The Methods for Studying Chylomicron Metabolism

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Introduction

Chylomicrons (CM) are major triglyceride (TG) -rich lipoproteins responsible for transporting dietary lipids to different tissues during postprandial state, thus playing essential roles in maintaining lipid and energy homeostasis. In response to lipid ingestion, CM are produced in enterocytes of small intestine, secreted to the lymph and finally drained into the circulation via thoracic duct. In the circulation, CM-associated triglycerides are hydrolyzed by peripheral lipoprotein lipase to generate monoacylglycerols and fatty acids, which are subsequently taken up by peripheral tissues, for example, adipose and muscle. Here we describe methods to study chylomicron metabolism in the body. These methods can be used to study how genetic and environmental factors contribute to diet-induced obesity in mice.

Schematic representation of the methods

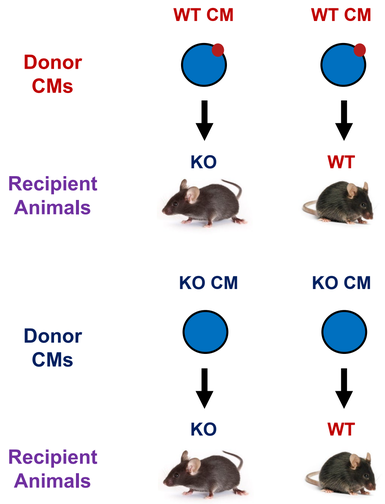


Figure 1. Methods to study chylomicron metabolism

Methodology

Donor Chylomicrons

Adult male or female Sprague-Dawley rats or mice were used to generate donor chylomicrons

Lymph Fistula Surgery

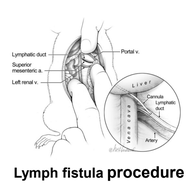
1.Cannulation of the intestinal lymph duct
2.Installation of a duodenal feeding tube
3. Recovery overnight

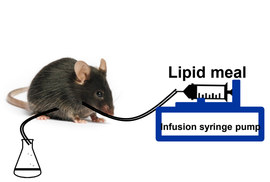
Infusion of Lipid Meal and Lymph Collection

1. A lipid meal containing ^{3}H-TG (to measure hydrolysis of TG in plasma and uptake into tissues) and ^{14}C-CHOL (to measure CM remnant removal from the plasma) was infused via feeding tube
2.Lymph was collected from the intestinal lymph duct

Chylomicron Preparation

Intestinal lymph was pooled and chylomicrons were isolated by flotation ultracentrifugation





Recipient Animals

Adult male or female genetically modified mice and Wild Type mice were used as chylomicron recipients

Delivery of Donor CMs to Recipient Animals (Fig.1)

1.Donor chylomicrons were injected into the jugular vein of recipient animals

Analysis of TG Hydrolysis and CHOL clearance from the Plasma

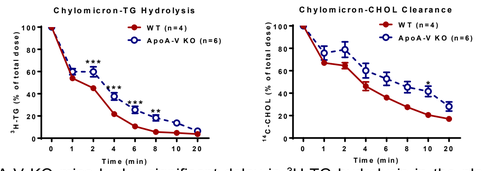
1.Tail blood was collected up to 20 minutes post chylomicron injection
2.Plasma was isolated and radioactivity was determined by scintillation counting

Analysis of Chylomicron Remnant Uptake by the Liver and Fatty Acid Uptake by Peripheral Tissues

1.At 20 minutes, animals were perfused with PBS
2.The liver, visceral fat, gastrocnemius muscle, and spleen were dissected
3.Tissue lipids were extracted by Folch Method and radioactivity determined

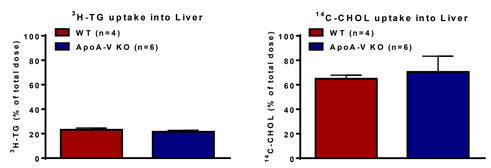
An example: ApoA-V chylomicron metabolism study using rat chylomicrons

Plasma clearance of CM-associated TG and cholesterol



ApoA-V KO mice had a significant delay in ^{3}H-TG hydrolysis in the plasma compared to WT mice. When measuring ^{14}C-CHOL removal from the plasma, ApoA-V KO mice had a slower clearance of cholesterol than WT mice. Data shown are means values ± SE. Two-way repeated-measures ANOVAs analysis were used.

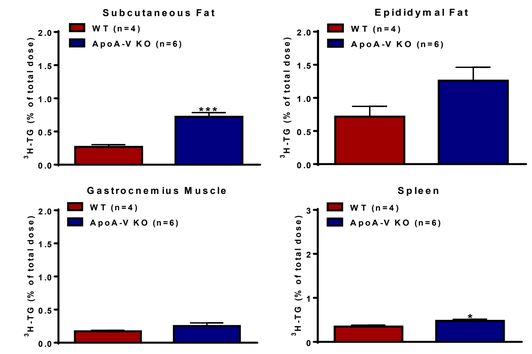
Chylomicron remnant uptake by the liver



There is no difference in ^{3}H-TG uptake or ^{14}C-CHOL uptake into the liver, suggesting no difference in chylomicron remnant uptake between apoA-V KO and WT mice. Data shown are means values ± SE. Two-tailed unpaired T tests were used.

ApoA-V study

Fatty acid uptake by epididymal fat, subcutaneous fat, gastrocnemius muscle, and spleen



ApoA-V KO animals have a greater uptake of ^{3}H-TG into the subcutaneous fat and the spleen. However, there was no difference in ^{3}H-TG uptake into the epididymal fat and the gastrocnemius muscle. The presence of ^{14}C-CHOL could not be detected (data not shown). Data shown are means values ± SE. Two-tailed unpaired T tests were used.

Conclusion and future application

- We found that apoA-V KO mice displayed a slower rate in TG hydrolysis in the circulation and an enhanced uptake of fatty acids by the subcutaneous fat and the spleen. Since the same apoA-V containing chylomicrons (from rats) were used for the metabolic studies in WT and apoA-V KO mice, our results suggest that apoA-V in the recipient animals affect chylomicron metabolism.
- We can apply these methods to determine if the differences in the metabolism of CM contribute to differences in body weight or body fat caused by genetic modification, diet-induced obesity, or other environmental factors.

References

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