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Using Sweat to Measure Cytokines in Older Adults Compared to Younger Adults: A Pilot Study

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Abstract

Background/Objectives—Current measures of cytokines involve urine, blood or saliva which have drawbacks including circadian rhythm variations and complicated collection methods. Sweat has been used to measure cytokines in young and middle-aged adults, but not older adults. We sought to determine the feasibility of using sweat to measure cytokines in older adults compared to younger adults.

Design—Two visit cross-sectional pilot study stratified by age group.

Setting—Independent living facility and Johns Hopkins University both in Maryland.

Participants—23 community-dwelling adults aged 65 and older and 26 adults aged 18–40 were included. Those with active cancer treatment or with a known terminal illness diagnosis were excluded.

Measurements—Sweat interleukin-6 (IL-6), interleukin-10 (IL-10), and tumor necrosis factor-alpha (TNF-α) were collected using a non-invasive sweat patch worn for 72 hours by each participant. Samples were measured with a single molecule array (SIMOA) technology for ultrasensitive, multiplexed detection of proteins.

Results—23 older adults and 26 younger adults with mean ages of 77±8.0 years and 28±5.5 years, respectively, completed the study. Both groups had high rates of compliance with patch wearing and removal. Higher concentrations of TNF-α, IL-6 and IL-10 were observed in older adults compared to younger adults, which remained significant after controlling for race, sex, body

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Author Contributions

SLS, MH and JG participated in the concept and design of the study. MH and CS participated in collecting data. LR, YC, CL, JG and MH extracted and analyzed the data. All authors contributed to the writing and approval of the final manuscript.

Conflict of Interest

Authors reports no financial, personal or potential conflicts of interest.

mass index, and chronic disease count (0.110±0.030 vs. 0.054±0.020pg/mL, 0.089±0.012 vs. 0.048±0.018pg/mL, and 0.124±0.029 vs. 0.067±0.025pg/mL, respectively).

Conclusion—These results suggest that sweat patches are a feasible method to collect cytokine data from older adults. Preliminary group differences in cytokine measurement between older and younger groups correspond with current literature that cytokines increase with age, suggesting that sweat measurement using the sweat patch provides a new method of exploring the impact of inflammation on aging. Further research using sweat and the sweat patch is recommended.

Keywords

Older Adults; Sweat Patch; Cytokines; Interleukin-6; Interleukin-10; Tumor Necrosis Factor-Alpha

INTRODUCTION

Inflammatory biomarkers are associated with aging[1,2] and are predictive of mortality.[3] Apart from aging itself, chronic inflammation is associated with many chronic disease processes that increase with age including cognitive impairment[4], heart disease[5], diabetes[6], and arthritis.[7]

Three prominent inflammatory biomarkers are Tumor Necrosis Factor-Alpha (TNF-α), interleukin-10 (IL-10) and interleukin-6 (IL-6). TNF-α and IL-6 are both pro-inflammatory cytokines related to aging, stress, and chronic disease.[5,8,9] IL-10 is an anti-inflammatory cytokine that down-regulates the expression of some pro-inflammatory cytokines, including IL-6.[10] The ratio of IL-6 to IL-10 has also been evaluated as a marker for systemic inflammatory balance and was shown to be predictive of outcomes for patients with cardiovascular disease[11] and depression.[12] Together these three cytokines give an overview of the pro- and anti-inflammatory status of each participant.

Inflammatory biomarkers are often difficult to study requiring body fluids like blood, saliva, or urine. Current collection for these fluids is cumbersome. Blood collection is invasive, and requires well-trained personnel and specific facilities which are not universally available.[13,14] In addition, urine specimens for inflammatory biomarkers usually require 24 hours of collection, must remain cold, and be transported to a processing facility by the participant.[15] Likewise, saliva must be collected multiple times each day and is influenced by gingival and dental disease, which is common in older adults.[16] Most inflammatory biomarkers also have diurnal rhythms of excretion, making standardization difficult to achieve[17] and requiring particular testing times that can inhibit study participation or increase study burden. Together these limitations in current collection methods likely result in a smaller pool of potential participants[18] which can lead to costly recruitment and biased outcomes. A reliable, non-invasive measure that overcomes circadian timing issues in older adults could greatly improve our ability to understand inflammation, aging and chronic disease and the effectiveness of interventions in this population.

One non-invasive method that could overcome these burdens is sweat. Sweat is a clear, odorless substance that is 99% water and rich with biomarkers from multiple parts of the

stress response system including electrolytes, cortisol, neuropeptide Y and Interleukin-6.[19] With over 2.5 million glands on the skin, eccrine glands are the main sweat glands found in all skin producing a little under 1 quart of sweat each day with the highest density on the palms and soles, followed by the head, the trunk and extremities.[20] Eccrine glands are important in thermoregulation. They are innervated by the sympathetic nervous system, and therefore, can also play a role in emotionally-induced sweating (fear, pain, stress and anxiety).[20] Emotionally-induced sweating tends to be limited to the eccrine glands of the forehead, soles and palms.[20] Therefore, collecting sweat from the abdomen or flank helps to avoid capture of emotionally-induced sweat excretion. Sweat collection is not painful nor invasive and bypasses diurnal rhythm challenges.

Sweat can be measured by a sweat patch, which is a non-occlusive, hypoallergenic collection device, like a small adhesive bandage, that uses sweat as its specimen source (PharmChem, Fort Worth, TX). Sweat patches have aided researchers in gathering inflammatory biomarkers, like cytokines, and correlate well with blood samples in small pilot studies of healthy young and middle-aged adults.[21,22] To the best of our knowledge, the sweat patch has not been tested to isolate cytokines with older adults. Therefore, the purpose of this pilot study was to determine the feasibility of using the sweat patch to measure cytokines in older adults and compare those results with findings from younger adults. We hypothesized that we would find cytokines in older adult sweat samples and that the older adults group would have higher pro-inflammatory cytokine levels and a higher pro-inflammatory to anti-inflammatory ratio compared to the younger adults.

METHODS

Design

This pilot study was conducted over two study visits. The first visit included screening for eligibility, obtaining consent, applying the sweat patch, and completing the questionnaire of demographic, health status, and psychosocial measures via interview. The second visit was 72 hours later and consisted of removing the patch, asking three questions to capture the practicality of wearing the patch, collecting the sweat patch and providing a $25 gift card for participation.

Participants

Two groups comprised this study. Group 1 consisted of community-dwelling adults aged 65 and older recruited from an independent living facility in Rockville, Maryland and screened for cognitive impairment and eligibility in May through June 2016. Group 2 consisted of young adults aged 18–40 recruited from Johns Hopkins University who were screened for eligibility in June 2016. Participants were asked for socio-demographic data, lists of chronic conditions and medications and weight and height measurements (Table 1). All data were obtained via self-report. The exclusion criteria included active cancer treatment or a terminal illness diagnosis. The Johns Hopkins Internal Review Board approved the study (IRB00095668).

Materials & Physiological Biomarkers

The sweat patch was used in this study to collect TNF-α, IL-10 and IL-6 cytokines. It is a sterile sweat collection device that is very similar to a band-aid (PharmChem, Fort Worth, TX). It is comprised of a white absorption pad covered with a polyurethane dressing. The absorption pad of the patch is protected from the environment by a layer of film composed of polyurethane coated with adhesive. The polyurethane film is a semipermeable membrane which allows the transfer of water vapor and gases. Sweat excretions are trapped by this dressing and retained on the white absorption pad. The sweat patch was approved by the FDA as a collection device in 1990 and has been used extensively in commercial drug screening.[23] It is currently classified as a Class I device, the lowest risk category, with the FDA.

Sweat Patch Placement, Removal and Transport

Each participant had the patch placed on their upper abdomen or flank (to avoid areas with excessive hair). The site was cleaned with alcohol and the patch was applied. Each patch was labeled with the participant's unique identifier along with the date and time of patch placement. Participants were asked to abstain from vigorous exercise, swimming and baths while wearing the patch. Vigorous exercise was avoided to circumvent the possible anti-inflammatory properties of IL-6 that have been shown during and following vigorous exercise in serum studies.[24] Showers and all other daily activity were permitted. A research team member removed the patch after 72 hours (+/− one hour). We previously piloted this 72 hour period in another cohort, and found that this timing period provided necessary insights into biological profiles that related to health and wellness. The patch was then placed in a plastic bag labeled with the participant's unique identifier and the date and time of patch removal. Within one minute of removal, the patch was deposited in a cooler of dry ice for transport. Patches were stored in a −80°C freezer until all patches were collected. Patches were subsequently transported on dry ice to the National Institutes of Health (Bethesda, Maryland) and stored at −80°C prior to extraction.

Sweat Patch Extraction Techniques

Utilizing the Gill Lab Sweat Patch Extraction Protocol[25], the sweat patches were thawed on ice in batches of 6. Each white absorption pad of the patch was removed using tweezers and placed in a 5mL conical tube along with 3mL of buffer. Buffer consisted of phosphate-buffered saline (PBS) with 0.1% Tween 20 and 0.2% bovine serum albumin (BSA). The 5mL conical tube was wrapped in parafilm and placed in a 50mL conical tube filled with ice and wrapped in parafilm again. The 50mL tube was shaken for 20 minutes. The patch and buffer solution were placed in a separate 50mL conical tube for centrifugation at 3000g for 3 minutes at 4°C. Three 250 microliter extract samples were aliquoted into 1mL conical tubes. The remaining extract was aliquoted into a 5mL conical tube for storage. All tubes were labeled using the participant's unique identifier. The white absorption pad of the patch and all conical tubes were then placed back into freezer storage at −80°C.

Laboratory Studies

TNF-α, IL-10 and IL-6 sweat concentrations were measured with an ultra-sensitive single molecule enzyme-linked immunoarray (Simoa,™ Quanterix Corporation, Lexington, MA) using a method previously described in Rissin et al., 2011.[26] This method utilizes both analogue and digital array equipment to detect much smaller quantities of solute compared to traditional ELISA methods.[27] We used the commercially-available Cytokine 3-Plex A kit, which is a 3-step multiplex digital immunoassay for the simultaneous quantitative determination of low-abundance levels of all 3 proteins. This specific Cytokine 3-Plex kit was chosen to determine if both pro-and anti-inflammatory cytokines could be found in the sweat of older adults. Currently, Quanterix does not have multiplex kits available with greater than three cytokines. All samples were run in duplicate. In order to measure the low concentration of each protein in sweat, the samples were diluted with 1 part diluent and 3 parts sweat sample. The lower limit of detection for TNF-α, IL-10 and IL-6 were 0.015 pg/mL, 0.003 pg/mL, and 0.008 pg/ml, respectively. Only intra-assay coefficients of variance (CVs) less than 20% were included in the analysis. The laboratory scientists who undertook this analysis were blinded to the participant groups.

Data Analyses

Descriptive statistics with means and proportions were used to describe categorical variables. Normality of cytokine distribution was tested using Shapiro-Wilk and Q-Q plots. Spearman correlation and multiple linear regression were used to test association of variables. Model building was aided by the use of the best subsets method of minimum AIC calculation. The final multiple linear regression model included age group, race/ethnicity, sex, BMI and medical problems count (Table 2).

RESULTS

Characteristics of the Study Subjects

The 49 participants included 23 community-dwelling adults aged 65 and older (mean age 77; range 66–90) and 26 younger adults (mean age 28; range 22–39). See Table 1. The older group had a higher number of medical diagnoses than the younger group as expected (4.70 vs. 0.35) as well as a higher number of medications (4.13 vs. 0.77). See Table 2 for self-reported chronic diseases by age group. Mean body mass index was also, as expected, different between the two groups (28.3 vs. 24.3 kg/m^2).[28]

Feasibility

In the older group, all participants (100%) wore the patch successfully for 72 hours and none reported any difficulties wearing the patch. All patches were successfully removed within ± 1 hour of 72 hours without incident. In the younger group, 25 (out of 26) participants successfully wore the patch for the 72 hours. One participant reported discomfort and itching and removed the patch prior to the 72 hours. Of the remaining 25 younger adult participants, 22 of the patches were removed within ± 1 hour of 72 hours. The remaining 3 participants' sweat patches were removed within ± 2.5 hours of the 72 hours due to scheduling discrepancies. Data was analyzed with and without the 3 younger group

participants with no change in results. Therefore, reported data includes the 3 participants with timing discrepancies.

Cytokines

All three cytokines (TNF-α, IL-10 and IL-6) were detected in all samples, including both the younger and older adult groups. Older adults had significantly higher sweat TNF-α, IL-10 and IL-6 quantities compared to the younger adults both before and after adjustment (Table 3 and Figure 1). The ratio of IL-6: IL-10 also increased in the older group compared to the younger group (Table 3) but was not significant after adjustment. One outlier in the older adult group was female with 2 medical problems and no prescribed medications with 0.30pg/mL, 0.023pg/mL and 0.032pg/mL for TNF-α, IL-10 and IL-6, respectively. Analyses were conducted with and without this participant with no significant change to results and, therefore, these results were included in all analyses.

DISCUSSION

We found that measuring cytokines in sweat is feasible in an older adult population. Additionally, this method provides promising quantitative data in both younger and older adult groups. As hypothesized, older adults consistently showed higher levels of inflammatory and anti-inflammatory cytokines compared to younger adults. Measuring cytokines in the sweat patch occurred with little difficulty or irritation suggesting that the sweat patch is wearable for all adult age groups, including older adults. Also, this study suggests that the methods presented here provide a platform for additional biomarker based studies in older adults.

Beyond feasibility, we found that the sweat patch cytokine data reflected trends expected with age in serum or plasma. For all three cytokines (IL-6, IL-10 and TNF-α), both adjusted and unadjusted models showed significantly higher levels of cytokines in the older group compared to the younger group. The ratios between pro-inflammatory cytokine IL-6 and anti-inflammatory cytokine IL-10 also changed with more IL-6 relative to IL-10 in the older group. Although not significant in this small sample, the increased IL-6: IL-10 ratio in older adults could indicate a change in the inflammatory response system itself where IL-10, which down regulates pro-inflammatory cytokines including IL-6, is no longer as responsive to increases in pro-inflammation in older adults. A higher pro-inflammatory state has long been considered an important consideration in aging research.[29–31] A higher pro-inflammatory state has also been associated with an increase in chronic disease count[2], which may explain why the outlier in the older adult group had lower cytokine levels compared to her peers. Our sweat patch results parallel those found in serum and plasma. This finding contributes to the potential validity and usefulness of sweat patch collection of cytokines.

Another strength of the current findings is that IL-10 and other small molecules are difficult to isolate in serum or plasma due to the amount of large proteins present (e.g. albumin). Sweat sampling may offer an interesting alternative to serum or plasma because of its ability to more accurately or readily isolate smaller molecules, such as IL-10. This finding indicates that the sweat patch might be not only more convenient, less invasive, and less sensitive to

circadian issues than other measurement techniques but also more sensitive to detection of some protein molecules, such as IL-10, that do not have a gold standard measurement.

Our study has a number of limitations. Sweat rates affect solute composition. A molecule or compound found in all sweat needs to be identified to help standardize future analyses by sweat rates. Also, the mechanism by which secretion of cytokines from blood into the sweat lumen remains largely unknown. Therefore, it is challenging to understand the clinical meaning of the data gathered. However, previous research on healthy adult women showed a direct correlation of cytokines from eccrine sweat glands to plasma levels.[22] This study does not allow us to evaluate circadian rhythm changes that occur with aging due to the continuous sampling method used. Finally, this is a pilot study with a small sample size recruited via convenience sampling, limiting the generalizability of findings.

There is a growing body of multi-disciplinary researchers exploring the use of wearable sweat sensors and patches in biological research. A number of research groups are investigating eccrine gland physiology, mechanisms of protein partitioning into sweat, and the technology needed to appropriately capture and measure sweat.[32–34]

One key advantage of sweat over other non-invasive sampling techniques is that the sweat patch captures biofluid directly on the same surface on which it is produced meaning that there is no sample loss and less possible adulteration compared to other biofluids including urine, tears or saliva.[22] Sweat sampling is also more ergonomic and convenient to the participant. It offers the option of continuous sampling bypassing the challenge of diurnal rhythms without physiological consequence to participation compared to collection of tears or saliva (eg. dry mouth or dry eyes).

In conclusion, wearing the sweat patch was feasible and allowed cytokine data to be collected without the use of a wet lab, participant travel, or invasive collection techniques. The possibility of obtaining cytokine data easily from participants could lead to sweat patch use in large cohort studies, allowing for an increased richness in data collection and biomarkers with substantial participant ease. Further research from our team is evaluating sweat measurement using the sweat patch and comparisons to other biofluids and collection methods.

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Sponsor's Role

The sponsors were not involved in the design, methods, subject recruitment, data collection, analysis or preparation of the paper.

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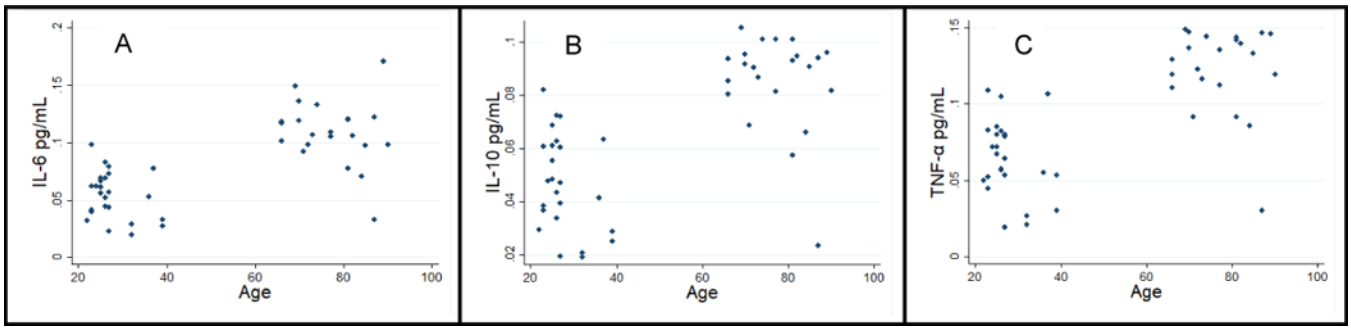

Figure 1. Scatter Plots of Individual Cytokines for Older vs. Younger Adults

A: Interleukin-6 by age; B: Interleukin-10 by age; C: Tumor necrosis factor alpha by age

Table 1

Characteristics of the Study Subjects and Sweat Patch Feasibility

Characteristic	Older Adults (n=23)	Younger Adults (n=26)	Total (n=49)
Age, mean ± SD	77 (8.0)	28 (5.5)	51 (25.4)
Gender, male: female (%female)	4:19 (82.6)	5:21 (80.7)	4.5:20 (81.6)
Race (%White)	78.3	61.5	69.4
Body Mass Index * (kg/m^2), mean (SD)	28.3 (8.3)	24.3 (5.7)	26.4 (7.4)
Education			
High School Graduate, Count (%Yes)	5 (21.7)	0	5 (10.2)
Associate Degree, Count (%Yes)	4 (17.4)	0	4 (8.2)
Bachelor Degree, Count (%Yes)	12 (52.2)	0	12 (24.5)
Graduate School or above, Count (%Yes)	2 (8.7)	26 (100)	28 (57.1)
Medical Problems Count, mean (SD)	4.70 (2.6)	0.35 (0.66)	2.4 (2.9)
Total Medications, mean (SD)	4.13 (3.0)	0.77 (0.76)	2.3 (2.7)
Feasibility			
Successfully worn for 72 hrs, (%Yes)	100	92	96
Removed within +/− 1 hr of 72 hr window (%Yes)	100	88.5	94
Patch Collection Occurred without Incident (%Yes)	100	96	98

* Based on self-reported height and weight

Table 2

Self-Reported Chronic Diseases by Age Group

Disease	Younger Adults (n=26)	Older Adults (n=23)	Total (n=49)
Hypertension	1 (3.9%)	14 (60.9%)	15 (30.6%)
High Cholesterol	0 (0%)	10 (43.5%)	10 (20.4%)
Diabetes	1 (3.9%)	1 (4.4%)	2 (4.1%)
Arthritis	1 (3.9%)	18 (78.3%)	19 (38.8%)
Stroke	0 (0%)	3 (13.0%)	3 (6.1%)
Heart Disease	0 (0%)	4 (17.4%)	4 (8.2%)
Renal Disease	0 (0%)	3 (13.0%)	3 (6.1%)
Chronic Lung Disease	0 (0%)	1 (4.4%)	1 (2.0%)
Liver Disease	0 (0%)	2 (8.7%)	2 (4.1%)
Depression	1 (3.9%)	6 (26.1%)	7 (14.3%)
Anxiety	1 (3.9%)	5 (21.7%)	6 (12.24%)
Other	2 (8.7%)	21 (91.3%)	24 (49.0%)

Table 3

Differences in Sweat Biomarker Means between Older and Younger Groups

Sweat Biomarker	Younger Mean (SD) pg/mL	Older Mean (SD) pg/mL	β* (CI) Unadjusted	P-Value	β (CI) Adjusted**	P-Value
Interleukin-6 (Y=23; O=20)***	0.054 (0.020)	0.110 (0.030)	0.055 (0.040 0.070)	<0.001	0.055 (0.027 0.083)	<0.001
Interleukin-10 (Y=24; O=21)	0.048 (0.018)	0.089 (0.012)	0.040 (0.031 0.050)	<0.001	0.032 (0.013 0.052)	0.002
TNF-α (Y=18; O=20)	0.067 (0.025)	0.124 (0.029)	0.057 (0.039 0.074)	<0.001	0.064 (0.042 0.087)	<0.001
IL-6/IL-10 Ratio	1.16 (0.11)	1.28 (0.17)	0.118 (0.028 0.208)	0.011	0.136 (−0.015 0.288)	0.076

* β represents the difference in means between the older and younger groups

** Adjusted model includes: Race, BMI, Sex, and Chronic Disease Count

*** Y and O represent the number of younger and older participants respectively included in each cytokine analysis with coefficients of variance less than 20%

CI: Confidence Interval; SD: Standard Deviation; IL-6: Interleukin 6; IL-10: Interleukin-10; TNF-alpha: Tumor Necrosis Factor-Alpha