Available online at www.sciencedirect.com

Metabolism

www.metabolismjournal.com

Triglycerides produced in the livers of fasting rabbits are predominantly stored as opposed to secreted into the plasma

Demidmaa Tuvdendorj[a,b,*], *Xiao-jun Zhang*[a,c], *David L. Chinkes*[a,c], *Lijian Wang*[b], *Zhanpin Wu*[a,c], *Noe A. Rodriguez*[a,c], *David N. Herndon*[a,c], *Robert R. Wolfe*[d]

[a] *Department of Metabolism Unit, Shriners Hospital for Children, University of Texas Medical Branch, Galveston, TX 77550, USA*
[b] *Department of Internal Medicine, University of Texas Medical Branch, Galveston, TX 77550, USA*
[c] *Department of Surgery, University of Texas Medical Branch, Galveston, TX 77550, USA*
[d] *Department of Geriatrics, Center for Translational Research in Aging & Longevity, University of Arkansas Medical School, Little Rock, AR 72205, USA*

ARTICLE INFO

Article history:
Received 30 June 2014
Accepted 5 January 2015

Keywords:
Rabbit liver
Stable isotopes
Mass spectrometry
Very low density lipoprotein
Triglyceride synthesis

ABSTRACT

Objective. The liver plays a central role in regulating fat metabolism; however, it is not clear how the liver distributes the synthesized triglycerides (TGs) to storage and to the plasma.

Materials and methods. We have measured the relative distribution of TGs produced in the liver to storage and the plasma by means of U-$^{13}C_{16}$-palmitate infusion in anesthetized rabbits after an overnight fast.

Results. The fractional synthesis rates of TGs stored in the liver and secreted into the plasma were not significantly different (stored *vs.* secreted: 31.9 ± 0.8 *vs.* 27.7 ± 2.6% · h^{-1}, $p > 0.05$). However, the absolute synthesis rates of hepatic stored and secreted TGs were 543 ± 158 and 27 ± 7 nmol · kg^{-1} · min^{-1} respectively, indicating that in fasting rabbits the TGs produced in the liver were predominately stored (92 ± 3%) rather than secreted (8 ± 3%) into the plasma. This large difference was mainly due to the larger pool size of the hepatic TGs which was 21 ± 9-fold that of plasma TGs. Plasma free fatty acids (FFAs) contributed 47 ± 1% of the FA precursor for hepatic TG synthesis, and the remaining 53 ± 1% was derived from hepatic lipid breakdown and possibly plasma TGs depending on the activity of hepatic lipase. Plasma palmitate concentration significantly correlated with hepatic palmitoyl-CoA and TG synthesis.

Conclusion. In rabbits, after an overnight fast, the absolute synthesis rate of hepatic stored TGs was significantly higher than that of secreted due to the larger pool size of hepatic TGs. The net synthesis rate of TG was approximately half the absolute rate. Plasma FFA is a major determinant of hepatic TG synthesis, and therefore hepatic TG storage.

Abbreviations: FSR, fractional synthesis rate; FFA, free fatty acids; FA-Cn, fatty acyl carnitine; FA-CoA, fatty acyl coenzyme A; GC-FID, gas chromatography flame ionization detector; GC-MS, gas chromatography mass spectrometry; LPL, lipoprotein lipase; MPE, mole percent excess; PalCn, palmitoyl-carnitine; PalCoA, palmitoyl-coenzyme A; PLs, phospholipids; TLC, thin layer chromatography; TGs, triglycerides; VLDL-TG, very-low density lipoprotein triglyceride.

* *Corresponding author at:* Division of Endocrinology, Department of Internal Medicine, The University of Texas Medical Branch, 8.138 Medical Research Bldg, 301 University Blvd, Galveston, Texas, 77555-1060. Tel.: +1 409 772 1962; fax: +1 409 772 8709.
E-mail address: detuvden@utmb.edu (D. Tuvdendorj).

http://dx.doi.org/10.1016/j.metabol.2015.01.002
0026-0495/

1. Introduction

The liver plays a central role in regulating fat metabolism [1–3]. An important function is to take up free fatty acids (FFAs) from the plasma, re-esterify them into triglycerides (TGs), and then secrete them back into the plasma as very low density lipoprotein triglycerides (VLDL-TGs) [4,5]. The FAs in VLDL-TGs can be used as an energy source by various tissues, or taken up and stored in adipose tissue [6,7]. Under normal conditions FA release from adipose tissues far exceeds the rate of fat oxidation, but the excess FAs provide a readily available substrate to enable a rapid increase in fat oxidation when required, e.g., at the onset of exercise [8]. The total amount of FFAs that can be transported in the plasma is limited because FFAs are insoluble and must be transported bound to albumin [2]. Secretion of FAs as TGs therefore serves as an additional potential energy source in the form of circulating lipids. On the other hand, TGs re-esterified in the liver can also be deposited as TG droplets in hepatocytes. Excess accumulation of hepatic TGs is associated with a variety of pathologies, including insulin resistance [1–3]. The regulatory mechanisms whereby the liver distributes re-esterified TGs to secretion *vs.* storage are unclear. Since hepatic steatosis is associated with insulin resistance and other pathologies [1–3], understanding the differentiation of TGs produced in the liver into storage *vs.* secretion is important.

The production of VLDL-TGs or liver-secreted TGs has been measured in several studies using tracer methodologies [9–11], and shown to be predominately regulated by the provision of plasma FFAs [12–14]. Under most conditions, the re-esterification of plasma FAs to TGs accounts for the majority of TGs synthesized in liver and the contribution of *de novo* synthesis of FAs to VLDL-TG production is minor [14]. Thus fairly extensive knowledge regarding regulation of the synthesis of VLDL-TGs secreted into the plasma is available. However, few quantitative data are available regarding the synthesis rate of TGs subsequently stored in the liver. This knowledge gap is largely due to the difficulty in measuring TG synthesis in liver tissue.

The present experiments were designed to simultaneously measure both the secretion and storage of TGs synthesized in the liver of rabbits after an overnight fast. We measured the isotopic enrichment of hepatic U-$^{13}C_{16}$-palmitoylCoA (PalCoA) and U-$^{13}C_{16}$-palmitoyl-carnitine (PalCn) to estimate the precursor pool [15,16], and the enrichment of U-$^{13}C_{16}$-palmitate in plasma and hepatic TGs for the product enrichments from the re-esterification pathway. We have previously shown that in men after an overnight fast there is essentially no *de novo* FA synthesis, meaning that all hepatic TG synthesis is derived from FAs taken up from the plasma [14]. We hypothesized that the same is true for fasting rabbits thus we assumed that the synthesized TGs were derived only from FAs taken up from the plasma. The methodology used in this study also enabled us to further compare the hepatic synthesis of TGs and phospholipids (PLs).

2. Materials and methods

2.1. Animal preparation

Adult male New Zealand White rabbits (Myrtle's Rabbitry; Thompson Station, TN), weighing 4–5 kg, were used. The rabbits were housed in individual cages and fed 150 g · day^{-1} of unpurified diet (Lab Rabbit Chow 5326, Purina Mills; St. Louis, MO) for weight maintenance. This protocol complied with the Public Health Service Policy on Humane Care and Use of Laboratory Animals, incorporated in the Institute for Laboratory Animal Research Guide for Care and Use of Laboratory Animals, and was approved by the Animal Care and Use Committee of The University of Texas Medical Branch at Galveston.

The animals were studied after overnight food deprivation with free access to water. Surgery was performed to insert catheters into the carotid artery and jugular vein under general anesthesia [17]. The arterial line was used for drawing blood and monitoring arterial blood pressure and heart rate; the venous line was used for infusing anesthetics and saline. An additional venous line was installed in a marginal ear vein via a Teflon-top needle (24 G 3/4 in Introcan® SafetyTM; B. Braum Medical; Bethlehem, PA), and used exclusively for tracer infusion. Tracheotomy was performed to place a tracheal tube that was connected to a hood filled with oxygen-enriched room air.

2.2. Stable isotope tracer infusion

After surgery, we observed blood pressure, heart rate and rectal temperature for 20–30 min to ensure stable physiological conditions before starting the tracer infusion. U-$^{13}C_{16}$-palmitate (99% enriched; Cambridge Isotope Laboratories), bound to 5% albumin [18] was infused at ~0.10 μmol · kg^{-1} · min^{-1} (15 ml/h) after a priming dose of 1.0 μmol · kg^{-1} (2.5 ml). Arterial blood (1.5–2.0 ml each) was drawn before and during the tracer infusion. At the end of tracer infusion 5 ml of saturated KCl was intravenously injected under general anesthesia, followed immediately by laparotomy. A piece of liver was collected by freeze-clamp technique to avoid any delay in sample processing that could decrease the precursor enrichment [17]. Another piece of liver tissue was then taken using scissors and washed thoroughly in a cup with ~80 ml ice-cold saline. The liver samples were frozen in liquid nitrogen before being transferred into cryogenic tubes and stored at −80 °C. The whole liver was then removed and weighed using a digital scale.

Two groups of rabbits received the above described palmitate tracer infusion. In group 1 (n = 7), the tracer was infused for 3 hours; arterial blood was drawn before the infusion and every 30 min during the infusion. In group 2 (n = 5), the tracer was infused for only 0.5 hour; arterial blood was drawn before the infusion and at 5, 10, 20 and 30 min during the infusion. Only group 1 was originally planned. However, the high enrichment of hepatic TG-bound palmitate measured from the liver samples taken at the end of the 3-hour infusion suggested possible overestimation of average precursor enrichment if there was tracer recycling. "Tracer recycling" means the release of labeled palmitate from both the plasma and intracellular lipid breakdown to the precursor pool for re-synthesis of lipids. Thus, group 2 was added; we assumed that any increase in precursor enrichment from 0.5 to 3 hours of tracer infusion could be attributed to tracer recycling (for details see Section 2.5). Clarification of the recycling allowed us to estimate the average precursor enrichment over the 3-hour tracer infusion, as we