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Review

Sweat metabolome and proteome: Recent trends in analytical advances and potential biological functions

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ABSTRACT

Metabolome and proteome profiling of biofluids, e.g., urine, plasma, has generated vast and ever-increasing amounts of knowledge over the last few decades. Paradoxically, omics analyses of sweat, one of the most readily available human biofluids, have lagged behind. This review capitalizes on the current knowledge and state of the art analytical advances of sweat metabolomics and proteomics. Moreover, current applications of sweat omics such as the discovery of disease biomarkers and monitoring athletic performance are also presented in this review. Another area of emerging knowledge that has been highlighted herein lies in the role of skin host-microbiome interactions in shaping the sweat metabolite-protein profiles. Discussion of future research directions describes the need to have a better grasp of sweat chemicals and to better understand how they function as aided by advances in omics tools. Overall, the role of sweat as an information-rich biofluid that could complement the exploration of the skin metabolome/proteome is emphasized.

1. Introduction

Sweat is a biological fluid involved in the thermo-regulation and host infection defense, produced by sweat glands, which are located within the dermal tissue rich in capillaries and nerve fibers, upon stimulation of the sympathetic nervous system. Sweat is slightly acidic (pH 4.0–6.8) consisting mainly of water (99%) [1]. Other components are also present including metal and nonmetal ions i.e., potassium, sodium, and chloride ions [2]; amino acids and urea; as well as other metabolites i.e., lactate and pyruvate; and xenobiotics such as drug molecules [3]. Nevertheless, the composition of sweat is very dynamic and significantly variable depending on skin type or disorder. For instance, in a disease state, sweat may include different components that can serve as biomarkers of that disease [2,3]. Interestingly, the composition of sweat can be affected by products secreted via the sebaceous gland. The latter is a holocrine gland that produces a viscous and lipid-rich fluid composed of cholesterol, cholesterol ester, triglycerides, and wax esters [4]. The role of such secretion has not been well-characterized, but it is believed that sebum secretions exhibit antifungal and antibacterial actions besides acting as a pheromone [5].

Abbreviations: 2-DE, two-dimensional gel electrophoresis; AD, atopic dermatitis; AMPs, antimicrobial peptides; CE-MS, capillary electrophoresis- mass spectrometry; CF, cystic fibrosis; CIL, chemical isotope labeling; DDA, data-dependent acquisition; DIA, data-independent acquisition; ED, ectodermal dysplasia; GC–MS, gas chromatography coupled to mass spectrometry; GC-O, GC-olfactometry; HS-SPME, headspace solid-phase microextraction; LC-MS, liquid chromatography-mass spectrometry; MS, mass spectrometry; MRM-MS, multiple reaction monitoring mass spectrometry; NMR, nuclear magnetic resonance spectroscopy; nLC-MS, nanoflow liquid chromatography-mass spectrometry; RP-LC, reversed-phase liquid chromatography; SWATH-MS, sequential windowed acquisition of all theoretical mass spectra; TB, tuberculosis; TOF, time of flight; VKH, Vogt–Koyanagi–Harada; VOCs, volatile organic compounds.

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Similar to other biofluids i.e. blood, urine, and saliva; sweat can be used for clinical analyses. Moreover, the properties of sweat make it easier to prepare and faster to analyze. Sweat also encompasses few impurities and is less prone to contamination. Consequently, sweat sampling, besides being noninvasive, can be stored for longer periods [6]. There has been an increasing interest in the use of sweat analysis as an anchor for disease biomarkers discovery [1,5,7]. The best example of diseases diagnosed through sweat analysis is cystic fibrosis (CF) [8], in which sweat test has been developed for newborn CF screening that allows for early identification and optimal treatment [9]. Due to mutation in cystic fibrosis transmembrane conductance regulator, chloride level is disturbed in CF serving as a biomarker for CF diagnosis. The chloride level in sweat is normally <30 mmol/L (borderline 30–59 mmol/L) and < 40 mmol/L (borderline 40–59 mmol/L) in elderly and infants [10]. Consequently, chloride level ≥ 60 mmol per liter of sweat indicates positive (typical) CF, whereas chloride concentration within a borderline range of 30–60 mmol per liter of sweat indicates atypical CF. Nevertheless, alteration in sweat gland functions is not limited to CF and has been observed in other abnormalities. Studies have reported that the protein composition of human sweat can significantly alter in several disorders including ectodermal dysplasia (ED) [11], atopic dermatitis (AD) [12], and schizophrenia [13]. More recently, sweat analysis has been implicated in the screening for lung cancer [14], tuberculosis (TB) [15], diabetes [16], and drugs of abuse [17].

In the last decade, advances in high-throughput omics approaches such as proteomics and metabolomics have provided valuable information regarding key biomolecules that could help to study the genetic and phenotypic variability with disease progression [18,19]. In-depth analysis of these biomolecules can provide an improved expedited route for deciphering the pathophysiological process of various disorders as well as for biomarkers discovery [20,21]. In addition, omics can also facilitate a comprehensive understanding of the crosstalk between skin microbiome-body relationship and the alteration of proteome-metabolome under various physiological conditions [22–24]. Although several studies have been reported on the potential applications of omics techniques in system biology and biomarkers discovery [25,26], most of which aimed to investigate the protein-metabolic alterations in common biofluids such as plasma [27–29], urine [30,31] and with less emphasis on sweat. In this review, we present state-of-the-art information about the currently available modern analytical tools

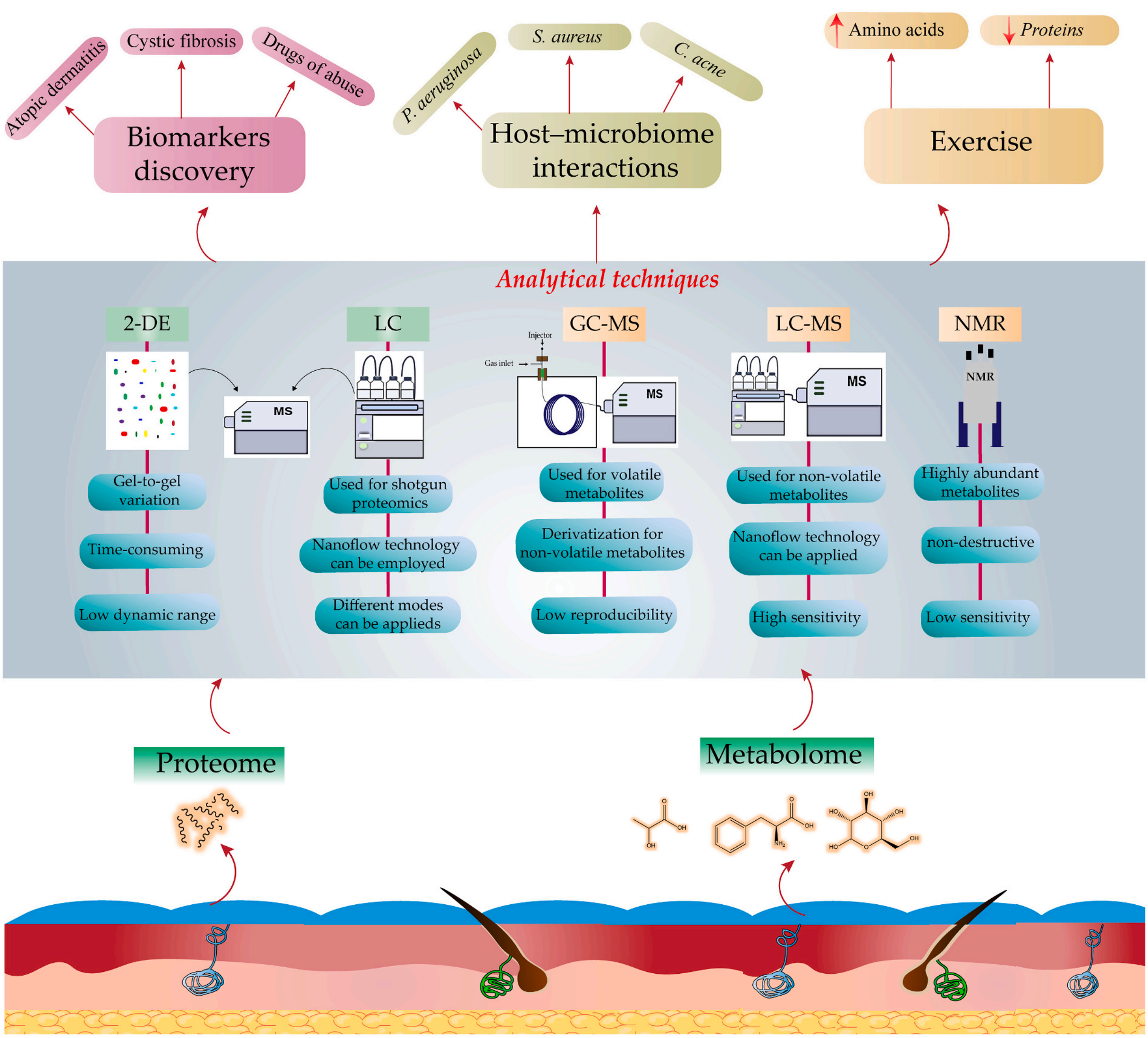

Fig. 1. Schematic representation summarizing the scope and outlines of this review. The main analytical approaches used in sweat metabolomics-proteomics experiments include nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS) hyphenated with other separation techniques such as gas-chromatography (GC), liquid chromatography (LC) and two-dimensional gel electrophoresis (2-DE) depending on the physicochemical properties of the analyte (s) of interest. Moreover, current biological applications of sweat omics such as the discovery of disease biomarkers, skin host-microbiome interactions and monitoring athletic performance are also presented.

used to measure the sweat metabolome and proteome. Asides, details of the sweat proteins and metabolites alteration due to various diseases, interaction with the skin microbiome, and different physiological conditions such as exercise are also presented for the first time. A graphical representation summarizing the scope and outlines of this review is depicted in (Fig. 1).

2. Current technologies for the analyses of sweat metabolites and proteins

Considering sweat complexity being made up of a large number of metabolites and proteins, utilizing high throughput technologies for its metabolome-proteome analyses seems imperative [32]. The main analytical approaches used in such experiments include nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS) hyphenated with other separation techniques depending on the physicochemical properties of the analyte(s) of interest.

2.1. Analysis of the sweat metabolome

NMR spectroscopy offers a prompt highly reproducible non-destructive platform for sweat metabolome analysis in addition to its simplicity and quantitative capabilities [33]. However, a major drawback in this technique lies in its lower sensitivity when compared to MS. Asides, peaks overlapping is observed particularly in ^{1}H NMR experiments which hinders the annotation of the detected metabolites. The latter problem could be overcome by using different 2D-NMR acquisitions via spreading the crowded signals in a second frequency domain. Compared to the several NMR-based metabolomics experiments reported for analyzing biofluids including serum [34], plasma [35], urine [36], and saliva [37], very scarce studies were devoted to analyzing sweat metabolome. These include a study in which NMR-based metabolomics has been applied to assess sweat metabolites variability within a population of healthy male and female subjects [38]. Besides, another study evaluated sweat metabolites from different body parts of healthy population identifying a unique metabolic pattern in the feet region [39]. Finally, sweat NMR-based metabolomics was reported to elucidate the pathogenesis of AD revealing that glucose might be a biomarker that indicates the severity of this disease [40]. These few reports suggest that more work ought to be devoted to employing this technique in future sweat metabolomics studies.

Contrary to NMR, several studies were reported for analyzing sweat metabolome using mass spectrometry usually preceded by a hyphenated technique to decrease the complexity of sweat metabolites [41]. The type of hyphenated technique used depends on the nature of the studied sweat analytes. For analyzing volatile organic compounds (VOCs) present in sweat, gas chromatography coupled to mass spectrometry (GC–MS) is the standard analytical approach employed in this case [42]. It has been applied to analyze the sweat volatile organic compounds for the assessment of cancer biomarkers [43] and also to distinguish sex differences of the human sweat volatilome [44]. Interestingly, high-resolution GC–MS using time of flight detector (TOF) has been also employed to analyze the sweat nonvolatile metabolome identifying 66 compounds predominated by carboxylic acids, amino acids and sugars after suitable sample pretreatment and derivatization steps for analysis of the non-volatile chemicals [45]. This method has been validated in terms of calibration, sensitivity, and recovery in addition to inter and intra-day variability posing it as the best protocol reported so far for analyzing sweat nonvolatile metabolome by GC–MS. Another hyphenated technology used primarily to analyze sweat nonvolatile metabolome is liquid chromatography-mass spectrometry (LC-MS) [46]. Interestingly, in a recent study, LC-MS has been coupled with chemical isotope labeling (CIL) using differential ^{12}C and ^{13}C dansylation to analyze the human sweat amine/phenol sub-metabolome at different time points during exercise [47]. Of ca. 2700 metabolite features detected in this study, only 83 metabolites were strictly identified with a high confidence level of annotation and with sweat sub-metabolome differences between early and late exercise conditions and further between gender. Another recent advancement in analyzing sweat metabolome by LC-MS lies in the application of nanoflow technology (nLC-MS) coupled with CIL to increase the coverage of the sweat metabolome analysis [48]. Capillary electrophoresis is another hyphenated technology that can be coupled with MS (CE-MS) and particularly targeting polar metabolites. It has been applied to characterize the sweat metabolome of screen-positive CF infants identifying metabolic changes associated with that disease [49]. In addition, it has been employed for measuring chloride ions level directly, which is a very useful approach in CF diagnosis [50].

2.2. Analysis of the sweat proteome

The general workflow in sweat proteomic analyses includes protein or peptide separation followed by mass spectrometry analysis for detection, identification, and finally quantification. The main purpose of protein separation is reducing the ion suppression effects often induced by coelution in addition to resolving the complex proteome for downstream mass spectrometric analysis. One of the earliest techniques used in sweat protein separation is two-dimensional gel electrophoresis (2-DE) [51]. This technique is based on separating sweat proteins according to their inherent charge, or isoelectric point in the first dimension, and proteins' molecular mass in the second dimension. However, 2-DE methods exhibit some limitations including significant gel-to-gel variation, time-consuming and low dynamic range warranting for other gel-free techniques of protein separation, such as liquid chromatography. Liquid chromatography techniques e.g. reversed-phase, affinity, ion exchange and size exclusion are well-characterized approaches of protein separation and have been extensively described in literature [52,53]. Of all these techniques, reversed-phase liquid chromatography (RP-LC) using silica-based particles modified by alkyl chains of varying lengths from C8 to C18 are the most popular stationary phases employed in sweat proteomics analysis [13]. These phases are usually packed in nanoflow high-performance capillary columns with an internal diameter of 75 to 100 μm and operated at a flow rate of 200 to 300 nL/min for ideal separation of sweat proteins [54]. This current state-of-the-art technology has been applied recently for the separation of eccrine sweat proteome of two pools samples from five male and female participants resulting in the separation of 311 proteins from the male samples and 189 proteins from the female samples [55].

Following sweat proteome separation, high-resolution mass spectrometry-based analysis is usually applied for protein identification and quantification. There are two approaches for proteome measurement via mass spectrometry. The first approach is the "top-down" proteomics which is characterized by measuring the intact proteins directly using high-resolution tandem mass spectrometers [56]. The second approach is the "bottom-up" analysis, also called shotgun proteomics, in which indirect measurement of proteins is achieved following their digestion into peptides using proteolytic enzymes [57]. The resulted peptide mixture is separated and subjected to MS/MS analysis for identification. The identification strategy is based on comparing the obtained MS/MS spectra of the digested peptides with theoretical MS/MS spectra generated from in silico digestion of a protein database. The latter approach exhibits some potential advantages since the analysis of the resulted peptides can be more achievable as they are easily fractionated, ionized, and fragmented in the gas phase compared to intact protein, thus it has been applied extensively in sweat proteomics studies [58]. Interestingly, the acquisition of the generated tandem MS/MS spectra in proteomics studies can also be acquired using several modes depending on the aim of the experiment. In the case of discovery-based studies, i.e., untargeted experiments, data-independent acquisition (DIA) such as the sequential windowed acquisition of all theoretical mass spectra (SWATH-MS) method is mainly employed to provide information about all detected proteins without prior knowledge or selection of certain proteins (peptides) for analysis [59,60]. In contrast, data-dependent acquisition (DDA) is usually employed for semi-targeted experiments in which a predefined number of precursor ions with the highest signal intensity are selected from a previous full MS scan (MS experiment) and then fragmented in MS/MS experiments without any targeted entity. Asides, performing proteomics analysis on sweat samples is also feasible via multiple reaction monitoring mass spectrometry (MRM-MS) which is a cost-effective technique used for selective detection and quantification of proteins to verify selected proteins as candidate biomarkers with higher sensitivity and specificity [61]. This approach is usually employed for targeted analysis and quantification of selected protein(s) using specific tandem MS instruments such as the triple quadrupole MS. LC-MS/MS under MRM-MS mode has been applied for proteomics analyses of eccrine sweat of healthy controls and individuals diagnosed with schizophrenia with 17 proteins showing a differential abundance of ca. two-folds or greater between the two groups [13].

3. Sweat metabolomics-proteomics for biomarkers discovery

3.1. Metabolomics for sweat biomarkers discovery

Metabolomics offered new avenues complementary to genomics and proteomics for the noninvasive diagnosis of diseases and biomarker discovery [62]. Most studies in this field have focused on metabolites profiling for biomarkers discovery using plasma [63], urine [31], or tissues; nevertheless, the complexity of these biological matrices hinders the search for a valid panel of diagnostic biomarkers . For instance, sweat is an unconventional biomatrix with less complexity than plasma when it comes to biomarkers discovery. In addition, alteration of the sweating process and its composition in different pathological conditions makes it useful for accounting of the pathophysiological alterations of various disorders via metabolomics (Fig. 2). For example, sweat metabolomics analysis of healthy subjects and AD patients revealed differences in glucose levels in sweat among patients according to the severity level of the disease [64]. Sweat with elevated glucose levels reflects progressive AD (Table 1). Moreover, the elevated glucose level may promote itching and delays the recovery of damaged skin barrier [64,65]. Additionally, lipid mediators of sweat such as ceramides and sphingoid metabolites showed an increase in AD patients compared with control subjects, with diagnostic potential for AD [66]. In autoimmune diseases, such as Vogt–Koyanagi–Harada (VKH) that leads to visual impairment, LC-MS-based metabolomics revealed significant alterations in 21 metabolites in the sweat of VKH patients compared to healthy controls. Aberrant amino acids metabolic pathways i.e., L-serine, L-tryptophan, choline, and betaine were proposed to play a critical role in the pathogenesis of VKH disease [58]. Furthermore, clinical evidence for lung cancer diagnosis has been provided through a panel of 5 sweat metabolites, including a trihexose, tetrahexose, suberic acid, monoglyceride (22:2), and nonanedioic acid, analyzed via LC-MS [14].

Moreover, sweat metabolites can be used further as diagnostic biomarkers for certain metabolic diseases. For instance, the glucose level in sweat has been strongly correlated with those in the blood posing it to be used as a non-invasive method to diagnose and control blood sugar in diabetic patients [67].

Sweat metabolites are also closely correlated with the clinical outcomes and may appear prior to disease progression, thereby providing potential biomarkers for the diagnosis of asymptomatic human diseases.

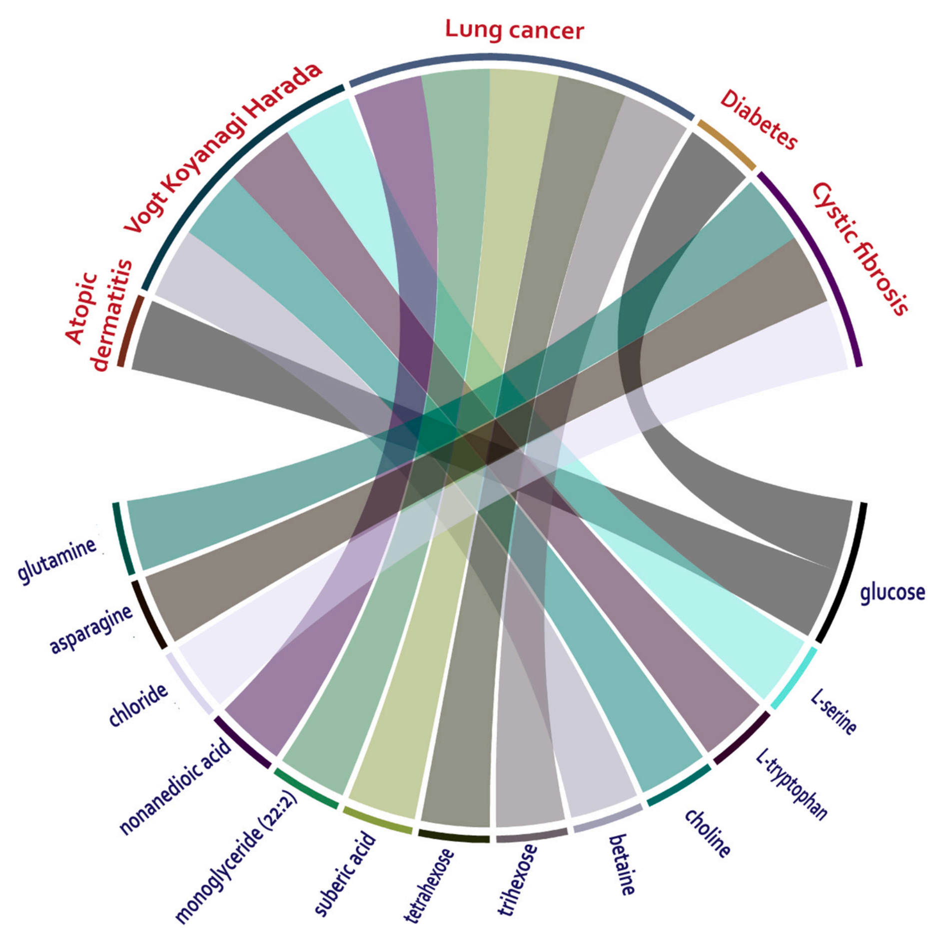

Fig. 2. Potential sweat metabolites that can be employed as biomarkers for various diseases as revealed via metabolomics including as glucose for diabetes and atopic dermatitis, some amino acids for Vogt–Koyanagi–Harada and cystic fibrosis and some hexoses and fatty acids for lung cancer.

Table 1
Summary of studies investigating the potential of sweat metabolomics-proteomics for biomarkers discovery.

Disease	Samples (patients & control)	Sample Collection	Technique Used	Biomarkers Identified	Ref
Sweat metabolomics studies for biomarker discovery					
Atopic Dermatitis	21 & 10	Sweat samples were collected from the backs of subjects in a sauna.	NMR	Significant Increase ($p < 0.05$) in glucose	[64]
Lung cancer	41 & 55	A Macroduct® Sweat Analysis System was used for sweat collection with Pilogel® Iontophoretic Discs as stimulant of sweat excretion. A total sweat volume around 50 μL was collected per individual.	LC-MS	A panel of five differential metabolites has been identified including suberic acid, tetrahexose, trihexose, monoglyceride (22:2), and nonanedioic acid.	[14]
Cystic fibrosis	18 & 50	A Macroduct® Sweat Analysis System was used for sweat collection with Pilogel® Iontophoretic Discs as stimulant of sweat excretion.	CE-MS	Significant decrease in pilocarpic acid and mono (2-ethylhexyl) phthalic acid and significant increase in glutamine and asparagin	[49]
Sweat proteomics studies for biomarker discovery					
Atopic Dermatitis	17 & 17	Sweat from the forehead was induced by physical exercise on a bicycle ergometer and collected and frozen immediately at −20 °C until analysis	Semi quantitative SELDI-TOF-MS followed by ELISA for protein quantification	Significant Decrease ($p < 0.001$) in some dermcidin-derived antimicrobial peptides.	[101]
Schizophrenia	23 & 55	Eccrine sweat from volar forearm was stimulated using pilocarpine and collected with the Macroduct Sweat Stimulation and Sweat Collection System	LC-MS/MS under MRM mode	Highly Abundant (2-fold or more) of 17 proteins e.g. Alpha-2-glycoprotein, corneodesmosin, dermcidin, keratin 10, peroxiredoxin 1, prostatic-binding protein and thioredoxin	[13]
Active Tuberculosis	32 & 24	Eccrine sweat from volar forearm was stimulated using pilocarpine and collected with the Macroduct Sweat Stimulation and Sweat Collection System	LC-MS/MS	Exclusive in TB: 26 proteins belonged mainly to auxiliary protein transport and immune response proteins.	[15]
Integrative studies for sweat biomarker discovery					
Vogt–Koyanagi–Harada	30& 30	Sweat samples were collected from the armpit of subjects in a sauna.	LC-MS/MS	116 proteins (99 decreased and 17 increased) and 21 metabolites (18 decreased and 3 increased) were significantly different in VKH patients when compared to controls.	[58]

For instance, sweat chloride level remains the golden standard for the diagnosis of CF for more than 60 years [68]. However, a new mechanism among CF patients beyond impaired chloride transport has been demonstrated. For instance, asparagine and glutamine are endogenous metabolites that can be used the confirm CF cases from asymptomatic CF carriers. These neutral amino acids were found to complement sweat chloride testing. Importantly, two other sweat metabolites i.e., pilocarpic acid and mono (2-ethylhexyl) phthalic acid were found to be secreted in the sweat of CF patients at significantly lower levels compared with asymptomatic CF carriers. These two exogenous metabolites are also positively correlated in sweat chloride and indicate a deficiency in human paraoxonase, which is known to play a major role in lung deterioration in affected CF children via mediating inflammation, bacterial biofilm formation, and recurrent bacterial infection [49]. The findings of the previous study indicated that the changes of sweat metabolites were not only used to confirm the diagnosis of CF, but rather to further differentiate between symptomatic and asymptomatic CF, ensuring early detection of the disease in order to reduce disease progression and death rates.

Sweat is also receiving increasing attention for the analysis of the xenometabolome such as drugs and their metabolites due to its ease of collection allowing on-site fast drug detection [17]. Sweat can be collected over longer periods of time permitting improved drug monitoring outcomes compared to other biofluids. Several methods were reported for the detectionof drugs' metabolites in sweat such as cannabinoids using GC–MS [69], cocaine, opiates, and nicotine metabolites using LC-MS/MS [70]. Moreover, sweat has been employed as a non-conventional biological matrix for the therapeutic monitoring of atomoxetine, a treatment for children with attention-deficit hyperactivity disorder, and its metabolites 4-hydroxyatomoxetine and *N*-des-methylatomoxetine using LC-MS/MS [71]. Interestingly, sweat has also been employed for identifying food biomarkers. For example, the strong "maple-syrup" odor which appears after fenugreek ingestion was investigated in the human male armpit sweat samples. A panel of 8 compounds appeared only after fenugreek intake analyzed using headspace solid-phase microextraction (HS-SPME) coupled to GC–MS and GC-olfactometry (GC-O), with 2,5-dimethylpyrazine as the main volatile component responsible for such aroma [72]. Still, food research area can benefit from sweat metabolomics in monitoring other key chemicals.

3.2. Proteomics for sweat biomarkers discovery

As mentioned earlier, human sweat is highly dynamic and not fully discovered. Consequently, the changes observed in sweat proteome and the underlying causes are not well-established [73]. Better understanding and in-depth analysis of the qualitative and quantitative alterations documented in the sweat proteome may lead to the identification of sweat-based disease biomarkers. The first proteomic analysis performed by Park et al. [74] recognized 115 proteins using LC-MS/MS in sweat obtained from healthy individuals. Antimicrobial peptides, such as lactoferrin were major forms suggesting that sweat contributes to skin defense against infection. Another study, utilizing LC-MS/MS operated in an MRM-MS mode identified 185 sweat proteins and compared the eccrine sweat proteome of healthy controls to individuals diagnosed with schizophrenia [13]. Interestingly, out of the proteins identified, 17 proteins showed a differential abundance of 2-folds or greater (Table 1). Furthermore, Burian et al. identified 544 proteins from skin wash samples obtained from both healthy volunteers and patients with ectodermal dysplasia (ED), in which 61 proteins were identified exclusively in healthy volunteers, whereas 100 proteins were exclusively detected in ED patients [11]. In another study conducted by Csősz et al. sweat samples from healthy individuals were analyzed via label-free MS where 95 proteins were identified, 20 of which were novel [73]. Most abundant proteins amounting to 91% of the secreted sweat proteins exhibited a protective role in maintaining the epidermal barrier integrity to include dermcidin (DCD), apolipoprotein D, clusterin, prolactin inducible protein, and serum albumin. Furthermore, Adewole et al. performed a global proteomic profile of eccrine sweat and compared sweat proteome between active-TB, other lung diseases (non-TB), and healthy control in order to identify potential biomarkers of active TB [15]. LC-MS/MS analysis identified 117 proteins among which 26 were exclusive for active TB (Table 1).

In the last decade, several studies investigated the role of peptidomics in biofluids due to its high significance [75–77]. Since endogenous peptidases are responsible for the proteolytic cleavage resulting in most peptides in biological systems, any dysregulation in the activities of such enzymes can lead to the formation of distinctive peptide patterns that can be identified by MS-based proteomics. The first human sweat peptidomics analysis was reported in a recent study conducted on healthy individuals, in which proteomic and peptidomic analysis identified a total of 861 unique proteins along with 32,818 endogenous peptides [1]. Among these, skin antimicrobial peptides, cystatins, and corneodesmosomal proteins were recognized to represent a major component of human skin physiology.

The sweat proteome analysis performed in the above-mentioned studies identified a wide range of proteins, between 95 and 861, displaying significant differences in the number of identifications. A pilot investigation conducted recently demonstrated several drawbacks associated with the use of sweat for biomarkers discovery which may explain the reasons behind the discrepancies observed [78]. Several factors influence proteomic identification in sweat such as the sample collection, locations on the body from where the sample is collected, sweat stimulation procedure, handling and pooling of sweat samples. Accordingly, Harshman et al. reported that for a proper discovery of biomarkers associated with sweat analysis, several factors must be considered including the optimization of enrichment methodologies to concentrate the low quantity of proteins available from a single sweat sample and outlining the definition of sample degradation and contamination for better metabolomics analysis from such medium. Hence, the methodological aspects affecting the analysis of sweat proteome should be controlled and defined for researchers in well-defined protocols [78].

4. Role of host-microbiome interactions in modifying sweat metabolic-proteomic composition

A wide range of microorganisms that function in a symbiotic and commensal fashion colonizes the skin, which represents the largest organ in the human body and provides the epithelial barrier to external factors. Such microbiota greatly affects the functions of human immunity thereby influencing normal skin homeostasis [79]. Accordingly, the imbalance between host and microorganisms can lead to skin disorders or infections. For example, the pathogenicity of AD, mentioned earlier, has been shown to be linked to dysbiosis of skin microbiota communities and the lack of its diversity [80–82]. It has been shown that in lesion AD, the skin is dominantly inhabited by *Staphylococcus aureus*, and thus decreases the diversity of other microorganisms [83]. However, in healthy subjects, coagulase-negative *Staphylococcus* species have been found to be the dominant strains. When these strains were isolated and introduced into subjects with AD, they exhibited a reduction in the colonization of cutaneous *Staphylococcus aureus* [84]. These findings illustrate that a properly functioning microbiome protects against pathogenic microorganisms, whereas disruption in the microbiota homeostasis encourages AD.

4.1. Sweat metabolomics for revealing host-microbiome interactions

Host-microbiome interaction has proved an impact on the type of

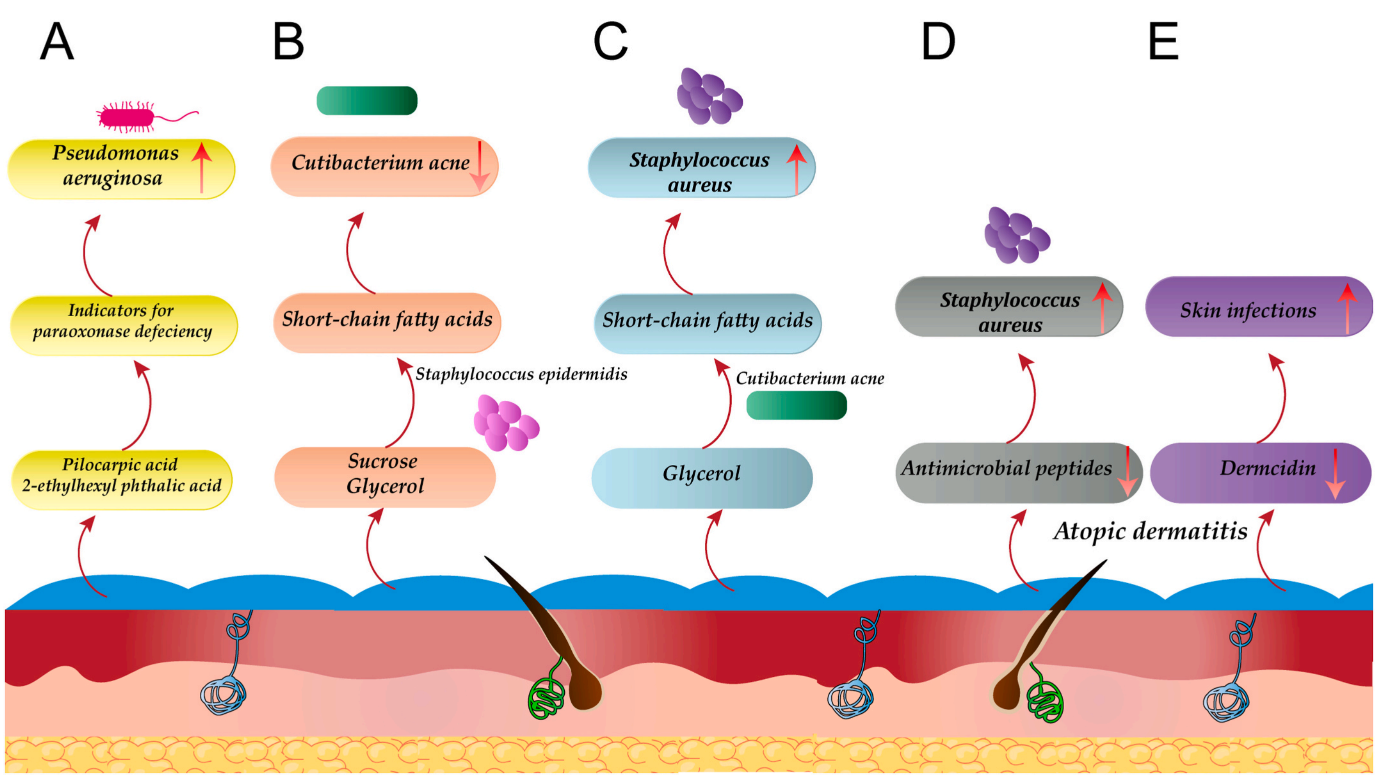

Fig. 3. Schematic shows the interactions between different sweat metabolites and proteins with the skin microbiome. Some metabolites such as pilocarpic acid and mono (2-ethylhexyl) phthalic acid (A) indicate deficiency of paraoxonase among CF infants, thereby increasing *Pseudomonas aeruginosa* infection. Other skin microbiome such as *Staphylococcus epidermidis* (B) and *Cutibacterium acne* (C) can ferment glycerol and other metabolites to form short-chain fatty acids that affect the growth of other bacteria. *Regarding interaction of sweat proteins with the microbiome,* a reduced number of antimicrobial peptides (D) and dermcidin (E) as in case of atopic dematities make individuals highly prone to *Staphylococcus aureus* and other skin infections.

produced metabolites (Fig. 3). Sputum analysis of CF patients revealed a higher level of proteolysis activity owing to the presence of neutrophil elastase and cathepsin G that is responsible for peptides and amino acids (e.g., phenylalanine and tryptophan) generation. Cystic fibrosis pathogen *Pseudomonas aeruginosa* has been proven to be significantly correlated with the abundance of these metabolites [85]. The authors of the latter study concluded that these amino acids and their breakdown metabolites, including indole and phenylacetic acid, can be used to identify patients with worsened disease progression [86]. However, in sweat analysis of CF patients, a different set of metabolites were found to be dysregulated and their presence is associated with CF disease status. For example, asparagine and glutamine have been identified in asymptomatic CF infants, while the lower levels of both pilocarpic acid and mono (2-ethylhexyl) phthalic acid in CF infants compared with unaffected CF infants, indicated deficiency of paraoxonase among CF infants [49], thereby increasing *Pseudomonas aeruginosa* infection and promote its biofilm formation [87].

Microbiota that colonizes the skin is in a dynamic interaction with the host physiology and thereby influences host immunity, which is maintained via the balanced interaction between the host and microbiota [88]. A study conducted by Sanford et al.revealed that skin microbiota takes part in maintaining health by protecting the host from offending pathogens via altering skin barriers or producing compounds that block the growth of competitors [89]. For example, *Staphylococcus epidermidis* suppresses the formation of *Staphylococcus aureus* biofilm via producing serine protease which is also involved in promoting the antimicrobial effect of human β-defensins [90]. Likewise, another study has shown that skin *Staphylococcus epidermidis* can ferment glycerol and sucrose to form short-chain fatty acids metabolites, including acetic acid, butyric acid, lactic acid, and succinic acid, thereby creating an inhibition zone to restrict the overgrowth of *Cutibacterium acne* [91,92]. On the other hand, *Cutibacterium acne* has been found to inhibit the growth of methicillin-resistant *Staphylococcus aureus* by changing skin acidity [93]. In short, *Cutibacterium acne* ferments glycerol, a natural metabolite, into short-chain fatty acids that preserve an acidic skin where *Staphylococcus aureus* cannot flourish at such pH [79,94]. The previous studies showed that *S. epidermidis* and *Cutibacterium acne,* but not *S. aureus* can fermentatively metabolize glycerol to produce short-chain fatty acids. Although the oppositional relationship has not been proven yet, it has been anticipated that during the development of acne vulgaris, both bacteria may utilize glycerol to produce different fatty acids that function as antimicrobial metabolites to compete against each other within the acne lesions [92]. The findings of this study demonstrate a precision microbe approach by utilizing sucrose as selective fermentation initiators for *Staphylococcus epidermidis* as a novel modality to rebalance dysbiotic acne.

Sweat secreted by the apocrine gland is also influenced by the host microbiota in which resident bacteria process and utilize the apocrine derived compounds. *Staphylococcus* and *Corynebacterium* spp. are the most abundant organisms as they colonize areas of high humidity [95]. Staphylococci, which is aerobic bacteria, grow on moist surfaces on the skin and most likely utilize the urea found in the sweat as a source of nitrogen [79]. On the other hand, *Corynebacteria* are aerobic and very fastidious. In fact, the processing of apocrine sweat by axillary microorganisms, mainly *Staphylococci* and *Corynebacteria,* is associated with the mal odor of sweat [96]. Ara et al. studied the foot odor in particular and showed that the odor was associated with the presence of isovaleric acid produced via the degradation of leucine in sweat by resident normal skin microbial flora, *Staphylococcus epidermidis* [97].

The effect of the host on the skin microbiota has been also explained during stress, where substance P is released in sweat and to provoke the virulence of gram-positive skin bacteria, namely *Staphylococcus epidermidis* and *Staphylococcus aureus* [98,99]. Thus, the effect of stress on the skin microbiota may attenuate the host defense and adapt the microenvironment for pathogens [100]. This finding may provide an explanation for the flare-up of certain skin diseases during stress. The application of omics technologies to monitor changes in sweat chemical makeup parallel with metagenomics sequencing of skin microbiota at different stress or diseases could aid in identifying exact microbial strains involved in these chemical changes.

4.2. Sweat peptides for revealing host-microbiome interactions

The eccrine sweat proteome is highly rich in antimicrobial peptides (AMPs), which may also influence the microbial colonization of the skin. In patients suffering from AD and ED, the eccrine sweat composition is characterized by a reduced number of AMPs associated with low immune response making such individuals highly prone to *Staphylococcus aureus* infections [11,101]. Although the relation between AMPs and immunity is well established, the exact mechanism by which sweat regulates skin homeostasis in health and disease remains poorly understood [102]. Rieg et al. investigated the expression of DCD in the sweat of patients with AD revealing that reduction of DCD was correlated with less innate defense [101]. DCD, which is expressed in eccrine glands and constantly secreted into the sweat, has been classified as an AMP with a broad spectrum of antimicrobial activity against gram-negative and gram-positive bacteria including *Escherichia coli, Enterococcus faecalis, Staphylococcus aureus,* and *Candida albicans* [103]. Subsequently, the reduced expression of DCD in AD patients resulted in a high susceptibility to frequent skin infections and modified skin colonization contributing to the chronic inflammation associated with this skin disease. Similarly, Burian et al. reported that reduction in defense-related proteins in eccrine sweat is associated with decreased immunity characterized in ED patients [11]. Therefore, the eccrine gland is not only involved in temperature regulation but rather plays a significant role in the skin immune defense by creating a niche that is not favorable for microbial colonization.

5. Role of exercise in modifying sweat metabolites and proteins

During exercise, the contraction of muscles produces a high amount of heat causing an increase in body temperature. This increase in body heat is detected by central and skin thermoreceptors and the stimulus is processed through the hypothalamus leading to the production of sweat to dissipate heat [104]. The cooling effect occurs through the transfer of heat from the body to the sweat released on the skin surface, which upon gaining sufficient heat is converted to water vapor. This process is further enhanced with exercise training resulting in earlier onset of sweat secretion, which in turn increases the total amount of sweat produced [104]. Accordingly, sweating rate may vary considerably in athletes.

Although sweat composition and sweat rates are known to be highly variable among individuals [105], exercise can act as an additional factor further modifying the sweat characteristics. Several studies investigated the effect of exercise on sweat rate and sweat electrolyte modifications, mainly sodium [106–109].

The potential of exercise in shaping sweat metabolites is one of the most interesting areas of sweat metabolomics investigations. Piling evidence has revealed that differences in sweat metabolites composition and concentration are frequently affected by the sweating stimulation techniques [110]. Studies have revealed that the intensity level of exercise has a potential effect on the types and levels of sweat metabolites [111,112]. For example, during moderate exercise, major sweat metabolites are identified as fatty acids, alcohols, carbohydrates, and non-proteinogenic amino acids [110]. Non-proteogenic amino acids are also collected at higher levels after exercise, the nature of these metabolites may provide information about the metabolism of amino acids in individuals after practicing moderate exercise [110]. In a study conducted to assess the characterization of sweat metabolites induced by exercise, pilocarpine, or those collected passively, practicing exercise seemed to increase several amino acids level i.e., alanine, serine, and valine in the female subjects compared with pilocarpine-induced sweating. However, these amino acids seem to have originated from the skin, and thus they may not reflect a good correlation with blood amino acid level [113].

The proteomic and metabolomics analysis of sweat-induced by exercise using a treadmill model showed a strong correlation in the metabolomics analysis in the overall abundance of sweat metabolites. In contrast, proteomic analysis reflected a low abundance of protein level in this medium, such low protein level in sweat indicates either a potential for protein degradation or a large number of low molecular weight protein/peptides [78]. A piece of evidence has shown that sweat induced by exercise demonstrated a large dynamic range among amino acids concentration. Additionally, the study showed a significant difference in the quantities of alanine, arginine, and threonine among individuals, and further among the same individual under different storage conditions [114].

Compared to the well-recognized role of exercise in sweat metabolome changes, the role of exercise in modifying sweat proteome is poorly discussed. Dunstan et al. reported that exercise at 32–34 °C resulted in significant loss of amino acids through sweat [115]. "Faux" sweat, which is sweat containing amino acids derived from the skin, was collected from male athletes over 40 min of exercise and to display high levels of serine, histidine, ornithine, glycine, alanine, and lysine. Interestingly, the study showed that after 35 min of exercise, total amino acids in sweat diminished thereby proving that sweat can leach amino acids from the stratum corneum [115]. In another study, amino acids lost through sweating were compared between males and females and associated with negative health outcomes [116]. It has been revealed that sweat collected from females had higher levels of 13 amino acids, including serine, alanine, and glycine, and that females are more susceptible to loss of essential amino acids during exercise. Moreover, amino acid loss through sweat was correlated to disturbances in nitrogen balance and collagen turnover, in addition to chronic fatigue in both males and females, contributing to a hypometabolic state [116].

6. Conclusion and future directions for sweat metabolomics-proteomics research

Sweat plays a crucial role in human thermoregulation through a cooling effect or evaporative cooling that maintains the body temperature. Nevertheless, sweat has been implicated in other roles that are of physiological significance including detoxification of heavy metals, elimination of chemicals, and bacterial cleansing. In fact, sweat proteome is among the key players in providing an anti-microbial defense since it has been proved that human sweat is rich in AMPs that protect the skin from microbial colonization. Besides, several sweat-associated metabolites and protein biomarkers have been proposed, allowing for faster screening and diagnosis through quick and non-invasive methods. This review summarizes the currently available analytical techniques used to decode for sweat metabolome and proteome. In addition, the potential of the sweat metabolites and proteins to function as biomarkers discovery and functional biology are investigated. Despite the relative value of sweat metabolome-proteome, studies performed are still rather limited and several aspects are not addressed yet. For example, integrative investigations are required to further elucidate the altered sweat protein-metabolic pathways during the pathogenesis of certain skin diseases such as psoriasis, vitiligo and eczema. Asides, longitudinal studies are still needed to investigate whether disease activity or treatment affects the sweat metabolite or protein profile. An area that is less explored on sweat applications involves the analysis of the xenometabolome such as drugs and their metabolites due to ease of collection allowing on-site fast drug detection. Drug doping detection from sweat samples has also yet to be explored similar to urine or blood specimens.

With regards to technology implementation for monitoring sweat changes, the major bottleneck in sweat metabolome analysis lies in the fact that no single platform can capture whole analytes. However, employing analytical techniques that target polar metabolites, the main constituents of sweat, should be emphasized such as capillary electrophoresis and hydrophilic interaction liquid chromatography. Another challenge associated with sweat metabolome-proteome characterization is the fact that it is not yet known whether the metabolites or proteins identified in human sweat have originated from the sweat per se or they are derived from proximal tissues such as the skin where they mix with the sweat. Labeling experiments analyzed using mass spectroscopy or NMR could aid in identification of metabolites origin. Moreover, the methodology followed in sweat analysis is not constant between different studies, which might lead to inconsistency in results and less significant findings supporting the use of sweat in biomarker discovery. Accordingly, it is of value that future studies employ well-established methods and standardized protocols related to sweat collection, sample preparation, and analysis. In general, further research is still needed for better elucidating the clinical significance of the sweat metabolome-proteome, their potential in biomarker discovery and moreover in the context of system biology to help identify how sweat is regulated in different phenotypes. The meta-analysis should also be recommended to evaluate studies with different protocols or subject numbers to be more conclusive regarding the biomarker significance of such rich chemical niche. The application of omics technologies to monitor changes in sweat composition parallel with metagenomics sequencing of skin microbiota at different stress or disease conditions could aid in identifying exact microbial strains involved in these chemical changes.

Declaration of competing interest

The authors have no conflicts of interest to declare.

Data availability

No data was used for the research described in the article.

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