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Micro-techniques for analysis of human adipose tissue fatty acid composition in dietary studies

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Abstract *Background and aims:* Adipose tissue (AT) fatty acid (FA) composition is considered to be the gold standard long-term biomarker of dietary fatty acid intake. Typically this measurement is made directly from samples collected via large-needle-biopsy or incision. However, with growing interest in the role of AT in relation to health, ideally the fatty acid composition would be analysed along with other measurements, such as gene expression or histology, on a single AT sample. Here we assess alternative ways of obtaining AT for measuring FA composition, in some cases in conjunction with other measurements.
Methods and results: The FA composition of tissue obtained via different methods was compared to that of tissue collected via large-needle or surgical biopsy. Fatty acid composition was not significantly different in AT collected by small-needle mini-biopsy ($n = 10$), from an RNA 'lipid layer' (obtained during RNA extraction, 2 sites, $n = 6$ for each), or from cryosectioned tissue prepared for histology ($n = 10$). We also assessed the usefulness of the composition of plasma NEFA as a surrogate marker of subcutaneous AT ($n = 58–80$). Most FAs in plasma NEFA correlated strongly with those in AT ($P < 0.05$).
Conclusion: It is feasible to measure the FA composition of AT on very small amounts of tissue. Additionally, it is possible to measure FA composition on the lipid rich 'by-product' of AT samples undergoing RNA extraction for gene expression. Samples sectioned for histology are

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also suitable. This provides further opportunities for multidisciplinary collaborations that may lead to a better application of dietary biomarkers.

Introduction

Traditional dietary assessment methodologies have a number of limitations that may influence the quality of the data collected [1]. A dietary biomarker provides objective information with which to examine diet and disease relationships. Adipose tissue (AT) is the main storage compartment for fatty acids (FA) in humans. Due to the slow turnover time of AT-triacylglycerol (TAG) ($t_{1/2}$ 9 months–2 years) [2–4], the FA composition of AT, in weight stable individuals is considered to be a gold standard as a biomarker of FA long-term intake.

Interest has increased in AT as key roles in metabolism and signalling have been identified. Thus, AT samples are now collected for gene expression [5], protein quantification [5], cell culture [6], histology [7], FA analysis [8], lipidomics [9], or proteomics [10]. Ideally, multiple measurements including FA composition would be made on a single AT sample.

AT biopsies are most commonly obtained with needle-biopsy [3,11], with or without skin incision [12]. Alternatively, AT samples may be obtained after a skin incision by using a scalpel or forceps [13], or from patients undergoing elective surgical procedures [14]. Collecting AT is often perceived to be invasive, requiring specialist equipment or skills [8]. As AT has an abundance of TAG [3] and FA composition analysis via gas-chromatography (GC) is very sensitive, it would seem reasonable that only a small amount of tissue would be required for analysis. This could be collected less invasively using a smaller needle than previously described [3,11]. Alternatively, small amounts of AT, such as those cryosectioned for histology, could be useful for measuring FA composition.

With growing interest in AT gene expression and health, samples may be prioritised toward such analysis. RNA extraction from AT generates a lipid layer by-product that is normally disposed of [15]. It is not known whether the FA composition of this layer is representative of the original tissue biopsy. Determining the usefulness of a lipid layer for measuring AT FA composition could allow multiple analyses of different parameters from a single tissue sample.

In some situations plasma samples rather than AT may be available. The systemic plasma non-esterified FA (NEFA) pool after an overnight fast mainly comprises FAs released by the action of intracellular lipases from AT-TAG. It would therefore seem reasonable that the FA composition of plasma NEFA (also known as free FAs) could potentially be a useful surrogate marker of AT FA composition [8]. The proportions of 18:2*n*-6, 18:1*n*-9, 20:5*n*-3 and 22:6*n*-3 in AT and in plasma NEFA have been reported to correlate, but these data are from dietary supplementation studies [16–18]. Whether the FA composition of the plasma NEFA pool reflects AT in individuals that have not increased intakes of specific FAs remains unclear.

As there is growing interest in using the FA composition of AT as a marker of metabolism or dietary intake, we had the following aims: 1) to validate the use of a mini-biopsy method for subcutaneous AT; 2) to explore the possibility of analysing the lipid layer produced as a 'by-product' during the extraction of RNA from AT for the measurement of FA composition; 3) to investigate if cryosections of AT are useful for measuring the FA composition; and 4) to determine if the FA composition of plasma NEFA is a good surrogate marker of AT FA composition.

Methods

Subcutaneous AT biopsies were collected as part of completed [7,19] or larger on-going studies [20]; thus, not all comparisons were undertaken on the same samples. Studies were approved by the Oxfordshire Ethics Committee or the Local Research Ethics committee NHS Greater Glasgow and Clyde. All subjects participating in the studies informed written consent.

Tissue collection

All needle biopsies were performed under aseptic conditions and local anaesthesia.

Large-needle subcutaneous AT (SCAT) biopsy

Samples were collected from healthy volunteers using a 14- or 18-gauge needle, either at the level of the umbilicus or the upper quadrant of the buttock (gluteal). The resulting AT was then washed in saline and stored at −80 °C before making a stock solution (see Supplementary data).

Surgical collection of AT

Samples were collected from non-labouring pregnant women undergoing elective Caesarean section. The surgeon obtained a sample of SCAT on entry into the abdominal cavity and a sample of visceral AT was obtained from the omentum after closure of the uterus and haemostasis was secured. Samples were "flash-frozen" in liquid nitrogen and then stored at −70 °C.

Mini-biopsy of SCAT

The biopsy was taken at the level of the umbilicus, using a 21-gauge butterfly needle, analogous to the method of Beynen and Katan [11]. The tubing (containing SCAT) was flushed with saline into a glass tube and centrifuged. The lipid layer was aspirated and used for lipid extraction (see below).

The lipid layer remaining after RNA extraction

AT collected from SCAT from abdominal and gluteal regions via a 14-gauge needle and homogenised in Tri-reagent®