolism with nutrient delivery. Similarly, 3-hydroxy-isobutyrate, a metabolite of valine catabolism, is secreted from skeletal muscle to promote transendothelial fatty acid transport, and it may contribute to muscle lipotoxicity and insulin resistance.

How does the endothelium handle and transport fats in response to these and other signals? Surprisingly little is known about how this process inside ECs occurs. Several mechanisms have been proposed: diffusion within endothelial membranes; paracellular transport (unlikely in continuous, unfenestrated endothelia); and transcellular transport, that is, transport across the EC. The identification of mammalian fatty acid transport proteins and associated acyl-CoA synthetases has strengthened the argument for protein-mediated transcellular fatty acid transport. The data in ECs, however, remain scant. siRNA experiments in cell culture have indicated that fatty acid transport proteins 3 and 4 are required for VEGFB and 3-HIB (hydroxyisobutyrate)–induced endothelial fatty acid uptake, but how these proteins function remains largely unknown.

An additional fascinating question is how do ECs mediate continuous transport, considering that delivery of fats from the blood seldom comes in a continuous flux? Circulating levels of glucose, for example, are tightly regulated by insulin and normally maintained within a narrow range. Not so for fats: levels of plasma triglycerides in chylomicrons can vary 10-fold between fasting and feeding in humans, and even more so in rodents. How does the endothelium handle this sudden surge? In this issue of Circulation Research, Kuo et al addressed this question by treating mice with oral gavages of olive oil, and by treating ECs in culture with various fats. Surprisingly, they observed that ECs dramatically induce the storage of fats into intracellular lipid droplets (LDs). In other words, the cells reesterify the free fatty acids liberated by lipoprotein lipase, and package them as triglycerides within the cell (Figure [A]). The process was observed both in vitro and in vivo, with striking accumulation of LDs observed in en face aortic wall whole mounts, 180 minutes after oral gavage with olive oil. And the process involved the expected repertoire of triglyceride-forming enzymes, including glycerol 3-phosphate acetyl transferase, acyl-glycerol 3-phosphate acetyl transferase, lipin, and diacylglycerol acetyl transferase. Moreover, the process was transient. Shortly after being formed, the triglycerides were again de-esterified, and fatty acids again liberated from the ECs, either to underlying tissue (eg, muscle) or back into the circulation (likely bound to albumin). This reversal process involved the expected repertoire of triglyceride-breakdown enzymes, including adipose triglyceride lipase, hormone-sensitive lipase, and monoacyl glycerol lipase. In sum, the authors beautifully demonstrated the pronounced and transient formation and dissolution of LDs within ECs in response to a lipid load, both in vitro and in vivo.

Why do ECs transiently store fats in this way? After all, the process is not free: each esterification comes with the cost of ligating CoA to a fatty acid chain, requiring conversion of ATP to AMP; a cost that is not recovered with de-esterification. At least 4 reasons come to mind. First, excess free fatty acids can be toxic, whereas neutral triglycerides in LDs are not. Kuo et al elegantly support this notion by demonstrating that storage of fats in LDs protects ECs from fatty acid-induced ER stress and apoptosis. Diverting fats into LDs when faced with a surge of fatty acid supply is, thus, a logical protective mechanism. Second, stored fats can, in principle, provide an energy source for endothelial ATP generation. Here,

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however, it is important to note that ECs likely derive most ATP from glycolysis, with little contribution from fats, and that ECs have constant access to plasma nutrients. Thus, it seems unlikely that ECs significantly rely on LDs to store fats for later use to generate ATP.

Third, storage of fats in LDs potentially provides a significant systemic buffer against fat overload. There are an estimated 1 to 3 trillion ECs in the human body, permeating nearly every organ, with an estimated total volume on the order of 1 L.\textsuperscript{7,8} The interface between ECs and plasma is between 4000 and 7000 m\textsuperscript{2}, equivalent to the surface of >20 tennis courts.

The endothelium is, thus, ideally suited for rapid buffering of plasma contents. Assuming that LDs can occupy 2% of endothelial volume, then up to 20 g of fat could be buffered in the endothelial compartment following, for example, a post-prandial surge of chylomicrons. Considering that the average consumption of fat in the United States is 100 g per day, and assuming 3 equivalent meals, this suggests that more than half of the fat content in a meal could transiently be buffered by the endothelium. Even if significantly imprecise, these coarse estimates highlight the impressive potential buffering capacity of LDs in the endothelium.

Figure. **A**, Fatty acids (FAs) transit through the endothelial triglyceride pool on their way to muscle. FAs are liberated from lipoprotein particles by LPL (lipoprotein lipase) and are then esterified by fatty acid transport proteins or other acyl-CoA synthases, at the cost of ATP to AMP conversion (1). Acyl chains are then esterified by glycerol 3-phosphate acyl transferase, acyl-glycerol 3-phosphate acyl transferase, and diacylglycerol acyl transferase 1 (2), and incorporated into lipid droplets (LDs). The process is then reversed by adipose triglyceride lipase, hormone-sensitive lipase, and monoacyl glycerol lipase (3). Newly liberated fatty acids can be released to the parenchyma (4), or back to the vascular lumen (likely to albumin). Paracrine factors from muscle, including VEGFB (vascular endothelial growth factor B) and 3-HIB (hydroxyisobutyrate), likely regulate this process, via still unclear mechanisms. **B**, Hypothetical kinetic buffering, by the endothelium, of FA delivery to underlying tissue. An acute postprandial surge of plasma triglyceride (TG) levels is temporarily buffered in endothelial LDs, and subsequently released to underlying skeletal muscle. The buffering allows smoother delivery of FAs to muscle and avoids crossing intramuscular lipotoxic thresholds.
Fourth, transient storage of fats in LDs could also provide a kinetic buffer to fatty acid delivery to underlying tissue, such as skeletal muscle. Muscle is prone to lipotoxicity, and excess lipids have been tightly associated with insulin resistance. Maintaining intramuscular lipids below a certain threshold is, thus, likely critical. Surges in lipid delivery, as observed postprandial, must, therefore, be tempered, and LDs in endothelium may do just that. Kuo et al. demonstrate that plasma concentrations of TGs peak 90 minutes after olive oil gavage, whereas accumulation of LDs in the aortic wall peak significantly later at 180 minutes. The authors then demonstrate in cell culture that ECs loaded with LDs subsequently unload their fat content back into the medium or adjacent cells such as muscle cells. Endothelium, thus, likely buffers and “smoothes out” the kinetics of transferring a plasma lipid surge to the underlying tissue (Figure B). Such kinetic buffering allows the fat to be absorbed into the parenchyma at a rate slow enough to reduce sudden excess accumulation, and may be critical to preventing acute lipotoxicity.

The study by Kuo et al. identifies exciting new biology in ECs. As with all such discoveries, numerous questions remain. How do fatty acid transport proteins interact with LDs, if at all? And are LD dynamics regulated by paracrine factors like 3HIB and VEGFB? For example, could LD formation be an important component of how 3HIB or VEGFB modulate fatty acid transport, a process that is currently not understood? Indeed, do all fatty acids that traverse the endothelium take an obligatory detour through the triglyceride pool, or does this occur only under specific circumstances, for example, postprandial lipid surges? And do all ECs have capacity for LD formation in vivo, or, in keeping with the tremendous heterogeneity of ECs, does LD formation capacity differ from one tissue endothelium to another? Addressing these questions experimentally will not be easy, but will have critical implications for any tissue that relies on fatty acid transport across its endothelium, which is to say the vast majority of tissues.

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