

# **3-D SEMIQUANTITATIVE MEASUREMENT OF INTESTINAL CHYLOMICRON PRODUCTION AND SECRETION**

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## **1. BACKGROUND**

In this study we developed a simple and quick method for semi-quantitative immunodetection of chylomicron production and secretion. Chylomicrons are synthesized in enterocytes of the small intestine in response to ingested fats. A significant pathway in chylomicron assembly is the lipidation of apolipoprotein B an obligatory structural component of chylomicrons. Cell culture studies suggest that primordial chylomicron assembly occurs with the ER with secretion occurring via the Golgi apparatus at the basolateral surface of the cell.

## **2. RESULTS**

Immunofluorescent microscopy was developed to investigate chylomicron assembly and secretion in wild-type mice given low-fat, or lipid supplemented diets. Following sacrifice and isolation of the upper small intestine, 10  $\mu$ m paraffin embedded sections were double labelled with anti-apo B and anti-Golgi apparatus antibodies. We found significant apo B in the apical perinuclear region of enterocytes colocalized with Golgi-apparatus, consistent with the site of biogenesis. A gradient pattern of staining for apo B was also found in the basolateral region of enterocytes and within the lacteals, clearly demonstrating the secretory pathway into the lymphatic architecture. For 3-D semiquantitative measurement, intestinal apo B was stained with polyclonal anti-apo B (1:500) and labelled with anti-rabbit IgG Alexa488 conjugate. 3-D fluorescent image was captured at x200 with Zeiss AxioVert 200M and ApoTome by capturing consecutive Z-stack optical sectioning images. The intensity of apo B fluorescence in the entire images was measured with automatic measurement module of AxioVision imaging software. In order to normalize the number of cells contained in each image, nuclei were counterstained with DAPI and the optical intensity was measured with the same method as apo B, then the intensity of apo B staining was expressed per DAPI intensity. Intestinal apo B staining was compared between control mice fed low-fat standard chow diet (4% w/w as total fat), a cholesterol supplemented (1% w/w) cholesterol, or a saturated-fat enriched diet (20% w/w) saturated fatty acid (SFA).

Semiquantitative analysis showed that the cholesterol and saturated fat fed mice had significantly greater production secretion of chylomicrons (determined as apo B). This finding is consistent with cell culture studies which demonstrate increased chylomicron production and secretion when supplemented with exogenous lipids.

## **3. CONCLUSION:**

The 3-D semiquantitative immunofluorescent method developed in this study was suitable for determining chylomicron homeostasis in mice given diets differentially enriched in fats or cholesterol.