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Review

Sweat: A sample with limited present applications and promising future in metabolomics

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ABSTRACT

Sweat is a biofluid with present scant use as clinical sample. This review tries to demonstrate the advantages of sweat over other biofluids such as blood or urine for routine clinical analyses and the potential when related to metabolomics. With this aim, critical discussion of sweat samplers and equipment for analysis of target compounds in this sample is made. Well established routine analyses in sweat as is that to diagnose cystic fibrosis, and the advantages and disadvantages of sweat *versus* urine or blood for doping control have also been discussed. Methods for analytes such as essential metals and xenometals, ethanol and electrolytes in sweat in fact constitute target metabolomics approaches or belong to any metabolomics subdiscipline such as metallomics, ionomics or xenometabolomics. The higher development of biomarkers based on genomics or proteomics as omics older than metabolomics is discussed and also the potential role of metabolomics in systems biology taking into account its emergent implementation. Normalization of the volume of sampled sweat constitutes a present unsolved shortcoming that deserves investigation. Foreseeable trends in this area are outlined.

Contents

Abbreviations: ANOVA, analysis of variance; CE, capillary electrophoresis; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; Da, Dalton; DAD, diode array detector; DCD, dermcidin; EI, electron impact ionization; EIA, enzyme immunoassay; ELISA, enzyme linked immunoassay; ESI, electrospray ionization; FID, flame ionization detector; GC, gas chromatograph/gas chromatography; GHB, gamma hydroxybutyrate; HMDB, human metabolome data base; ISE, ion selective electrode; LC, liquid chromatograph/liquid chromatography; LLE, liquid–liquid extraction; LOQ, limit of quantitation; METLIN, metabolite and tandem MS data base; MS, mass spectrometer/mass spectrometry; MRMMS, multiple reaction monitoring mass spectrometry; NMR, nuclear magnetic resonance spectroscopy; PCA, principal components analysis; PIP, prolactin inducible protein; PLS-DA, partial least squares-discriminant analysis; pROC, partial receiver operating characteristics; R, Robert and Ross programme language; RIA, radio immunoassay; SPE, solid-phase extraction; TOF, time-of-flight; UV, ultraviolet.

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1. Introduction

Sweat is a clear, hypotonic biofluid produced by eccrine and procrine glands located in the epidermis [1]. This slightly acidic biofluid (pH range 4.0–6.8) is composed mainly by water (99%), containing the so-called electrolytes (*e.g.* sodium, chloride, and potassium), urea, pyruvate and lactate; but proteins, peptides, amines, amino acids and metal ions in smaller concentrations are also found in this biofluid in addition to inhibitors, antigens, antibodies and a variety of xenobiotics such as drugs, cosmetics, and ethanol. These substances are stored in the sweat glands, secreted into the sweat and finally transported to the epidermis surface with partial selective reabsorption of sodium and chloride during transportation, which results in hypotonicity of the secreted sweat in healthy individuals [1]. Diseases can change sweat composition either by altering the concentration of common components or reporting new components that, in any case, could act as biomarkers of the given disease.

Except for the case of some high molecular weight proteins, which reach sweat by different intracellular storages in particular situations [1,2], most sweat components are small molecules resulting from metabolic pathways; therefore, their study pertains to the metabolomics field, the omics of small molecules typically <1000 Da or <1500 Da), defined in 2002 by Fiehn as "a comprehensive analysis in which all the metabolites of a biological system are identified and quantified" [3].

The scant traditional use of sweat as clinical sample and the present incipient interest in this biofluid make convenient to review the state-of-the-art of well-established clinical uses of sweat, of those that have not been assessed yet, and those that foreseeably can be developed in the near future by taking advantage of present cutting-edge analytical technology. With this aim, traditional and recent sampling approaches, equipment for sample analysis, fields of application of sweat as clinical sample and its role in omics studies, mainly in the search for biomarkers, are discussed.

2. Sweat sampling and analysis

The characteristics of sweat sampling and the special samplers it requires deserve discussion separated from analysis that can be either different or similar to that in other biofluids.

2.1. Sweat sampling

Sweating is naturally increased by nervousness, exercise, stress and nausea, and decreased by cold. Sweat excretion is also affected by other factors, such as ambient temperature, relative humidity, body location (in general, sweat glands are distributed over the entire body, except for the lips, nipples and external genital organs), hormonal imbalances, overactive thyroid gland and the sympathetic nervous system, and certain foods and medications.

With sampling purposes, sweating must be stimulated (usually by heat or chemicals such as pilocarpine) to obtain a given volume of this biofluid enough for subsequent analysis. Stimulation of perspiration by pilocarpine requires impregnation of the zone (usually arm or leg) with this compound accompanied by a low intensity electrical current (2.5–3.0 mA) for a short time (about 5 min). The minimum sweat rate demanded to obtain a valid sweat sampling is 1 g/m^2 per min [4].

Initially, sweat collection devices consisted of an occlusive bandage formed by one-to-three layers of filter paper or pieces of cotton, gauze or towel [5]. However, this kind of patch was time-consuming to apply, uncomfortably large, prone to detachment and yielded a small volume of sweat for analysis. In addition, it was found to alter the steady-state pH of the skin, the types of bacteria that colonize the skin and the transport characteristics of the skin, producing skin irritation [6]. To overcome these difficulties, non-occlusive sweat collection devices were developed, consisting of an adhesive layer on a thin transparent film of surgical dressing to which a rectangular absorbent pad was attached (the simplest pad is a filter paper – *e.g.* a 42 ashless Whatman filter paper, no. 1442070, 70 mm diameter – in contact with the adhesive patch) the overall being attached to the arm radial region. Non-volatile substances from the environment cannot penetrate the transparent film, which is a semipermeable membrane over the pad that allows oxygen, water and carbon dioxide to pass through the patch, leaving healthy the underneath skin [5]. During wearing of the patch, as sweat saturates the pad and slowly concentrates it, sweat components are retained, while water evaporates from the patch, thus misleading results of chloride concentration when used for the diagnostic of cystic fibrosis (CF). Therefore, this non-occlusive design does not permit to quantitate the concentrations of analytes in sweat, since the whole volume of secreted sweat is unknown; nevertheless, some advantages of this sampling device are a unique identification number, which aids with chain of custody and identification, and its design that makes sample adulteration difficult because attempts to remove it before the end of the collection period or tamper with it are readily visible to personnel trained to monitor the sweat patch. An improved, commercial version of this sampler is shown in Fig. 1A, in which the "native" sweat collection system is brought up from polypropylene copolymer bag and adhesive rubber, which is airtight and watertight in such as way that none of its components has influence on the sweat composition for the period of use. The overall sampler includes a window covering a skin surface of 150 cm^2, and a bag part to accumulate sweat [7]. Some authors have designed tools for removal of sweat from the bag depending on the collected volume. Thus, Kamei et al. have designed glass rollers with dull surfaces and holders for sample collection when the sweat quantity is limited (Fig. 1B), and special pipettes with reverse capillaries for sweat drop collection in the case of the profuse sweat secretion (Fig. 1C) [8].

One of the most common commercial samplers at present is the Macroduct (Fig. 2A). It consists of a concave disk and a spiral plastic tube where the sweat is collected. The minimum