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Review

New methodologies for studying lipid synthesis and turnover: Looking backwards to enable moving forwards☆

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ABSTRACT

Our ability to understand the pathogenesis of problems surrounding lipid accretion requires attention towards quantifying lipid kinetics. In addition, studies of metabolic flux should also help unravel mechanisms that lead to imbalances in inter-organ lipid trafficking which contribute to dyslipidemia and/or peripheral lipid accumulation (e.g. hepatic fat deposits). This review aims to outline the development and use of novel methods for studying lipid kinetics in vivo. Although our focus is directed towards some of the approaches that are currently reported in the literature, we include a discussion of the older literature in order to put "new" methods in better perspective and inform readers of valuable historical research. Presumably, future advances in understanding lipid dynamics will benefit from a careful consideration of the past efforts, where possible we have tried to identify seminal papers or those that provide clear data to emphasize essential points. This article is part of a Special Issue entitled: Modulation of Adipose Tissue in Health and Disease.

1. Introduction

Adipose tissue is broadly divided into two major categories, white adipose provides insulation, cushions vital organs and acts as an endocrine organ whereas brown adipose tissue is generally thought to contribute to thermogenesis. As small molecules, lipids are an excellent fuel source, in addition, they play a critical role in subcellular signal transduction. The accumulation of lipids (or adipose tissue) during growth is comparable to the accumulation of lean body mass, for example, a 3.5 kg newborn baby will accumulate ~12 kg of fat vs ~12 kg of (dry) lean mass as they transition to a healthy 75 kg adult, reminding us that "getting fat" is part of being normal (obviously, too much of a good thing is bad). Note that a majority of weight gain is simply water mass, a relatively small amount of fat-free lean mass reflects true nitrogen accretion whereas fat mass has very little hydration making the accumulation of lipid and muscle comparable.

The aim of this review is to outline methods that are available for quantifying lipid flux, although attention will be directed towards new advances we intend to highlight selected examples from the older literature since this may help to identify gaps for future investigations. The perspective used in discussing these methods is based on our experience studying the biochemical basis of triglyceride accumulation in adipose tissue and the role of adipose dysfunction in the pathogenesis of dyslipidemia and cardiometabolic disorders, comparable methods can be used for both purposes.

Although numerous investigators have studied de novo lipogenesis [3,38,65,155], most experiments do not allow conclusions regarding net lipid balance. We had suspected that in order to develop a comprehensive understanding of lipid accumulation, investigators should consider a tool(s) that yields an estimate of triglyceride synthesis and degradation including the contribution of de novo lipogenesis to the triglyceride-bound fatty acids [20]. Perhaps the most straightforward approach for examining lipid deposition is to determine the change in pool size over time (which equals the synthesis minus the degradation) while quantifying the rate of lipid synthesis via a tracer method, one can solve the equation to estimate the degradation rate [11,12,20].

In our experience, the use of ^{2}H-labeled water offers a number of advantages as compared to other tracers. In addition to the fact that labeled water rapidly and evenly distributes throughout body fluids, it is incorporated into multiple end-products which enables comprehensive studies of metabolic flux while requiring a minimum of resources (Fig. 1). Typical tracer protocols require catheterized subjects, while this is somewhat trivial to setup in a clinical setting the routine catheterization of animal models is labor intensive. Although there are no formal rules regarding how long animals should recover following a surgical manipulation, investigators often allow several days prior to initiating a tracer study in rodents [6]. To expand on this example, assume that one aims to test a hypothesis in a study requiring ~24 animals, how long would it take one FTE to conduct the study and collect

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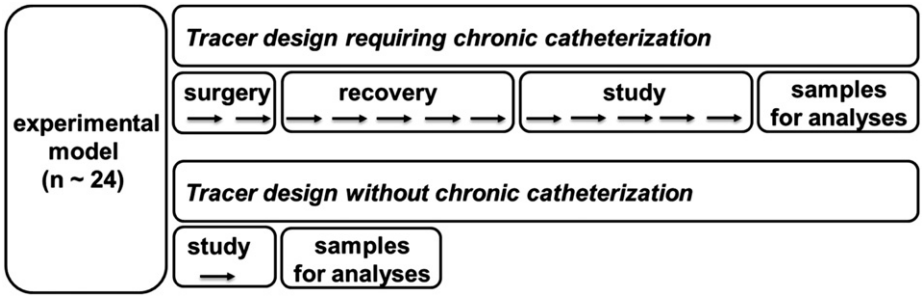

Fig. 1. Design considerations for tracer studies. Assuming a single FTE were available, it would require considerably more time to run a study involving ~24 rodents if catheterization is required; it requires ~2 days to implant catheters, ~4–5 days of post-operative recovery and ~5–6 days to perform tracer infusions vs ~1 day if catheters are not required. Note the each arrow (→) represents the equivalent of one day.

samples? Based on our experience it would take ~2 days to place catheters in rodents, there may be a recovery period of several days followed by several more days of effort to perform the tracer infusions [74,104,119,123,157]. In contrast, using ^2H_2O it is possible to dramatically reduce the time it takes to move from hypothesis generation to sample collection. In addition, if it is necessary to scale up the number of studies, it is possible to run more water-based studies with a minimum of extra effort and in a shorter amount of time as compared to studies that require catheterization. To circumvent the use of chronically catheterized animal models, Kurland and colleagues have used mini-osmotic pumps to deliver tracers whereas Bateman and colleagues have administered a single intraperitoneal bolus of labeled leucine to study protein synthesis [10,159]. While those approaches are somewhat easily implemented in rodent models, it is also possible to administer a more standard intravenous bolus which is suitable for studies in higher species as well [100,101].

We recognize that lipids may mean glycerides to some and sterols to others, the focus here is on triglycerides and cholesterol with a consideration of some related topics. We aim to provide examples of how tracers can and have been used to quantify these flux rates, we hope that our discussion will help guide and expand the use of tracers in future applications [93,113]. Finally, in order to gain a comprehensive understanding of metabolic regulation it is important to attempt linking changes in gene expression with alterations in biochemical flux. Unfortunately there can be disproportionate and/or opposing changes between expression profiles and flux [59,154]; although tracer studies can be used to make statements regarding biochemical flux they cannot necessarily explain points of control just as expression profiles may be useful for identifying potential regulatory sites yet they cannot be used to infer metabolic rates.

2. Why consider lipid flux in white adipose tissue?

A general misconception may be that lipids are stored in adipose tissue to support periods of prolonged fasting/starvation. In fact, the regulation of lipid homeostasis by adipose tissue was rigorously examined nearly 80 years ago [141]. Studies suggested that a large portion of dietary fat passed through adipose tissue each day (presumably, if a subject was in energy balance then equivalent amounts of fat would be stored after a meal and later released during the postabsorptive period). This point seemed so obvious in 1936 that Schoenheimer and Rittenberg [141] wrote:

> "*Instead of comparing the fat tissues to a cellar in which food is stored for times of emergency, it seems more correct to compare them to an ice box in which a part of the food is kept during the short intervals between meals. The fat tissue can therefore be regarded as an energy buffer. During absorption it takes up in the form of fatty acids excess of food material not immediately used for the energy requirements. Conversely, during the postabsorptive period it supplies fatty acids to make up the energy deficit.*"

It is of interest to note that this statement was italicized in the original text, presumably to convey the importance of the observation. This concept was recently revisited by Frayn and colleagues, it is safe to say that the conclusions drawn nearly 80 years ago remain true today despite the marked differences in the methods and models [45,141]. Other studies have reported temporal changes in the activity of lipases in response to changes in glucose and insulin vs fasting suggesting a transient divergence in lipid trafficking away from muscle and towards adipose tissue in humans [42]. A final example regarding the importance of adipose tissue in maintaining daily lipid flux is found in the work of Samuel et al. [134], this indirect evidence serves as an interesting cross-validation. Briefly, they examined how diet-induced fat accumulation affects hepatic signal transduction related to glucose homeostasis. We draw the reader's attention the degree of fatty liver that was observed (see Figure 1, ref [134]), we estimate that the total difference in liver triglyceride is ~0.3 g. Although there were clear differences in the amount of fat in the liver, we believe that that change was trivial in the context of daily lipid flux. We estimate that rats on a high-fat would eat ~15 g of fat per day and that the total lipolysis (i.e. glycerol flux) might be ~5 g of triglyceride per day. Based on these estimates one expects that ~20 g of fat are trafficking through the circulation per day, of which, the slightest imbalance leads to an immediate and profound impairment in normal insulin signaling. In total, these observations further support a critical role for adipose tissue in preventing peripheral lipotoxicity.

The observations noted above raise a final question regarding adipose tissue biology, If a substantial amount of circulating lipids are removed by adipose tissue as part of normal physiological homeostasis are there conditions in which the ability of adipose tissue to clear fat may be limiting? For example, the removal of triglyceride from blood appears to be saturable in vivo [51,128]. Considering the need for adipose tissue to tightly regulate its own lipid flux and its participation in inter-organ lipid trafficking it should come as no surprise that there are a number of approaches for quantifying lipid flux in/out of adipose tissue. One will appreciate that tracer methods per se are helpful in addressing questions but it is often necessary to couple their use with ancillary techniques, e.g. tissue biopsies and/or surgical manipulations [15,18,130].

3. Quantifying triglyceride kinetics in adipose tissue: direct or inferred via plasma measurements

3.1. Dietary interventions

In the classic work performed by Hirsch, subjects were fed modified diets for weeks or months and biopsies of adipose tissue were obtained at various intervals, triglyceride turnover was estimated by