Measurement of endogenous and exogenous triacylglycerol kinetics in the fed and fasted states

F. Sun1, M. Stolinski, F. Shojaee-Moradie and A.M. Umpleby
Department of Diabetes & Endocrinology, Postgraduate Medical School, University of Surrey, Guildford, Surrey GU2 7XH, U.K.

Abstract
Emerging evidence has shown that an abnormal postprandial accumulation of dietary fat is atherogenic. However, there is a lack of data describing the mechanisms for accumulation of triacylglycerol (TAG) in the postprandial period. There is therefore a need to establish a specific measure of the kinetics of endogenous and exogenous TAG in the postprandial period.

Background
Abnormally elevated blood TAG (triacylglycerol) levels in the postprandial period are predictive of an increased risk of CVD (cardiovascular disease) [1]. Abnormal triglyceridaemia is due to the presence of excess TRL (TAG-rich lipoproteins) which consist of endogenously derived TRL, VLDL (very-low-density lipoprotein) and CMs (chylomicrons). VLDL is secreted continuously by the liver and transports TAG formed from fatty acids derived from peripheral TAG storage pools, from dietary TAGs which have incorporated into endogenous pools or from DNL (de novo lipogenesis). All endogenously derived lipoprotein particles contain apo (apolipoprotein) B100, a large protein containing 4536 amino acid residues. In the fed state, dietary TAGs are transported in CMs which in humans are synthesized by enterocytes. ApoB48, the major apolipoprotein of CMs, is a protein containing 48% of the N-terminus of apoB100 and is distinct from intestinally derived particles. TAGs from both CMs and VLDL undergo lipolysis by LPL (lipoprotein lipase) and release fatty acids for uptake into peripheral tissues. As a result, these particles become reduced in size and develop into cholesteryl ester-enriched remnants which are mostly taken up by the liver.

Currently, our understanding of postprandial TAG metabolism is inadequate due to an inability to simultaneously quantify endogenous and exogenous TAG kinetics in vivo. A pathological increase in fasting plasma TAG concentration or an increase in the area under a TAG versus time curve following a meal cannot be used to determine whether abnormal accumulation is due to the overproduction of TRLs, impaired clearance or a combination of both. Stable isotopic methodology is an established technique that enables determination of the kinetics of lipoproteins in vivo.

Measurement of TAG kinetics in the fasted state
TAG kinetics can be measured by infusing or injecting intravenously isotopically labelled glycerol or fatty acids which become incorporated into TAG molecules which then become integrated into lipoprotein particles. By taking repeated blood samples over many hours after administration of the tracer and measuring the glycerol or fatty acid enrichment of the TAG isolated from lipoproteins, the production, clearance and turnover rates of TAGs in TRL can be calculated.

Early studies using radioactive tracers [2] demonstrated that glycerol is a more accurate tracer than palmitate because it recycles to a lesser extent. Zech et al. [3] pointed out that hydrogen-labelled glycerol recycles less than carbon-labelled glycerol because only material transformed directly to $\alpha$-glycerol phosphate for TAG synthesis retains the $^3$H label, whereas $^{14}$C-labelled glycerol could be transformed to gluconeogenic precursors and still retain its label. Stable isotopes are now more widely used than radioactive isotopes for the measurement of TAG kinetics in humans [4,5]. Lemieux et al. [5] compared the stable isotopic form of $[2H_5]$glycerol to the radioactive isotope $[2H_3]$glycerol and showed that $[2H_5]$glycerol is a convenient and safer tracer that can be used to measure VLDL-TAG turnover over a shorter study duration (12 h). Patterson et al. [6] have demonstrated that a bolus injection has an improved ability to resolve hepatic glycerolipid tracer recycling when compared with a continuous infusion of tracer. Therefore, in the fasted state, $[2H_5]$glycerol administered as a bolus is considered to give the most precise measure of VLDL-TAG kinetics. Isotopic techniques which can provide an accurate quantitative measure of TAG kinetics in the fed state are yet to be established.

Measurement of TAG kinetics in the fed state
One of the major difficulties in measuring TAG kinetics in the fed state is that the fed state is a non-steady-state
condition, whereas a steady state is required for isotopic quantitative measurements. A number of studies using stable isotopic methodology have attempted to overcome this issue by using a multiple meal feeding approach to provide a pseudo-steady state. Cohn et al. [7] and Lichtenstein et al. [8] fed an initial large meal consisting of 4/23 of a daily caloric intake followed by a series of hourly meals containing 1/23 of daily intake over 20 h. Each of the 1/23 meals was identical and was composed of spaghetti, meat, bread, butter and milk. These protocols were used to minimize the time taken for TAG levels to plateau and achieved a TAG steady state for a period of 15 h. The former study [7] reported that plasma TAG concentrations were significantly greater in the fed than fasted state (1.74 ± 0.2 compared with 0.72 ± 0.079 mmol/l, P < 0.001). The latter study [8] showed that plasma TAG levels were variable during the first quarter of the infusion period, but remained constant for the remaining study period. Infusion of isotope was begun 5 h after the first meal to avoid variations in plasma TAG levels. Most continuous meal feeding protocols consist of hourly feeding regimens [9,10], however reporting of plasma TAG and apo concentrations to show achievement of a steady state is limited. Pont et al. [11] have used a protocol of feeding every 2 h for 22 h. These authors infused isotope 6 h after giving the first meal and reported that plasma lipid and apolipoprotein values remain constant throughout the kinetic study. The continuous feeding protocols described above have all used an infusion of labelled amino acids [7–11] to measure the kinetics of the proteins specifically associated with either endogenous or exogenous particles (apoB100 and apoB48). These can be separated by SDS/PAGE. Although this provides a measure of particle kinetics, it does not provide a measure of the kinetics of TAGs within the particle itself. A methodological difficulty in measuring TAG kinetics in the fed state is that endogenously derived TRL needs to be purified from exogenously derived TRL if the kinetics of the two populations of TRL are to be determined.

An approach which allows the separation of endogenous from exogenous lipoproteins as intact particles is immuno-affinity chromatography. CNBr-activated Sepharose 4B has been widely used for the immobilization of proteins because of the gentle conditions employed to obtain stable multipoint attachment sites with large ligands containing primary amino groups such as antibodies. This method, using different combinations of monoclonal antibodies specific for apoB100, has been generally used to purify TRL [12–14]. Antibodies such as 4G3 and 5E11 which are mapped to residues 2980–3080 and 3441–3568 respectively [15] are utilized by coupling to CNBr-activated Sepharose 4B. Once separated, TAG can be extracted from the isolated lipoprotein particles. This technique has been used to quantify exogenous and endogenous TRL. However, the recovery and the efficacy of separation of this purification vary enormously and the relatively high cost of antibodies limits the application of this method. The recovery measured by protein concentration using SDS/PAGE combined with scanning densitometry is reported to be 90% in some studies [12,13]; however, when measured as TAG concentration, recovery can be as low as 77% [14]. This may imply that the unbound fraction is not pure apoB48 and is mixed with as much as 30% of apoB100-containing lipoprotein particles.

Conclusion

Further development of methods which allow the measurement of endogenous and exogenous TAG kinetics in the fed state is necessary to advance our understanding of postprandial hypertriglyceridaemia. These would include improved immunoaffinity chromatography methods for separating apoB100- and apoB48-containing lipoproteins, the development of more physiological feeding protocols to achieve a steady state in the production of exogenous lipoproteins and the investigation of isotopic techniques which can provide an accurate quantitative measure of TAG kinetics.

References

5 Lemieux, S., Patterson, B.W., Carpentier, A., Lewis, G.F. and Steiner, G. (1999) J. Lipid Res. 40, 2111–2117

Received 11 January 2007