Rapid sampling and long-term storage of subcutaneous adipose-tissue biopsies for determination of fatty acid composition[1–3]

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ABSTRACT The fatty acid composition of fat tissue is a valid indicator of the fatty acid composition of the diet over the preceding 1 to 3 yr. Here we describe a rapid method for sampling of buttock fat without anesthesia. On average, 35 mg can be obtained using a common blood-sampling system. Sampling of 40 subjects takes about 3 h. The procedure caused no more anxiety or discomfort than a routine venipuncture. Application in about 500 subjects has as yet yielded no infectious or other complications. With regard to the fatty acid composition of the biopsy, sampling site (left versus right buttock) was not found to be a source of error. The biopsies could be stored for periods up to 1.5 yr without changes in the fatty acid profile. With this method one can obtain biopsies from a large number of subjects and determine objectively the long-term fatty acid composition of their diet. *Am J Clin Nutr* 1985;42:317–322.

KEY WORDS Fatty acid composition, diet, adipose tissue, biopsy method, dietary studies

Introduction

The fatty acid composition of subcutaneous adipose tissue in humans is an objective, valid index of the fatty acid composition of the habitual diet over the past 1 to 3 yr (1, 2). The fatty acid spectrum of adipose tissue has been used as a criterion of adherence to experimental diets (3–5). We have recently used the linoleic acid content of adipose tissue in order to compare linoleic acid intake in the USA and UK (6). Furthermore, there is evidence that a low percentage of linoleic acid in subcutaneous adipose tissue correlates with a high risk for coronary heart disease (7, 8) and a low risk for malignant melanoma (9). Thus, it appears that the fatty acid composition of subcutaneous adipose tissue could be an important tool in various types of nutrition research.

Sampling of biopsies of fat tissue is often considered difficult and also to be distressing for the subjects. As an alternative, a noninvasive method to obtain human cheek epithelial cells has recently been developed (10, 11). However, as yet the value of the fatty acid composition of cheek cells in nutritional studies has not been fully established. In this communication we show how easy it is to obtain microbiopsies of buttock adipose tissue with minimal discomfort for the subjects. In addition, we show that such microbiopsies can be stored for prolonged periods without changes in the fatty acid pattern. This method

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deserves consideration in large-scale cross-sectional or prospective studies in which objective information on the fatty acid composition of the diet is required.

Materials and methods

Sampling

Our method is a modification of the method described by Hirsch et al (12). The subject lies face down on an investigating table or a bed. The subject's trousers or skirt are slightly lowered, so as to bare the upper half of the buttocks; complete undressing is not necessary. The skin is disinfected with 70% (v/v) ethanol in water. The subject is instructed to tense his/her muscles, so that muscle and fat tissue are clearly recognized. A skinfold from the upper outer quadrant of a buttock is gripped between two fingers and the thumb of one hand; using this part of the buttock minimizes the risk of hitting the ischiatic nerve. Subsequently, the needle of the vacuum tube assembly (**Fig 1**) is inserted under an angle of about 45° with the other hand (**Fig 2**). A needle of 1.5 mm diameter (between Gauge 16 and 17) is required, because thinner needles will not allow the biopsy to pass. No anesthetics are used. After insertion the evacuated tube is pressed forward to connect the vacuum with the needle, which is then gently pushed back and forth a few times in the adipose tissue layer. The angle between needle and buttock should not be too shallow because the tip might then touch the inside of the skin, which is highly sensitive. Fat tissue will collect in the top of the connector (Luer adaptor) between the needle and the tube. The evacuated tube is now disconnected and the needle is removed from the tissue. The sampling site is sealed with a piece of plaster (Leukosilk®, Beiersdorf AG, Hamburg, FRG). Subjects are instructed to leave this in place and not touch the site so as to avoid infections. The protocol was approved by the human subjects committee of the Department of Human Nutrition.

The biopsy is left in the connector; the connector is stored in its original disposable container at −20°C (Fig 1). The entire procedure of sampling takes about 4.5 min per subject.

Fatty acid analysis

The fat biopsies were removed from the connector and weighed. About 15 mg (sometimes less had to be used) was transferred to a 5-ml flask. Methyl esters of the component fatty acids were synthesized according to Metcalfe et al (13), without prior extraction of the lipids. Methyl esters were separated by gas liquid-chromatography on a Packard Model 433 (United Technologies, PO Box 519, 2600 AM Delft, The Netherlands), using a 1.8-m glass column (inner diameter, 2 mm) filled with 10% Silar 5CP on 100–120 mesh Chromosorb WHP packing (Supelco Inc, Bellefonte, PA). For separation of fatty acid geometric isomers a 6-m glass column (inner diameter, 2 mm) filled with 15% silicone OV 275 on 100–120 mesh Chromosorb P AW-DMCS packing (Supelco Inc) was used. The injection volume was 1 μl (containing 2 to 4 μg of individual fatty acid), and the carrier gas was helium (25 ml/min; about 200 kPa). The oven temperature was programmed to increase from 120°C to 215°C in 20 min (or from 180°C to 215°C in

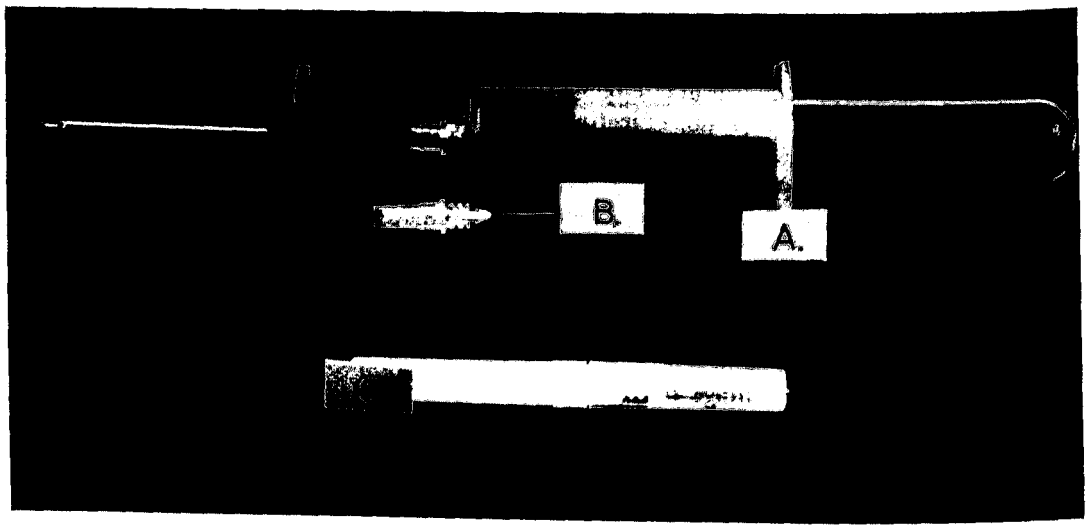

FIG 1. Equipment used. *A* is the vacuum tube assembly consisting of an evacuated 10-ml tube (Terumo Venoject VT100 PZ, Terumo Corporation, Tokyo, Japan or Becton Dickinson Vacutainer 606430, 100 × 16 mm) tube holder (Venoject holder® or Vacutainer 4893), connector (Venoject multi-sample Luer adaptor® Code XX-MN 2000 or Vacutainer 7290) and Luer butterfly-needle (Strausskanüle, length 43 mm, outer diameter 1.5 mm, inner diameter 1.1 mm; Laméris Instrumenten BV, 3572 AW Utrecht, The Netherlands. 1.5 mm outer diameter is intermediate between Gauge 16 and 17.) *B* is the connector (Luer adaptor®) alone, and *C* is the connector in its original disposable container.

FIG 2. The technique of sampling subcutaneous adipose tissue.

28 min for the separation of geometric isomers) and then held at 215°C for 40 min; the injector was kept at 205°C and the detector at 240°C. The gas chromatograph was equipped with a hydrogen flame ionization detector, and the peaks were integrated by a Spectra Physics Integrator SP 4100 (San Jose, CA). Data are presented in terms of mass percentage of the methyl esters of the component fatty acids. When flame-ionization detectors are used to measure the amount of fatty acid methyl esters from C 10 to C 20 the response is proportional to the weight of methyl ester within the limits of accuracy of the method (14).

As quality control check, two samples of a commercial frying fat were analyzed in each run. This fat was stored at 4°C, and for fatty acid analysis samples were only taken from the core of the package. The combined within- and between-run variation for this material over a 1.5-yr period, expressed as relative SD, was 1.4% for major and 2.5% for minor peaks.

Effect of storage

To study the effect of storage on fatty acid composition, biopsies were taken from one piece of subcutaneous, abdominal adipose tissue exactly as described above. This tissue (weighing about 500 g) had been removed from a patient undergoing surgery at the Pieter Pauw Hospital, Wageningen. Within 15 min after the removal of the tissue at the hospital, the first biopsies were taken at the Department of Human Nutrition. The first three biopsies were immediately subjected to methyl-ester formation as described above. Other biopsies were stored in the connector (in triplicate) for various periods and at various temperatures.

Results

Effect of sampling site

The yield of adipose tissue per aspiration was 36 ± 20 mg (mean ± SD; n = 44), with individual values ranging from 1.5 to 83 mg. In our hands even the smallest samples still allowed fatty acid analysis. Sampling site as a source of error was investigated by taking biopsies from both the left and right buttock in 15 subjects. Mean percentages (±SD) of linoleic acid (*cis,cis* C 18:2 n-6) were 14.70 ± 3.51 and 14.85 ± 3.49 mass % for the left and right buttock, respectively. The difference was 0.15 ± 0.30 mass %, this did not reach statistical significance.

Effect of storage

Table 1 presents the fatty acid composition of fat biopsies taken from one piece of freshly excised human adipose tissue and stored

TABLE 1
Stability of fatty acid composition of fat biopsies taken from a fresh piece of human fat tissue and stored at various temperatures for various periods

Storage		Total polyunsaturated fatty acids	Total monounsaturated fatty acids	Total saturated fatty acids	*Cis,cis* linoleic acid (*cis,cis* C 18:2 n-6)	α-Linolenic acid (*cis,cis, cis* C 18:3 n-3)	Elaidic acid (*trans* C 18:1 n-9)	*Trans,trans* linoleic acid (*trans,trans* C 18:2)
Temperature	Duration	*g/100 g fatty acid methyl esters*						
Ambient	±15 min	20.2	49.9	27.7	18.3	0.7	2.7	0.4
	1 day	20.1	49.9	27.9	18.5	0.5	2.8	0.4
	1 wk	19.6	49.0	27.8	18.0	0.5	2.8	0.4
	1 mo	19.9	50.5	27.4	18.5	0.5	2.6	0.3
	3 mo	20.5	50.3	27.5	18.9	0.5	2.4	0.3
4°C	1 wk	19.9	50.5	28.1	18.2	0.6	2.9	0.4
	1 mo	19.7	50.4	27.9	18.4	0.5	2.6	0.4
	3 mo	19.4	49.5	28.3	18.4	0.5	2.4	0.3
	18 mo	19.6	48.6	28.3	18.2	0.6	2.5	0.3
−20°C	1 wk	19.2	49.9	28.1	18.0	0.4	2.8	0.4
	1 mo	19.4	50.6	28.1	18.1	0.5	2.7	0.4
	3 mo	19.6	50.4	27.6	19.0	0.5	2.5	0.2
	18 mo	19.4	49.0	28.1	18.1	0.5	2.4	0.3
−80°C	1 wk	19.2	50.6	28.0	17.8	0.5	2.7	0.4
	1 mo	19.2	51.3	27.4	17.8	0.6	2.6	0.4
	3 mo	19.6	51.1	26.9	18.6	0.5	2.6	0.4
	18 mo	19.3	49.5	28.1	17.7	0.5	2.4	0.4

Each result is the mean of three samples. Unknown peaks in the chromatograms corresponded to about 2 g/100 g of fatty acid methyl esters.

under various conditions. When compared to the values obtained at time zero, storage at the various temperatures and for the various periods up to 1.5 yr did not affect fatty acid composition. This holds not only for total saturated, mono- and polyunsaturated fatty acids, but also for the individual fatty acids such as *cis,cis* linoleic acid (*cis,cis* C 18:2 n-6), elaidic acid (*trans* C 18:1 n-9) and *trans,trans* linoleic acid (*trans,trans* C 18:2). The decrease was also minimal for α-linolenic acid (*cis,cis,cis* C 18:3 n-3) which is known to be particularly susceptible to oxidation.

Subject acceptance of the procedure

An anonymous questionnaire was used to assess anxiety and discomfort associated with the fat biopsy procedure. In this questionnaire 41 participants in a dietary trial (15) were asked to compare the fat biopsy procedure with a routine venipuncture. The questionnaire was administered about 4 wk after the biopsy. At that time each subject had undergone at least 15 venipunctures over a period of 5 mo. **Table 2** shows that about half of the subjects experienced equal anxiety, or the lack of it, prior to undergoing the fat biopsy and a venipuncture; the other half were more anxious about the fat tissue sampling. Actual discomfort during the biopsy procedure was judged similar to that during venipuncture. The majority of the subjects experienced neither more nor less discomfort after the biopsy compared with a venipuncture.

Medical complications

Over the past few years we have obtained fat biopsies from about 500 subjects, including university students, Seventh-Day Adventists (2), and housewives. To our knowledge, none of these subjects developed an infection or other medical complication. There have been some complaints about pain or tenderness during or after the procedure; this inconvenience disappeared within a few days.

Discussion

Dietary surveys, if carefully performed, can provide data on the mean fatty acid composition of the diet of groups of subjects.

TABLE 2
Results of anonymous questionnaire

Before	
• "I was distinctly more anxious about undergoing the fat biopsy than about a venipuncture"	44%
• "I was distinctly less anxious about undergoing the fat biopsy than about a venipuncture"	0%
• "I was neither more nor less anxious about undergoing the fat biopsy than about a venipuncture"	56%
During	
• "I found a venipuncture distinctly more unpleasant than the fat biopsy"	5%
• "I found a venipuncture distinctly less unpleasant than the fat biopsy"	41%
• "I found a venipuncture neither more nor less unpleasant than the fat biopsy"	54%
Afterwards	
• "The fat biopsy caused me distinctly more discomfort than a venipuncture in the subsequent days"	18%
• "The fat biopsy caused me distinctly less discomfort than a venipuncture in the subsequent days"	0%
• "The fat biopsy caused me neither more nor less discomfort than a venipuncture in the subsequent days"	82%

Response rate was 39 out of 41 participants in a dietary trial.

Estimates for individuals however, are highly variable from day to day (16), and the memory of the subjects is usually not reliable enough to yield valid data on the integrated consumption over periods of several years. The fatty acid composition of body fat reflects the mean fatty acid pattern of the diet over a period of 2 to 3 yr (1, 4, 12). The composition of adipose tissue has been shown to be an objective, valid index of the qualitative dietary fatty acid intake not only of groups (1), but also of separate individuals (2). In light of the potential importance of the use of fat biopsies in nutritional investigations, we studied various aspects of the biopsy method.

In their original description of the fat tissue biopsy method (2), Hirsch et al expressed the expectation that cases of procaine sensitivity or adverse psychic reaction might be encountered. The risk for this is less in our method, as no anesthetic is used, and the equipment used may look less intimidating than the 50-ml syringe used by Hirsch et al.

Our study shows that subcutaneous adipose tissue biopsies can be taken routinely from large numbers of persons with minimal discomfort for the subjects. It can be done by paramedical personnel with little extra training. The method of sampling is easy and rapid, and therefore allows application on a large scale. With regard to the fatty acid composition, sampling site is not a source of error, and the biopsies can be stored without any precautions for periods up to at least 1.5 yr without introducing bias. This indicates that samples can be collected in the field situation without special precautions as to storage conditions.

Stability of fatty acids in biopsies could also be demonstrated for individual fatty acids which may be of special interest, such as linoleic acid and *trans*-fatty acids. The adipose tissue content of *cis,cis* linoleic acid appears to be related to the risk for coronary heart disease (7, 8) and perhaps to malignant melanoma (9). The *trans* isomers of oleic acid and linoleic acid are of interest since they may be an indicator for the intake of hydrogenated oils and fats (17).

Methods for determining average long-term food intake in individuals have been a bottleneck in epidemiological research on the relation between dietary fatty acids and health for a long time. The method described above may circumvent these methodological problems.

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