

Non-invasive metabolite profiling using
sweat sensing wearable platforms leveraging
continuous enzymatic reactions

Thesis by
José A. Lasalde-Ramírez

In Partial Fulfillment of the Requirements for
the degree of
Doctor of Philosophy

The Caltech logo, featuring the word "Caltech" in a bold, orange, sans-serif font.

CALIFORNIA INSTITUTE OF TECHNOLOGY
Pasadena, California

2027
(Defended June 26, 2026)

© 2027

José A. Lasalde-Ramírez
ORCID: 0000-0003-2834-3120

ACKNOWLEDGEMENTS

I dedicate this work to the mentors, family members, and friends who have provided unconditional support throughout this journey. I consider this accomplishment as much theirs as it is mine.

I would like to extend my deepest gratitude to Professor Wei Gao. As a young researcher in 2018, (also young) Prof. Gao gave me the unique opportunity to work as a WAVE summer research student in his group, where I got to celebrate the lab's first-year anniversary. Wei has provided unwavering support throughout my undergraduate and graduate endeavors. It was an incredible opportunity to return as a PhD student and work toward improving patient health through medical device innovation. It was also a wonderful experience to see us both grow as professionals and people, and an honor to be a member of the Gao Group.

I would like to thank the members of my thesis committee, Professor Anqi Zhang, Professor Henry Lester, and Professor Morteza Gharib, for their time and guidance throughout this process. Your feedback and insights have significantly enriched this work.

Additionally, I would like to thank all of my lab colleagues and peers, especially Julianne Sempionatto, Chihyeong Won, Soyoung Shin, Moon-Ju Kim, Yu Song, and Sijie Ji. Your contributions to this work and your encouragement were invaluable.

Special thanks to my colleagues and mentors at Samsung and MedTech Innovator for supporting my professional development and helping me gain a unique perspective on medical device development.

I would also like to thank the Student-Faculty Programs team at Caltech. Without their continued efforts to provide research opportunities to underrepresented students in STEM, I would not have found my way to Caltech. Thank you to Maria Manzanares, Candace Rypisi, and Carol Casey. It has been a pleasure working alongside you to open the door for more students to access these incredible research environments.

Special thanks to the pickup basketball community and my golfing buddies for providing friendship, balance, and many welcome breaks throughout this journey. I also want to especially thank my friends and family: Luis, Miguel, Javier, Gabriel, Andres, and my brother from another mother, Kevin. I will forever cherish your constant support, motivation, and spontaneous visits to keep me company.

Thank you to my parents, Rosaura and José, for being a guiding light and my source of inspiration. Your sacrifices and commitment to our growth have not gone unnoticed. Thank you to my brother, César. I could not be more grateful to share every stage of life, including the PhD, alongside you.

Lastly, I want to acknowledge my wonderful partner, Alondra. I will forever be grateful that we have grown together into the people and professionals we dreamed of becoming. Thank you for moving all the way to California and sacrificing time with your loved ones back in Puerto Rico so that we could be closer to one another.

Los quiero, los amo, gracias.

ABSTRACT

Continuous lipid profiling offers a powerful approach to reveal dynamic lipid fluctuations underlying cardiometabolic health. While enzymatic reactions offer great promise for continuous metabolic tracking, existing technologies cannot sustain the multi-enzyme reactions required for continuous lipid detection, particularly those dependent on non-regenerable cofactors. Here, we introduce a wearable epidermal lipid profiler that enables noninvasive, continuous monitoring of cholesterol and triglycerides through a combination of dual-step and cascading enzymatic reactions supported by continuous refreshment of adenosine triphosphate (ATP). The system integrates a polymeric cofactor-releasing (Poly-CORE) module composed of polypyrrole–ATP nanostructures that provide controlled, sweat-triggered ATP replenishment for long-term, continuous biosensing. Complementary glucose, pH, and sweat rate sensors were integrated to offer individualized calibration and contextual metabolic information. We demonstrate real-time lipid tracking during dietary challenges and habitual activities, capturing distinct postprandial responses to macronutrient composition. In a preclinical study, simultaneous sweat and blood analyses coupled with causal machine learning revealed key physiological confounders and established individualized mappings between sweat and blood lipids. This cofactor-refreshing biosensing framework extends continuous monitoring to complex enzymatic systems and couples dynamic lipidomics with causal inference, establishing a foundation for noninvasive, longitudinal cardiometabolic health assessment.

PUBLISHED CONTENT AND CONTRIBUTIONS

1. J. A. Lasalde-Ramírez, C. Won, S. Ji, S. Shin, R. M. McDonald, K. Karish, G. Kim, H. Hang, D. Mukasa, B. Arianpour, Y. Song, J. Yang, T. K. Hsiai, Z. Li, T. Lee, J. R. Sempionatto, W. Gao, Noninvasive continuous lipid profiling via cofactor-refreshing cascading enzymatic reactions and causal machine learning, *Nature Sensors*, 2026, Article In Press.

W.G., J.L.R. and J.R.S conceived the project. J.R.S. and J.L.R. led sensors and platform development. J.L.R, C.W. and J.R.S. led platform characterization and human studies. C.W., S.J. and D.M. contributed to data processing. R.M. contributed to sensor development. S.S., K.K., G.K., R.M. and Y.S. contributed to sensor characterization and testing. S.S. and B.A. contributed to the human studies' sample collection. W.G., J.L.R., J.R.S., C.W., and S.J. cowrote the paper. All authors contributed to the data analysis and provided feedback on the manuscript.

2. M.-J. Kim, J. A. Lasalde-Ramírez, W. Heng, W. Gao, Biomolecular profiling for noninvasive health monitoring, *Nature Biotechnology*, 2026. <https://doi.org/10.1038/s41587-026-03050-2>.

M.-J.K. and W.G. conceived the study and designed the outline and scope. M.-J.K., J.A.L.R., W.H. and W.G. wrote the manuscript.

3. S. Shin, R. Liu, Y. Yang, J. A. Lasalde-Ramírez, G. Kim, C. Won, J. Min, C. Wang, K. Fan, H. Han, C. Uwakwe, W. Heng, T. K. Hsiai, Z. Li, J. D. FitzGerald, W. Gao, A bioinspired microfluidic wearable sensor for multi-day sweat sampling, transport, and metabolic analysis, *Science Advances*, 2025, 11, eadw9024.

Conceptualization: W.G., S.S., Y.Y., and Z.L. Methodology: W.G., S.S., R.L., and Y.Y. Investigation: S.S., R.L., Y.Y., J.A.L.-R., G.K., C.Won, J.M., C.Wang, K.F., H.H., C.U., W.H., T.K.H., Z.L., and J.D.F. Funding acquisition: W.G., T.K.H., J.D.F., and Z.L. Validation: S.S., R.L., Y.Y., C.Wang, J.A.L.-R., and Z.L. Data curation: S.S. and R.L. Resources: T.K.H., Z.L., and W.G. Formal analysis: S.S. and R.L. Visualization: S.S. and R.L. Software: J.M. and W.H. Supervision: W.G. Project administration: W.G. Writing—original draft: W.G., S.S., and R.L. Writing—review and editing: S.S., R.L., J.A.L.-R., G.K., C.Won, J.M., C.Wang, K.F., H.H., C.U., W.H., T.K.H., Z.L., J.D.F., and W.G.

4. C. Wang, K. Fan, E. Shirzaei Sani, J. A. Lasalde-Ramírez, W. Heng, J. Min, S. A. Solomon, M. Wang, J. Li, H. Han, G. Kim, S. Shin, A. Seder, C.-D. Shih, D. G. Armstrong, W. Gao, A microfluidic wearable device for wound exudate management and analysis in human chronic wounds, *Science Translational Medicine*, 2025, 17, eadt0882.

C.W. and W.G. conceived the project. C.W., K.F., and W.G. developed the methodology. C.W., K.F., E.S.S., J.A.L.-R., S.S., M.W., and A.S. developed and characterized the iCare systems. C.W. performed characterization and tests on cells, bacteria, and animals. C.W., K.F., and C.-D.S. performed patient studies. C.W., W.H., and J.M. developed the PCB and evaluation. J.L. performed simulations. K.F., H.H., and G.K. assisted in the SEM imaging. C.W. and S.A.S. performed machine learning analysis of patient data. W.G. and D.G.A. acquired funding. W.G., C.-D.S., and D.G.A. supervised the whole project. C.W., K.F., and W.G. wrote the original draft. C.W., K.F., E.S.S., J.A.L.-R., W.H., J.M., S.A.M., M.W., J.L., H.H., G.K., S.S., A.S., C.-D.S., D.G.A., and W.G. edited the manuscript for review.

5. A. Childs, B. Mayol, J. A. Lasalde-Ramírez, Y. Song, J. R. Sempionatto, W. Gao, Diving into sweat: Advances, challenges, and future directions in wearable sweat sensing, *ACS Nano*, 2024, 18, 24605-24616.

All authors contributed to the writing of the manuscript.

6. J. R. Sempionatto, J. A. Lasalde-Ramírez, K. Mahato, J. Wang, W. Gao, Wearable chemical sensors for biomarker discovery in the omics era, *Nature Reviews Chemistry*, 2022, 6, 899–915.

All authors contributed to researching data for the article, and writing and review/editing of the manuscript before submission.

TABLE OF CONTENTS

Acknowledgements.....	iii
Abstract	v
Published Content and Contributions.....	vi
Table of Contents.....	viii
List of Illustrations and/or Tables.....	x
Abbreviations.....	xv
Chapter I: Introduction.....	1
1.1 Wearable chemical sensors for biomarker discovery.....	4
1.2 Wearable chemical sensing strategies	9
1.2.1 Electrochemical chemical sensing strategies	9
1.2.2 Optical biosensing strategies	13
1.3 Emerging wearable sensing strategies.....	14
1.4 Materials and wearable chemical sensing	18
1.4.1 Material considerations.....	19
1.4.1.1 Electrode materials.....	19
1.4.1.2 Electrode surfaces	20
1.4.1.3 Surface coatings	20
1.4.1.4 Skin Conformability	20
1.4.2 System level considerations.....	21
1.4.2.1 Fluid extraction and sampling	21
1.4.2.2 Wearable sensor platforms	21
1.4.2.3 Wireless and continuous data collection	23
1.4.2.4 Power.....	23
1.5 Data-driven biomarker discovery	23
1.5.1 Multiplexed multimodal wearable sensing.....	24
1.5.2 Machine learning-driven biomarker discovery	28
1.6 Outlook.....	31
1.7 Bibliography of Chapter 1	33
Chapter II: Wearable electrochemical lipid biosensing.....	48
2.1 Introduction.....	48
2.2 Biosensor characterization.....	51
2.3 Sweating-enabled Poly-CORE module.....	55
2.4 Continuous epidermal lipid profiling	59
2.5 Conclusions.....	62
Appendix A	63
Bibliography of Chapter 2	89
Chapter III: Machine learning-driven lipid biomarker validation.....	94
3.1 Introduction.....	94
3.2 Lipid biomarker validation via causal machine learning	95
3.3 Conclusions.....	100

Appendix B	101
Bibliography of Chapter 3	104
Chapter IV: Summary and Outlook	106
4.1 Overall Summary	106
4.2 Limitations	107
4.3 Future Outlook	108
4.3.1 MS-Wearable Convergence.....	108
4.3.2 Sensor-Ready Biomarkers	111
4.3.3 Platform Development and Validation.....	114
4.3.4 Dynamic Health Monitoring.....	117
Bibliography.....	119
Materials and Methods.....	125

LIST OF ILLUSTRATIONS AND/OR TABLES

<i>Number</i>		<i>Page</i>
Figure 1-1.	FDA categories of biomarkers	2
Figure 1-2.	Biomarker discovery via wearable chemical sensors	3
Table 1-1.	Non/Minimally invasive metabolites of interest	6
Figure 1-3.	Wearable electrochemical and optical biosensing strategies	10
Figure 1-4.	Bioaffinity-based molecular switches and in situ sensor regeneration	15
Figure 1-5.	Functional materials for wearable chemical sensors	18
Figure 1-6.	Wearable platforms for biofluid extraction, sampling, and data collection	22
Figure 1-7.	Multiplexed multimodal wearable sensing and machine learning for biomarker discovery	27
Figure 2-1.	Epidermal lipid profiling	48
Figure 2-2.	Enzymatic sensing strategy for continuous epidermal lipid profiling	49
Figure 2-3.	Wearable patch design for continuous epidermal lipid profiling	50
Figure 2-4.	Biosensor characterization for continuous lipid monitoring	52
Figure 2-5.	Characterization of the Poly-CORE module	55

<i>Number</i>		<i>Page</i>
Figure 2-6.	Continuous epidermal lipid profiling	60
Figure A-1.	Images of the wearable cardiometabolic tracking patch	66
Figure A-2.	Structural layout of the wearable sweat-sensing patch	66
Figure A-3.	Fabrication process of the wearable sweat-sensing patch	67
Figure A-4.	Stepwise electrode surface modification	68
Figure A-5.	Calibration and reproducibility of enzymatic biosensors	69
Figure A-6.	Low-concentration sensitivity of CH and TG sensors	70
Figure A-7.	Shelf stability of enzymatic biosensors	70
Figure A-8.	Selectivity of electrochemical sensors in the absence of target analytes	71
Figure A-9.	pH dependency of electrochemical biosensor performance	71
Figure A-10.	Temperature dependence of enzymatic sensors	72
Figure A-11.	Operational stability of glucose and CH sensors over 8 h	72
Figure A-12.	Effect of ionic diffusion and drift in the sweat rate sensor	73
Figure A-13.	Selectivity of the ionic strength sensor	73
Figure A-14.	Calibration of sweat rate sensor	74
Figure A-15.	pH dependence of the sweat rate sensor	74

<i>Number</i>		<i>Page</i>
Figure A-16.	Temperature dependence of the sweat-rate sensor	75
Figure A-17.	Characterization of the sweat rate sensor	75
Figure A-18.	On-body evaluations of the sweat rate sensor	76
Figure A-19.	Validation of the TG enzymatic sensing mechanism	77
Figure A-20.	ATP release kinetics from polymer matrices under matched electropolymerization conditions	77
Figure A-21.	ATP release kinetics from representative polymer entrapment matrices	78
Figure A-22.	Electrochemical impedance characterization of ATP-releasing electrodes	78
Figure A-23.	ATP-dependent TG sensor response under skin-temperature conditions	79
Figure A-24.	Reproducibility of ATP release from PPy/ATP electrodes	79
Figure A-25.	Influence of electrodeposition parameters on ATP release from Poly-CORE electrodes	80
Figure A-26.	Effect of flow-rate variations on ATP release from the Poly-CORE module	81
Figure A-27.	ATP release kinetics of the Poly-CORE module under repeated hydration–dehydration cycles	81
Figure A-28.	Passive and electrochemically stimulated ATP release from PPy/ATP structures	82
Figure A-29.	SEM imaging of the ATP sensor surface	82

<i>Number</i>		<i>Page</i>
Figure A-30.	Reproducibility of the ATP sensor response	83
Figure A-31.	pH dependence of the ATP sensor	83
Figure A-32.	Selectivity of the ATP sensor in the presence of sweat interferents	83
Figure A-33.	Calibration of TG sensing based on ATP concentration	84
Figure A-34.	TG sensor response at elevated ATP concentrations	85
Figure A-35.	Skin-temperature validation of TG sensing based on ATP concentration	85
Figure A-36.	System-level block diagram of the wireless wearable patch for lipid profiling	85
Figure A-37.	Printed circuit board (PCB) circuit design of the wearable patch for lipid profiling	86
Figure A-38.	Computed TG calibrations from on-body meal studies	87
Figure A-39.	Fasting and post-meal comparison of sweat and blood lipid levels	87
Figure A-40.	Baseline and postprandial biomarker responses following different meals	88
Table A-1.	Interpolation table for determination of triglycerides	88
Figure 3-1.	Validation of sweat lipid biomarkers using causal machine learning	96
Figure B-1.	Linear regression results of non-causal ML models	101

<i>Number</i>		<i>Page</i>
Table B-1.	Patient demographics for lipid biomarker validations	102
Table B-2.	Causal ML performance compared with negative control (nonsensor covariates only)	103
Table B-3.	Causal ML performance against conventional baselines in sweat-to-blood lipid inference	103
Figure 4-1.	Wearable platform for continuous epidermal lipid profiling	105
Figure 4-2.	From episodic diagnostics to continuous molecular monitoring	108
Figure 4-3.	Mass spectrometry-based untargeted omics for biomarker discovery	111
Figure 4-4.	Architecture of wearable biochemical sensors	113

ABBREVIATIONS

AFE. Analog front end.

ATP. Adenosine triphosphate.

Au. Gold.

BMI. Body mass index.

BMR. Basal metabolic rate.

BSA. Bovine serum albumin.

CH. Cholesterol.

ChEs. Cholesterol esterase.

Chi. Choline.

ChOx. Cholesterol oxidase.

CNT. Carbon nanotube.

CR. Creatine.

CV. Cyclic voltammetry.

DAG. Directed acyclic graph.

DPV. Differential pulse voltammetry.

FDA. US Food and Drug Administration.

G3P. Glycerol-3-phosphate.

G3PO. Glycerol-3-phosphate oxidase.

GK. Glycerol kinase.

GOx. Glucose oxidase.

H₂O₂. Hydrogen peroxide.

HRP. Horseradish peroxidase.

IP. Iontophoresis.

IRB. Institutional Review Board.

ISF. Interstitial fluid.

LIP. Lipase.

MAE. Mean absolute error.

MIP. Molecularly imprinted polymer.

ML. Machine learning.

MS/MS. Tandem mass spectrometry.

MWCNT. Multi-walled carbon nanotube.

NCEP. National Cholesterol Education Program.

NiHCF. Nickel hexacyanoferrate.

PAM. Polyacrylamide.

PANI. Polyaniline.

PAPBA. Poly(3-aminophenylboronic acid).

PASEF. Parallel accumulation–serial fragmentation.

PB. Prussian blue.

PBS. Phosphate-buffered saline.

PCB. Printed circuit board.

PDA. Polydopamine.

PDMS. Polydimethylsiloxane.

PEDOT:PSS. Poly(3,4-ethylenedioxythiophene):poly(styrenesulfonate).

PI. Polyimide.

PMAA. Polymethacrylic acid.

PmPD. Poly(m-phenylenediamine).

Poly-CORE. Polymeric cofactor-releasing.

PPy. Polypyrrole.

PVB. Polyvinyl butyral.

SBS. Poly(styrene–butadiene–styrene).

SEM. Scanning electron microscopy.

SHERLOCK. Specific high-sensitivity enzymatic reporter unlocking.

SR. Sweat rate.

ssDNA. Single-stranded DNA.

SVM. Support vector machine.

TBW. Total body water.

TG. Triglycerides.

TMB. 3,3',5,5'-Tetramethylbenzidine.

UA. Uric acid.

WE. Working electrode.

INTRODUCTION

Biomarkers are important biophysical and biochemical parameters, detected as an indicator of certain biological, physiological and pathogenic processes or as pharmacological responses to therapeutic interventions^{1,2} (**Fig. 1-1**). The levels of certain biomarkers will fall above or below the norm in individuals who are sick. Advances in medicine related to the ability of precise diagnosis, treatment and prevention of diseases are directly related to the biomarkers. Therefore, the discovery of new biomarkers is very important to predict the onset and course of a disease and to develop effective therapeutic drugs. When identifying a potential biomarker, the lack of standardized protocols for sample collection, storage and analytical procedures makes it difficult to assess and verify the performance of the biomarker candidate in a specific health condition. The research for biomarker discovery usually requires participants to attend frequent hospital visits and tolerate invasive procedures (for example, blood draw or tissue biopsy), which increases the drop-out rate and hinders the study of biomarkers for diagnostics and prognostics of asymptomatic diseases¹ (**Fig. 1-2a**). Because of this, when compared with episodic and invasive laboratory blood or tissue analyses, wearable chemical sensing is becoming an emerging technology for the screening of potential biomarkers in alternative body fluids, such as sweat, saliva, tears and interstitial fluid (ISF), as it can capture rich molecular information non-invasively and in real time³⁻⁶ (**Fig. 1-2a**). Discovering new non-invasively accessible biomarkers could be realized through continuous monitoring of the analytes using suitably designed wearable sensors coupled with data processing (**Fig. 1-2b**).

Classification of biomarkers

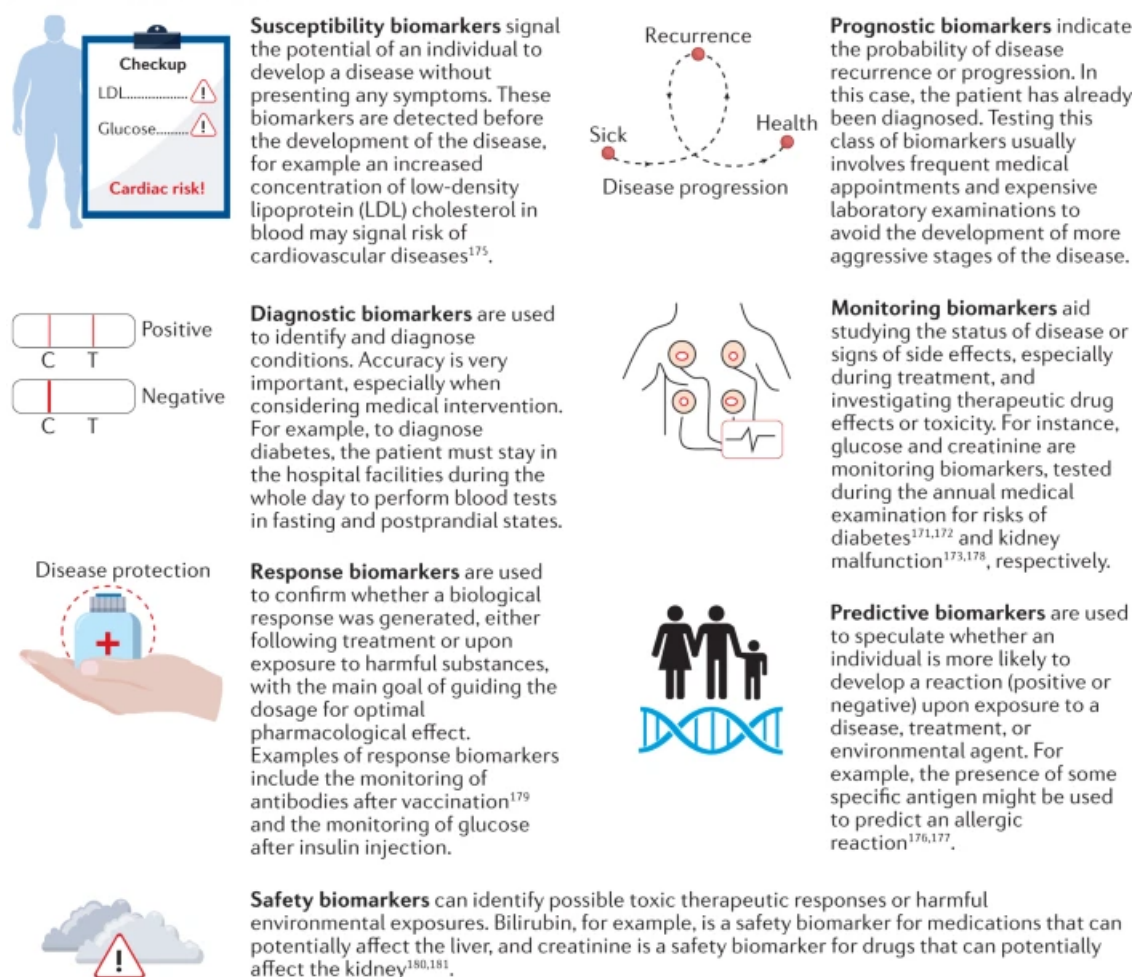


Figure 1-1. FDA categories of biomarkers. According to the definition by the US Food and Drug Administration (FDA)¹⁷⁴, there are seven categories of biomarkers: susceptibility, prognostic, diagnostic, prediction, monitoring, response and safety. Understanding these definitions allows the implementation of standard protocols by matching a biomarker with its appropriate purpose and condition, increasing the efficiency in the development of precise diagnostic and therapeutic approaches. This, in turn, facilitates the introduction of new strategies and tools for novel biomarker discovery.

The emerging Internet of things, a network of connected objects (for example, sensors) that could exchange data over the Internet, promises to revolutionize traditional medical practices in centralized clinical settings. The Internet of things also presents a tremendous opportunity for developing wearable sensor technologies through integrating and miniaturizing analytical

tools into a body-worn platform which may enable remote, wireless personalized health monitoring (**Fig. 1-2c**). Commercially available health monitors (for example, smartwatches) are already capable of autonomously monitoring several physiological parameters, such as heart rate, respiration rate, oxygen saturation, blood pressure and skin temperature. Wearable chemical sensors, such as continuous glucose monitors, convert the levels of various analytes in body fluids into measurable electrical, optical or piezoelectric signals. Recent advances in wearable biosensors have enabled continuous measurement of chemical biomarkers, including dynamic levels of electrolytes, metabolites, nutrients, drugs, hormones and proteins in blood surrogate biofluids^{4, 6-8,10}. This unique ability provides wearable chemical sensors with great value in the era of multi-omics (for example, genomics, proteomics, metabolomics, pharmacogenomics), enabling myriad molecular signatures to be monitored simultaneously and in real time, offering great advantages over conducting multiple single analyte assays¹¹. Such wearable chemical sensors, in combination with information gathered from wearable physical sensors (that monitor biophysical parameters such as temperature, heart rate and motion), modern data processing and prediction algorithms, allow investigation into the roles of the distinct molecular signatures underlying disease prognosis. In this regard, wearable sensors could serve as a powerful tool for discovering novel biomarkers through identifying intricate correlations between analytes and disease status.

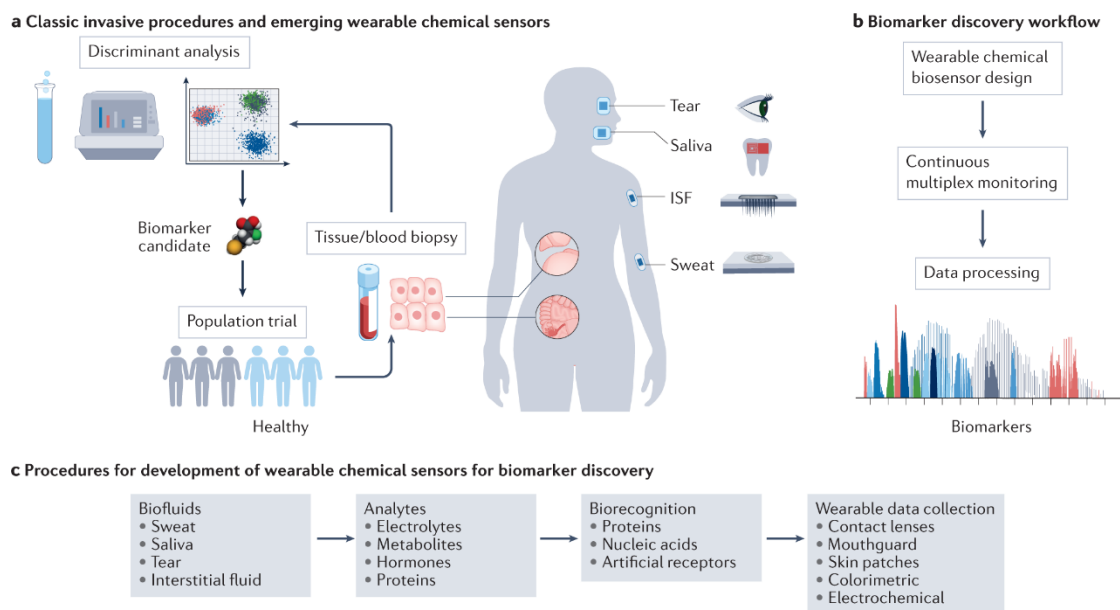


Figure 1-2. Biomarker discovery via wearable chemical sensors. **a**, Biomarker discovery in precision medicine via classic tissue or blood biopsy procedures and via emerging wearable chemical sensors. **b**, General workflow for wearable chemical sensor-enabled biomarker discovery. **c**, Workflow procedures for the development of wearable chemical sensors towards biomarker discovery. ISF, interstitial fluid.

This section provides an overview of wearable chemical sensor research and sheds insight into future directions of wearable sensor-based biomarker discovery. Firstly, the classification and chemical properties of potential biomarker candidates required for sensor design and fluid sampling are introduced. This is followed by a discussion of electrochemical and optical transduction techniques used by existing wearable chemical sensors as well as emerging methods for the continuous monitoring of a broad range of molecular signatures. Finally, a summary of the rich molecular data obtained continuously under normal and abnormal conditions with powerful computational tools for identifying new biomarkers is provided. Together, rapidly emerging wearable chemical sensors, in conjunction with modern data analysis tools, can expand our understanding of biomarkers from the bench to the body.

1.1 Wearable biosensors in biomarker discovery

Despite the significance of the biomarkers in clinical assessment, it is challenging to discover new, specific, single-molecule biomarkers. Deep prior knowledge and understanding is required of a disease's biological pathways and the pharmacology of new medications. However, most diseases are complex with intricate progressions depending on the patient's underlying conditions, lifestyle and nutrition. Glucose, for example, can be used as a diagnostic and monitoring biomarker for diabetes, but can be also related to stress and other pathological conditions¹². Moreover, electrolyte imbalance can be linked to dehydration, hyperkalaemia, kidney disorders and other pathologies¹³. The vast number of metabolites and small molecules linked to different medical conditions demonstrate the important role that these compounds play across processes of the human body^{14,15}. Thus, the specificity of key biomarkers is a challenge, as it is likely that several known and unknown conditions might

be related to any new compound. However, trial and error testing of the concentration of every biomarker for every disease is expensive and time-consuming.

Wearable chemical sensors offer an attractive approach to address these challenges by simultaneously monitoring a broad spectrum of molecular signatures towards multi-omics analysis⁷. The unique ability to perform continuous monitoring and identify temporal patterns can be a great asset in the discovery of biomarkers that vary rapidly within a short period of time. Examples of such important applications could include the use of wearable biosensors to monitor cardiac, epilepsy or other acute diseases, towards understanding the physiological changes in the body prior to such events. Currently, there are still no devices or tests able to predict life-threatening events such as heart attack, epilepsy episodes or heat stroke. Moreover, numerous health conditions, such as Alzheimer disease or long COVID-19, do not yet have standard alarming biomarkers. Continuous and real-time monitoring of multiple biomolecules during an individual's daily activities could provide invaluable information about the chemical signature of the abnormal health conditions. Such an ability offers the fast screening of molecular signatures, and upon coupling to data-mining tools leads to a more effective biomarker discovery process which cannot be achieved with any other analytical tool.

To be suitable for wearable-based biomarker discovery, the molecule or molecular fragment candidates should be present in alternative biofluids such as sweat, saliva and ISF. Considering that they commonly originate from either blood or secretion glands, the biomarker candidates should be stable, preferably charged species, have small molecular weight for easy partition from blood and have potential links with their blood counterpart or targeted health condition⁸. These commonly include various metabolites, electrolytes, nutrients, hormones and therapeutic drugs^{7,8,16-25} (**Table 1-1**). The stability of these molecules could be impacted directly or indirectly by various parameters including the testing temperature and the pH and reactive oxygen species levels in the body fluids in situ. These parameters could vary with the activities and pathophysiological conditions. This can cause a shift in the chemical properties of the biomarker such as ionization states or surface

charges, which may eventually impact the performance of wearable sensors. It should also be noted that most of the analytes in non-invasively accessible biofluids are not clinically approved biomarkers, although many of their counterparts in the blood are currently considered biomarkers. For example, sweat or saliva glucose are not used in a clinical context, whereas blood glucose is the well-recognized biomarker for diabetes.

Target		Reported range				Molecular weight (Da)	Health association examples	Refs.
		Blood (μM)	ISF (μM)	Sweat (μM)	Saliva (μM)			
Metabolites	Glucose	3.9×10^3 – 5.5×10^3	0.8×10^3 – 6×10^3	6–300	30–140	180	Diabetes, metabolic syndrome	3,4,5,6,7,8,9,10,12,15,22,24,34,38,39,40,41,49,50,86,102,103,104,105,109,111,116,124,140,144,171,172
	Lactate	0.5×10^3 – 10×10^3	2.5×10^3 – 6.6×10^3	3.7×10^3 – 50×10^3	$<3.5 \times 10^3$	90	Lactic acidosis	3,4,5,6,7,8,9,10,15,28,29,33,116,124,131,134,140
	Uric acid	100–400		4–100	100–250	168	Gout, cardiovascular diseases	43,123
	Creatinine	65–140		9–18	7–17	113	Kidney diseases	22,60,162,173
Electrolytes	Na^+	135×10^3 – 145×10^3		10×10^3 – 100×10^3	27×10^3 – 217×10^3	23	Hydration, heart failure, liver disease	3,4,5,6,7,8,9,10,18,19,20,33,42
	Cl^-	96×10^3 – 106×10^3		10×10^3 – 100×10^3	7×10^3 – 18×10^3	35	Cystic fibrosis	3,4,5,6,7,8,9,10,18,19,20,33,42,106,107
Hormones	Cortisol	0.7×10^{-4} – 690×10^{-3}	Similar to blood plasma	1×10^{-4} – 20×10^{-3}	1×10^{-4} – 20×10^{-3}	362	Stress, anxiety, Cushing syndrome	16,17,18,27
	Testosterone	52×10^{-5} – 24×10^{-4} (females) 97×10^{-4} – 382×10^{-4} (males)		Lack of report	19×10^{-6} – 35×10^{-6} (females) 263×10^{-6} – 544×10^{-6} (males)	288	Heart muscle damage, fertility issues, irregular menstrual periods	7,8
Nutrients	Tyrosine	200–400	Similar to blood plasma	66–300	0.07–205	181	Metabolic disorders, gout	7,43

Target		Reported range				Molecular weight (Da)	Health association examples	Refs.
		Blood (μM)	ISF (μM)	Sweat (μM)	Saliva (μM)			
	Tryptophan	55–80		20–91	Not reported	204	Anxiety, nerve damage	7,22
	Vitamin C	2.8–98		<36	45–62	176	Connective tissue defects, joint pain	6,7,9,10,44
Proteins	Cytokines	Picomolar to nanomolar	15-42% of blood plasma	1.97×10^{-5} (IL-1α) (mean)	2.8×10^{-7} – 2.2×10^{-6}	>5,000–10,000	Immunodeficiency disorders, cytokine storm	8,21,158
	C-reactive protein	7×10^{-3} – 29×10^{-3}	Lack of report	4.2×10^{-6} – 2.5×10^{-4}	3.3×10^{-6} – 1.8×10^{-4}	120,000	Coronary heart disease, obstructive pulmonary disease	121
Drugs	Levodopa	0.5–15	Similar to blood plasma	Similar to plasma		197	Parkinson’s disease	23
	Acetaminophen	66–132		<50		151	Fever, pain	

Of all the criteria in biomarker discovery, the most critical is to confirm that the target molecule is present in the biofluid. Most analytes detected in alternative biofluids are small molecules as they can partition easily through tissue cells, whereas large macromolecules such as proteins may not be readily partitioned to these biofluids^{22,26}. Once the presence of the molecule in the fluid is confirmed, the molecule needs sufficient stability to be detected. Although detecting radicals and unstable complexes is possible, it is not very accurate because of the short half-life of the analyte. Moreover, the molecule may react chemically with other compounds (for example, oxygen) in the body fluids, resulting in misleading lower concentrations^{5,8,10}.

Assuming that a target is stable in the biofluid, its levels need to be correlated with its blood concentrations. For sweat, saliva and tears sensors, the molecule can either be partitioned from blood, where the correlation can usually be achieved successfully, or be excreted by their respective glands^{8,26} where the overall correlation with blood will be affected by the mixed molecules secreted from the glands. Even if a molecule's secretion to these biofluids

is primarily based on diffusion, there is still a time lag to be considered. For example, although blood glucose peaks within 10–15 min upon food ingestion, a wearable sensor can only detect the peak in an alternative biofluid after 15–20 min. Dilution effects are another major factor that needs to be considered as biofluid secretion rates could influence the final secreted molecular concentrations. Potentially, internal calibration methodologies²⁷ can be introduced to obtain a normalized and accurate response.

To facilitate biomarker discovery, a wearable chemical sensor can be developed to analyze the selected molecular signature continuously in real time (**Fig. 1-2c**). To prove the effectiveness of a new chemical sensor, the biofluid is analyzed simultaneously in situ and via external validation using benchtop laboratory analyses, to ensure similar trends are observed. The sensor operation range must cover the physiologically relevant concentrations, which usually requires a very low detection limit and high sensitivity along with a wide linear range. For wearable use, the desired sensor must be fully biocompatible and selective to the target molecule in the complex biofluid matrix without additional sample processing steps. This high selectivity is realized by using immobilized recognition receptors such as enzymes, aptamers or molecularly imprinted polymers (MIPs) that selectively bind to the target molecules. By following these criteria, and with the introduction of novel detection methodologies, it would be expected that a wearable sensor may be able to perform multi-omic analysis using non-invasive biofluids. Wearable sensors that can detect DNA and RNA molecules, fragments, hormones, proteins and whole small organisms, including viruses, will be of utmost importance for public health monitoring and surveillance.

Efficient biomarker discovery requires robust molecular collection in participants. The main challenges of wearable biosensors for collecting reliable data include the noise signal imposed by human motion, influences of the pH, temperature, conductivity and refreshing of the body fluids on sensing responses. Suitable filtering and calibration mechanisms are required, alongside selection of the platform/system for fluid extraction and sampling (**Fig. 1-2c**). To enable biomarker validation, longitudinal and cross-sectional studies need to be performed to collect large sets of data; modern data-mining techniques (such as machine

learning) can facilitate biomarker identification and generate predictive algorithms for disease monitoring, diagnosis, treatment and prevention.

1.2 Wearable chemical sensing strategies

Wearable sensors have attracted tremendous attention over the past decade^{6, 28-31} as they are able to perform real-time detection in situ directly on the body in a reagent-free and continuous manner. Protocols requiring additional washing or chemical addition steps are generally not suitable for on-body operation of wearable chemical sensors. Currently, wearable sensors are primarily electrochemical sensors^{21, 32, 33} or optical sensors³⁴⁻³⁷ based on signal transduction mechanisms.

1.2.1 Electrochemical biosensing strategies

Electrochemical sensors convert target analyte information into a measurable electrical signal in real time with high sensitivity. For this, bioreceptor (such as enzyme, antibody, DNA and aptamer) molecules are usually immobilized on the surface of the conductive electrode material to selectively interact with the target molecules. Amperometry, potentiometry and voltammetry are the most common electrochemical detection techniques^{32, 33}.

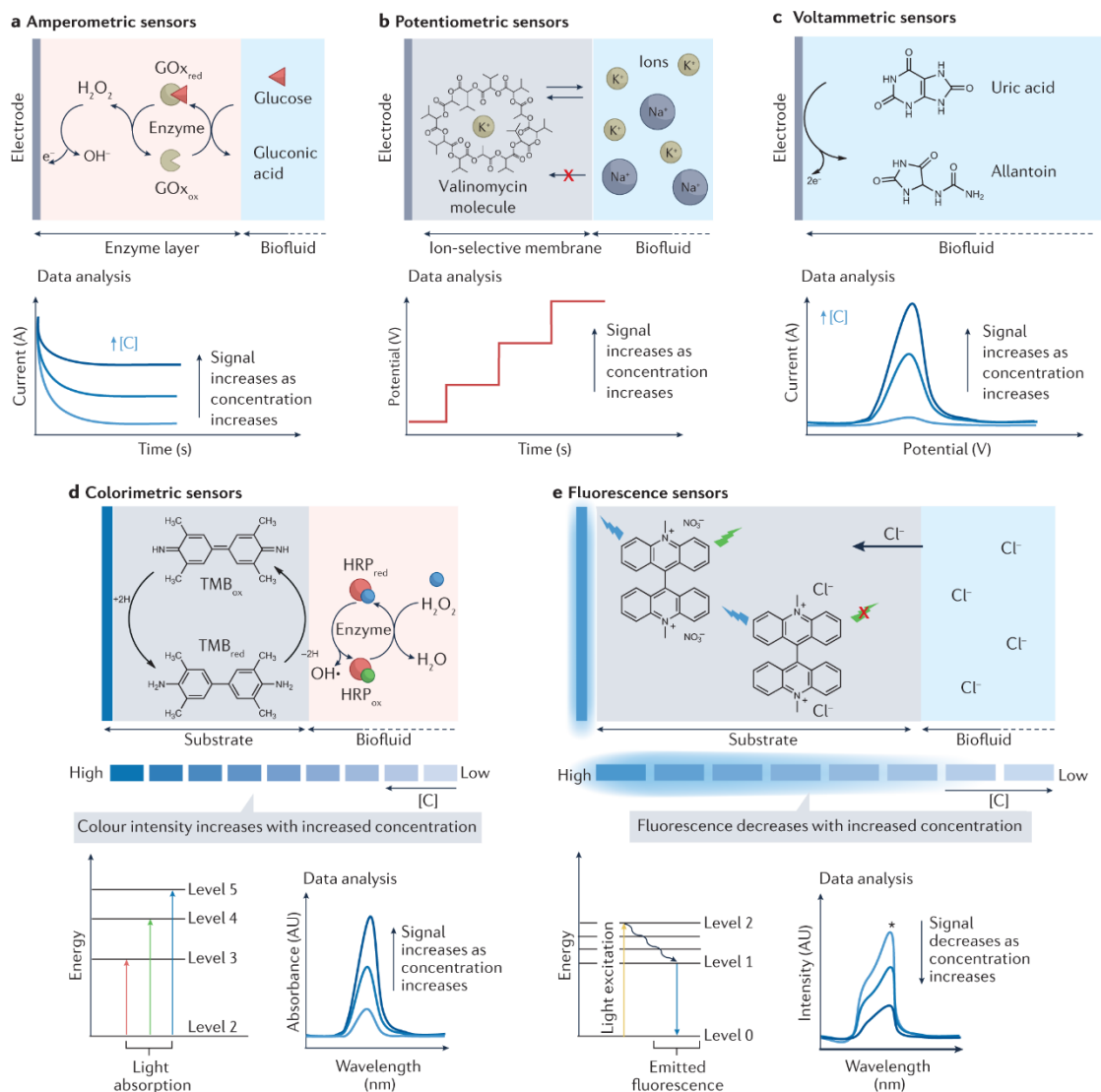


Figure 1-3. Wearable electrochemical and optical biosensing strategies. a–c, Recognition and signal transduction for wearable electrochemical biosensors. **a,** Amperometric enzymatic electrodes: for glucose sensing, the working electrode is modified with glucose oxidase (GOx) and the glucose molecule is oxidized to gluconic acid. Glucose is detected by applying a fixed potential to measure the hydrogen peroxide (H₂O₂) by-product or to monitor the reaction of a mediator with low redox potential. The generated current signal increases with the increase of glucose concentration. **b,** Potentiometric ion-selective sensors: the working electrode is modified with an ion-selective membrane containing the ionophore (for example, valinomycin for potassium

ion sensing) molecules with cavities allowing only ions with specific charge and size to interact. The measured potential has a log-linear relationship with the target ion concentration. **c**, Voltammetric sensors: an electroactive molecule (for example, uric acid) can directly lose or donate electrons on the electrode surface when a redox potential is applied to the sensing electrode. The measured oxidation or reduction peak current height is directly proportional to the concentration of the target analyte. **d,e**, Recognition and transduction for wearable optical biosensors. **d**, Colorimetric sensors: the color indicator molecule is immobilized on a substrate and upon contact with the analyte it changes colour. For hydrogen sensing based on horseradish peroxidase (HRP), the color change is promoted by changes in the redox state of the chromophore molecule 3,3',5,5'-tetramethylbenzidine (TMB) during the enzymatic degradation of H_2O_2 ; the measured light absorbance or color intensity directly correlates with the concentration of target analyte. **e**, Fluorescence sensors: fluorometric sensing relies on a high-energy light source (for example, ultraviolet light or blue light) to excite the fluorophore molecule so it emits light with a longer wavelength through radiative electron transitions. For example, lucigenin can be used as a fluorescent indicator for chloride ion sensing; the chloride ion (Cl^-) reacts with lucigenin by replacing NO_3^- , resulting in the quench of lucigenin fluorescence intensity through a collisional mechanism. The change of fluorescence intensity is directly proportional to the change in analyte concentration. **red**, reduced analyte; **ox**, oxidized analyte; **[C]**, analyte concentration.

In the amperometric sensors, an applied potential facilitates the redox reaction of the electroactive molecule, and electron transfer between the molecule and the electrode is measured as the current signal. Enzymes are typically used in wearable amperometric sensors as the biocatalytic reaction can confer great selectivity and reversibility to the sensor system towards continuous analysis of target molecules (for example, glucose and lactate) in biofluids^{38, 39}. In **Fig. 1-3a**, the amperometric enzymatic detection mechanism is exemplified for the glucose sensor whose sensing electrode is modified with an enzyme (that is, glucose oxidase (GOx))-loaded biorecognition layer. Glucose molecules from the biofluid are oxidized to gluconic acid, catalysed by GOx. The detection of glucose is achieved by

amperometric techniques. This is done by applying a fixed potential to detect the enzymatically generated hydrogen peroxide (H_2O_2) by-product (first-generation glucose biosensor) (**Fig. 1-3a**), to monitor the reaction of a mediator with low redox potential (second-generation glucose biosensor), or to monitor the direct electron transfer from the enzymatic reaction without any intermediate stage (third-generation glucose biosensor)^{38, 39}. The measured response in current is proportional to the concentration of the target substrate. Given the ability of performing continuous measurements with a fast response, amperometric enzymatic sensors have been successfully applied in commercial continuous glucose monitors^{40, 41}.

In contrast to amperometric sensors, there is no applied potential or current flow involved in potentiometric sensors. Instead, these sensors rely on the potential difference between a reference electrode and a working electrode. Solid-state ion-selective electrodes are typically employed in wearable biosensors to monitor the levels of electrolytes or ions (for example, Na^+ , K^+ and Ca^{2+}) in biofluids⁴² (**Fig. 1-3b**). The working electrode, or ion-selective electrode, is usually modified with an ion-selective recognition membrane composed of ionophore molecules that form cavities in the polymer film, allowing only ions with specific charge and size to interact. This selective recognition process creates an electrochemical phase boundary potential that is translated by the transducer into the voltage signal⁴². The potential signal measured between the ion-selective electrode and a solid-state reference electrode (for example, Ag/AgCl) has a log-linear relationship with the target ion concentration according to the Nernst equation³.

Another versatile electrochemical detection approach is voltammetric detection where the applied potential varies with controlled steps and speed, whereas the alternations in the resultant current signal are monitored. There are various approaches to sweep the potential including cyclic voltammetry, differential pulse voltammetry and square wave voltammetry. Voltammetric detection is often applied to monitor electroactive molecules (for example, uric acid and vitamin C) by detecting their direct oxidation/reduction reaction around a specific redox potential on the electrode surface under an applied potential waveform^{43, 44}.

(Fig. 1-3c). Compared with amperometry or cyclic voltammetry, differential pulse voltammetry and square wave voltammetry are able to minimize the background charging current and achieve highly sensitive electrochemical measurements. Similarly, stripping voltammetry, involving an analyte preconcentration step followed by a voltammetric scan, is able to detect ultra-low levels of heavy metals in body fluids⁴⁵⁻⁴⁷. In the absence of specific bioreceptors (such as enzymes), the selectivity of voltammetric electroactive molecule detection is limited as different molecules may have a similar redox potential.

In addition to these electrochemical signal transduction techniques, numerous other approaches involving the measurement of resistive, capacitive and impedance changes of the sensing electrode upon selective analyte biorecognition event could also be used for wearable electrochemical biosensing⁴⁸. It should also be noted that several key challenges for wearable electrochemical sensors have yet to be fully addressed, including electrode biofouling, electrical noise, electronic backbone integration and power supply.

1.2.2 Optical biosensing strategies

Optical sensor systems rely on chemical or biological reactions capable of modifying optical detection elements, including changes in light absorbance (for example, colour) or emission (for example, fluorescence or luminescence)³⁴⁻³⁶.

In wearable colorimetric sensors, chromophore molecules are usually used as colour indicators. External stimuli can change the electron state of the chromophore molecule, resulting in the absorption of photons with different wavelengths (visualized as colour changes). Common examples of such stimuli for wearable colorimetric biosensors include gain or loss of electrons (electrochromism) or ions (ionochromism), or changes in pH (halochromism). A common colorimetric mechanism coupled with enzymatic reaction as the target biorecognition event is illustrated in **Fig. 1-3d**. The colour change of the chromophore 3,3',5,5'-tetramethylbenzidine (TMB) is promoted by the reaction of the chromophore molecule with H₂O₂; the measured colour intensity directly correlates to the concentration of target analyte

In wearable fluorescence sensors, fluorophores are commonly used for signal transduction. Under specific light excitation, the fluorophore molecules can absorb the light energy and re-emit at a longer wavelength. An ionochromism process that alters the fluorescent light signal intensity is demonstrated in **Fig. 1-3e**. The main difference between a colorimetric and a fluorescence sensor is the light source necessary to observe the phenomenon. Qualitative analysis of colorimetric sensors can be performed by the naked eye or directly using a phone camera, whereas additional optical accessories are often needed to accurately acquire fluorescence or luminescence outputs. As a result, fluorescence sensors are usually more sensitive than colorimetric sensors.

The main advantages of these optical biosensors are the simple fabrication process and system integration on the skin as no additional electronic components are needed on the body. However, high sensitivity, high long-term stability and high-frequency data collection are difficult to achieve with a miniaturized external wearable system (for example, cell phone or hand-held optical reader)⁴⁹⁻⁵¹.

1.3 Emerging wearable sensing strategies

Despite the great promise for the existing wearable sensing strategies, enzymatic reactions, ionophore–ion interactions and direct molecular oxidation/reduction can only be used to directly monitor a limited number of targets^{4, 5, 8}. The majority of body fluid analytes, such as circulating metabolites, nutrients, hormones and proteins, play important roles in clinical risk assessment and diagnosis (**Table 1**) but cannot be monitored using these wearable sensing approaches. For example, the concentrations of melatonin, prolactin and reproductive and growth hormones could be used for the prediction of seizures⁵², and troponin I and brain natriuretic peptides are closely related to the severity of heart failure⁵³. Selective detection of analytes in biofluids usually requires bioaffinity receptors, such as antibodies, DNAs/RNAs, aptamers and MIPs. However, existing bioaffinity sensors are often one-time use, and require multiple incubations and washing processes for regenerating their receptor, making it challenging to apply such sensors towards continuous biosensing in situ^{48, 54}.

In this direction, a very promising approach for affinity sensors, towards detection of several classes of molecules, is the so-called ‘switch biosensor’⁵⁵. Switch sensors mimic natural bioprocesses using artificial or modified biomolecules to undergo conformational changes upon binding the target molecules. Commonly used biomolecular switches include proteins⁵⁶, oligonucleotides^{57,58} and synthetic receptors (MIPs)⁵⁹⁻⁶¹ (**Fig. 1-4a**). The structural changes are based on a specific interaction such as hydrogen bonding, van der Waals force and hydrophilicity between the target and bioreporter, conferring selectivity and reversibility to the system. To achieve in situ reagent-free molecular detection, biomolecules can be coupled with either optical tags (for example, fluorophores or quantum dots) or electrochemical tags (for example, redox or enzyme molecules) for signal transduction⁶² (**Fig. 1-4b**).

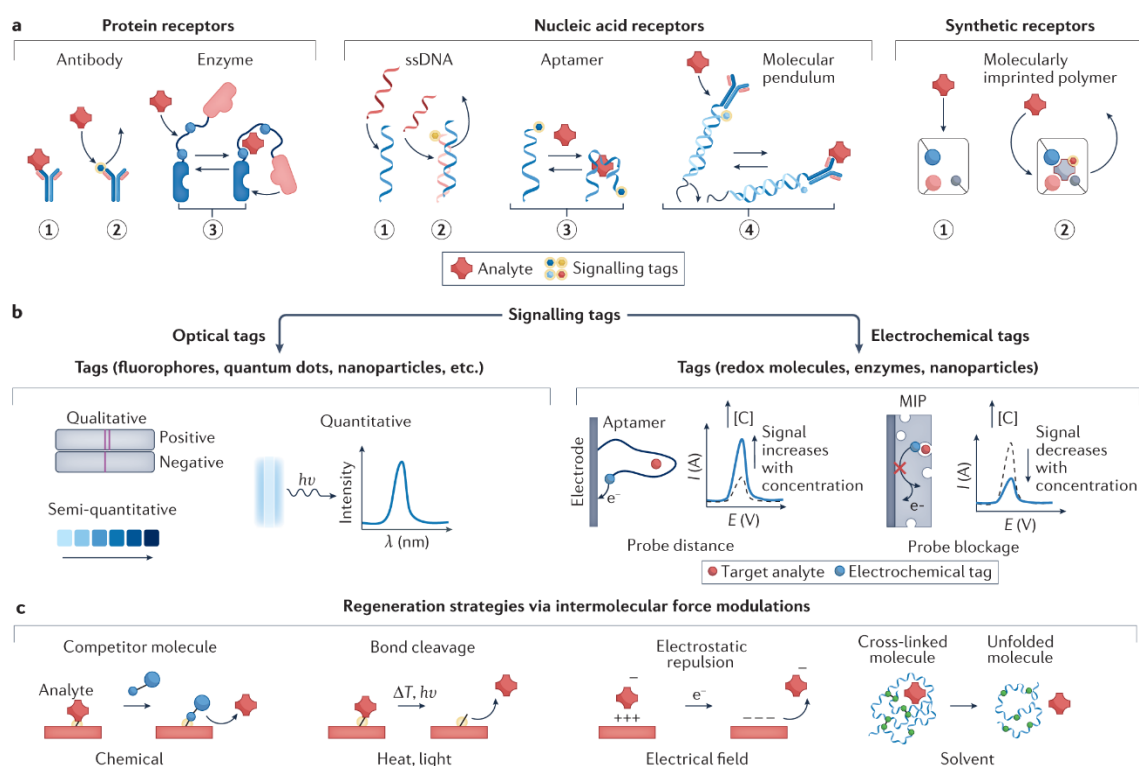


Figure 1-4. Bioaffinity-based molecular switches and in situ sensor regeneration. a, Target recognition based on bioaffinity receptors and molecular switches for wearable biosensing. Protein receptors include antibodies for target recognition through direct binding (panel 1) or displacement of a signalling probe (panel 2) and enzyme switches that

change molecular configuration upon target binding (panel 3). Nucleic acid receptors include single-stranded DNA (ssDNA) used for target recognition through direct DNA hybridization (panel 1) or displacement of another DNA strand with a signal probe (panel 2), aptamer switch with shape change upon binding of the target molecule (panel 3) or a DNA-based molecular pendulum (coupled with a redox probe and a protein receptor) that changes the probe–electrode distance upon target binding (panel 4). Synthetic receptors such as molecularly imprinted polymers (MIPs) can perform target recognition through direct target binding (panel 1) or displacement of molecules with a signal probe (panel 2). **b**, Optical and electrochemical signalling tags used in bioaffinity sensors for in situ signal transduction. Optical tags such as fluorophores, quantum dots and nanoparticles can be used to transduce the target recognition to measurable optical signals. The resulting optical signal could offer qualitative analysis (yes/no response), semi-quantitative sensors based on colour change and quantitative analysis via absorbance or luminescence intensities. Electrochemical tags such as redox probe molecules, enzymes and nanoparticles are used to transduce the target recognition to measurable electrical signals; for example, the recognition of targets with aptamers decreases the distance of the tagged redox probe from the electrode, leading to increased redox signal; binding of target molecules with MIP sensors reduces the exposure of the redox probe to the biofluid, leading to a decreased redox signal. **c**, In situ sensor regeneration strategies based on intermolecular force modulations. Biosensors can be regenerated chemically, by introducing a competitor molecule that has a stronger binding affinity to the receptor than the target molecule; with heat and light, as external energy to cleave the bond between the receptor and target molecule; by electrical fields, which remove the target molecule by electrostatic repulsion; or by solvent effects, varying solution properties (for example, pH and ionic strength). λ , wavelength; E , potential; I , current; $[C]$, analyte concentration.

Aptamers are molecules exhibiting target-induced conformational changes, and are highly attractive structures when analysing the presence of analytes by distance modulation between the electrode and the signalling redox indicator (for example, methylene blue)⁶³⁻⁷⁰ (**Fig. 1-4b**). A major advantage of aptamers is the ability to engineer their desired nucleic-base

sequences specifically for the target analytes through an aptamer designing process called systematic evolution of ligands by exponential enrichment⁷¹. Electrochemical aptamer-based sensors have been exemplified in the continuous detection of multiple chemotherapeutic drugs, including doxorubicin, kanamycin and tobramycin in human whole blood and in live rats⁷². Binding of the target small molecules with the aptamer receptor triggered reversible conformational changes, resulting in an increased redox current correlating with the target concentration (**Fig. 1-4b**). Similarly, fluorometric switches are also commonly used in aptamer or protein biosensors in which the aptamer conformation modulates the quenching of the probe's fluorescence signal^{73,74}; the measured decrease in fluorescence intensity is proportional to the increased concentration.

In addition to aptamers, electrochemical DNA switches coupled with antibodies have recently been demonstrated for reagentless monitoring of proteins (for example, troponin I) in multiple biofluids (that is, saliva, sweat, tears and blood) based on the motion of an inverted molecular pendulum⁷⁵. For the detection, a positive potential is applied to electrostatically attract the redox reporter-tagged DNA molecule to the surface, to decrease the probe-electrode distance; the binding of the target molecule increases the weight on the pendulum body, leading to a signal change that is proportional to the analyte concentration. In another example, wearable sensing of nucleic acids, such as SARS-CoV-2 guide RNA, was achieved in a breath sample via freeze-dried reactions in the face mask including lysis, reverse transcription-recombinase polymerase amplification and CRISPR-Cas12a-specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) sensing⁷⁶. The presence of pieces of SARS-CoV-2-derived amplified RNAs, amplicons, leads to the cleavage of quenched single-stranded DNA (ssDNA) fluorophore probes which can be detected via a colorimetric lateral flow assay strip.

Although these emerging bioaffinity sensing technologies hold great promise in the detection of a broad spectrum of analytes in biofluids, there are many challenges related to stability and reversibility that need further attention. In particular, continuous detection is strongly dependent on the ability to regenerate the active sensor surface and realize reversible target

bonding in situ without extra washing steps or chemical addition. Such sensor regeneration could be achieved by modulating the intermolecular forces between the bioreceptor and the target molecule (for example, hydrogen bond, van der Waals force and electrostatic interaction) through various stimuli such as heat, light, electrical field, ultrasound field, competitive binding and ionic strength or pH⁷⁷⁻⁷⁹ (**Fig. 1-4c**).

1.4 Materials and wearable system design

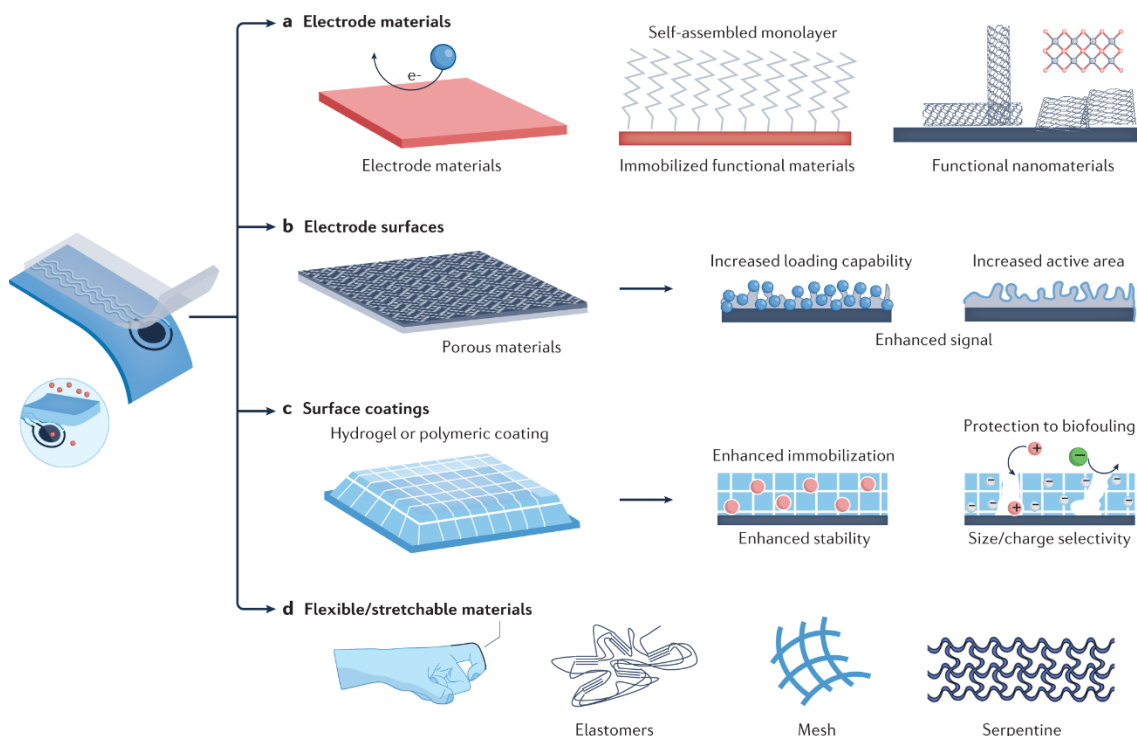


Figure 1-5. Functional materials for wearable chemical sensors. A sensor immobilized with functional materials could result in enhanced wearable sensing performance (left). **a**, Conducting and functional electrode materials for wearable chemical sensing. Gold transducer surfaces are suited for modification with self-assembled monolayers (for example, alkanethiol) for anchoring the biorecognition elements to introduce selectivity. Due to the excellent electrochemical and optical properties, functional nanomaterials (for example, carbon nanotubes, graphene, MXenes, metal–organic framework) are also used for highly sensitive transducer surfaces. **b**, Porous materials with high electrochemically active areas and high bioreceptor loading abilities towards enhanced in situ chemical

sensing. **c**, Surface coatings (for example, hydrogel or polymeric coating) towards enhanced in situ chemical sensing. The properties of these coatings (for example, surface charge, hydrophilicity or porosity) can be tailored to increase receptor loading capabilities and resist the adhesion of fouling/interferent molecules. **d**, Soft flexible and biocompatible materials for enhanced wearable sensing. Flexible and stretchable materials can be used to realize enhanced skin conformability. The flexibility and stretchability of the sensors can be realized by using intrinsically stretchable materials (for example, elastomers) or through structure designs (for example, mesh or serpentine configuration designs).

Flexible materials and miniaturized devices play an important role in developing wearable chemical sensors for enhanced, reliable and in situ molecular data collection. There are considerations both in materials and at the system level to effectively develop wearable sensors in biomarker discovery.

1.4.1 Material considerations

Electrode materials, the electrode surface and surface coatings, and skin conformability are key considerations for optimizing the sensitivity, stability and longevity of wearable sensors.

1.4.1.1 Electrode materials

Common wearable electrochemical transducers rely on conducting carbon materials or gold surfaces owing to their high electrochemical stability, electrical conductivity^{80,81} and ability to anchor biorecognition elements efficiently using materials such as self-assembled monolayers⁸². Owing to their excellent electrochemical and optical properties, functional nanomaterials (for example, carbon nanotubes, graphene, MXenes, metal–organic framework) are also potential candidates for developing highly sensitive transducer surfaces⁸³ (**Fig. 1-5a**). Moreover, carbon-based nanomaterials show great promise for wearable sensing electrodes as they can be mass produced at a low cost and have a wide operational potential window.

1.4.1.2 Surface coatings

Prolonged on-body monitoring of different biofluids is often hindered by gradual surface passivation in protein-rich biofluids. Anti-biofouling protective hydrogels or polymeric coatings, such as chitosan⁸⁸, polyvinyl chlorides⁸⁹, Nafion⁹⁰ or serum albumin⁹¹, are widely used for extending the operational stability towards continuous reliable molecular profiling (**Fig. 1-5c**). The use of these surface-coating materials can tailor the surface charge, surface hydrophilicity or porosity to resist the adhesion of fouling/interferent molecules onto the electrode. For example, Nafion is a negatively charged polymer used as an anti-fouling coating against negatively charged proteins whereas some hydrogels consist of porous networks that allow only analytes with smaller sizes to reach the electrode surface.

1.4.1.3 Skin conformability

Conformal contact between wearable chemical sensors and the human body is realized by matching the mechanical properties of the device with those of the human tissues, through use of skin-friendly, soft and stretchable elastomers such as polyimide, polydimethylsiloxane (PDMS) or poly(styrene-butadiene-styrene) (SBS)⁹² (**Fig. 1-5d**). In addition, carbon nanomaterials, metal-carbon nanocomposites or metallic nanomaterials are commonly employed for developing stress-enduring carbon inks along with silver-based interconnect circuitry. Compared with highly elastic substrate materials, the conducting materials usually have low stretchability. A trade-off between the conductivity and stretchability is achieved by adopting an optimal ratio of conductive and elastomeric materials and design-based techniques (for example, mesh-like or serpentine-like structures), resulting in an attractive sensor performance along with comfort^{81,92} (**Fig. 1-5d**).

It should be noted that, in addition to the above factors, biocompatibility of the transducer and substrate materials is always crucial for wearable chemical sensors; biocompatible polymers, carbon materials and noble metals are preferred for meeting this requirement⁹³⁻⁹⁶.

1.4.2 System-level considerations

To ensure high performance for on-body analyte monitoring, the integrated wearable system must be carefully designed to allow efficient fluid sampling, reliable in situ sensing, wireless data collection and communication, and can be powered sustainably.

1.4.2.1 Fluid extraction and sampling

During blood circulation, the transcapillary filtration of the blood forms ISF, which has similar composition and levels of biomarkers to blood^{97,98}. For efficient ISF profiling of disease-related biomarkers, microneedle-based electrochemical sensing systems have received considerable attention owing to their painless, minimally invasive, transdermal access^{97,99-101} (**Fig. 1-6a**). ISF can also be accessed non-invasively using reverse iontophoresis, a technique that facilitates the transport of charged molecules by applying an electric current. This approach allows the extraction of the ions and neutral molecules from the ISF to the skin surface¹⁰²⁻¹⁰⁵ (**Fig. 1-6b**). Potential challenges here are interpersonal variations due to differences in the skin properties. Direct iontophoresis can also initiate localized sweating without the need for heat or vigorous exercise, particularly in connection with transdermal delivery of muscarinic agonists (for example, pilocarpine, acetylcholine, carbachol) to induce localized sweating¹⁰⁶⁻¹¹¹. The sweat-stimulating electrodes (cathode and anode) are thus interfaced with the skin by a separating thin layer of the drug-loaded hydrogel (**Fig. 1-6c**). In addition, the microfluidics-based sample handling modules can be used for in situ intermittent storage and controlled handling of the secreted sweat without dilution, mixing or cross-contamination^{110,112-115} (**Fig. 1-6c**).

1.4.2.2 Wearable sensor platforms

Various skin-worn wearable sensors have been developed over the past decade for detecting and assessing the sweat-based biomarkers non-invasively, including epidermal tattoos⁹⁸, patches¹¹⁶, flexible smart bands^{3,117} or smart textiles⁸⁶ (**Fig. 1-6d**). Microfluidic wearable sensors can be developed to achieve fully autonomous sweat extraction, sampling and multiplexed sensing⁶¹. Such microfluidic wearables could minimize sweat evaporation to

enable rapid sweat analysis. Tears and saliva are other important biofluids that contain a plethora of biomarkers of clinical importance¹¹⁸⁻¹²¹. Wearable biosensors based on contact lenses⁴⁹, dental chips¹²² and mouthguard-based¹²³ devices have thus been recently developed but need more evaluation in participants. Wearable microneedle arrays show considerable promise for realizing continuous monitoring of multiple biomarkers beyond glucose in ISF¹²⁴. Owing to the close analyte correlation between blood and ISF, minimally invasive wearable ISF monitors are expected to provide the most accurate metabolic profiling compared with other wearables.

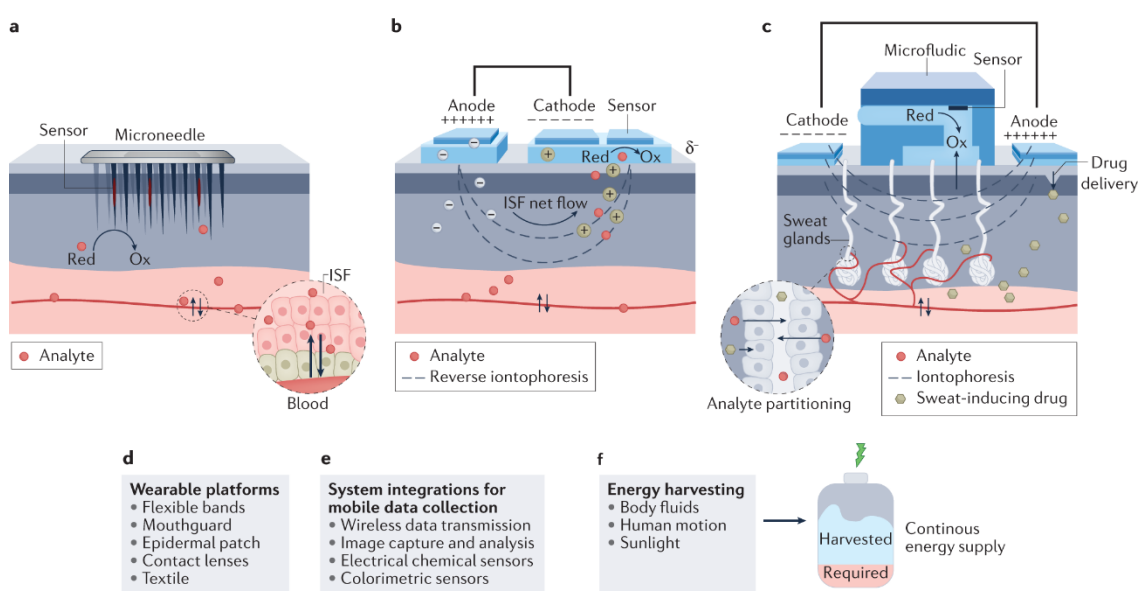


Figure 1-6. Wearable platforms for biofluid extraction, sampling, and data collection.

a, Minimally invasive microneedle-based interstitial fluid (ISF) extraction and chemical sensing. **b**, Non-invasive reverse iontophoresis-based ISF extraction and chemical sensing via a wearable device. **c**, Non-invasive iontophoresis-based sweat extraction, microfluidic sweat sampling and chemical sensing via iontophoresis using a wearable device. The sweat-stimulating electrodes are interfaced with the skin via a thin layer of the hydrogel loaded with agonists (for example, pilocarpine or carbachol). A small current is applied to transdermally deliver agonist molecules that stimulate the sweat gland to trigger local sweating. The microfluidics-based sample handling modules can be used for in situ intermittent storage and controlled handling of the secreted sweat. **d**, General wearable

platforms. **e**, Wireless wearable system integration for mobile data collection. **f**, Wearable energy harvesting systems. These systems convert chemical energy in body fluids, mechanical energy from human motion and light into electricity to continuously power wearable sensors. **Red**, reduced analyte; **Ox**, oxidized analyte.

1.4.2.3 Wireless and continuous data collection

Wearable devices continuously collect biomarker profiles from different biofluids of the wearer, accompanied by real-time wireless signal transmission to mobile devices (**Fig. 1-6e**). Pre-calibrated wearable sensor arrays may accurately profile the level of multiple chemical biomarkers from different biofluids to create specific signature patterns. Similarly, in colorimetric-based optical devices, the signal can be directly seen in the chromogenic bands and these colour changes can be quantified for the patches using a pre-calibrated app-based data processor for finding the biomarker levels⁸⁸ (**Fig. 1-6e**). Combining rich biomarker temporal profiles based on wearable electrochemical and optical sensing, and relating these profiles to abnormal body conditions, would serve to pinpoint the symptom-specific physicochemical fluctuation of the individual towards creating biomarker signatures.

1.4.2.4 Power

Complex wearable biosensing systems require suitable power sources to reach their full ability. Existing systems are commonly powered by batteries or through near-field communication but suffer from limited long-term continuous usability. Self-powered wearables, harvesting energy from body fluids, human motion or sunlight, are attractive candidates for next-generation wearable biosensors for biomarker discovery¹²⁵⁻¹³⁰.

1.5 Data-driven biomarker discovery

The emergent multiplexed wearable chemical sensors have broadened the spectrum of biomarkers available to diagnose, monitor and treat diseases. The continuous monitoring of multiple analytes via wearable biosensors has resulted in the collection of large sets of molecular data able to facilitate the assessment of a patient's health status^{131, 132}. Alongside developing novel approaches to enhance the sensors' sensitivity, selectivity and stability and

creating smart bioelectronics for in situ measurements, systematic analysis of the chemical data provides insightful information and broader data sets of biomarkers related to a specific health condition⁴³. In this regard, implementing modern data analysis tools such as machine learning algorithms to interpret and predict patterns in vast amounts of measurements can aid in the identification of robust and accurate biomarkers¹³³.

1.5.1 Multiplexed multimodal wearable sensing

Physiological monitoring of a single chemical analyte has been proven ineffective for disease diagnostics as, in most cases, they are affected by a combination of other chemical and physical markers¹³⁴. Although there are several models proposed for realizing multi-omics analysis¹³⁵⁻¹³⁷, they usually rely on molecular data from different techniques collected at different times, which increases the errors and challenges the implementation of a precise health condition prediction/correlation. Advances in the miniaturization of wearable bioelectronic devices, through modern fabrication protocols, have allowed the integration of several sensors in a confined space. This has allowed the development of microsensor arrays able to perform detection of different analytes from the same biofluid sample^{86, 134, 138-140} (**Fig. 1-7a**). Multiplexed wearable sensors can monitor multiple analytes simultaneously and in real time, giving them a unique potential in chemical biomarker discovery. Moreover, other physical biomarkers (that is, vital signs) contain rich information which can be included in the diagnosis and prognosis of numerous diseases (**Fig. 1-7a**).

Recently, continuous wearable monitoring of cardiac and respiratory-related signals such as coughing, body temperature and patterns of activity (movements) was successfully demonstrated for detecting early symptoms and evolution of COVID-19 (ref.¹⁴¹). Moreover, a multiplex physical sensor was demonstrated for neonatal and paediatric critical care applications where physiological sensors (for example, blood pressure, heart rate and oxygenation) were integrated with movement, orientation and vocal sensors for tracking the tonality and temporal characteristics of crying¹⁴².

Within this context, multimodal wearable sensors that simultaneously monitor chemical and physical health features could offer more powerful and comprehensive information of an individual's health state (**Fig. 1-7a,b**) than single-modal wearables, increasing diagnostic accuracy⁸⁹. Integrated wearable chemical and physical sensors have been demonstrated for the simultaneous monitoring of chemical analytes and blood pressure: a wearable skin patch capable of monitoring sweat caffeine, alcohol, lactate and ISF glucose was used to deconvolute the additive effects on blood pressure and heart rate throughout daily activities¹¹⁶. This ability is crucial to improve the reliability during daily tracking and avoid erratic interpretation of biomarker behavior. Such combinations are significantly important when applied to the early detection of sepsis, characterized by a surge in lactate levels and a drop in blood pressure¹⁴³, and for the daily supervision of biomarkers in patients with diabetes who are obese and hypertensive.

Owing to the unique complexities of biofluid secretion and the uncontrolled operational conditions of wearable sensors, it is also important to create multiplexed and multimodal sensors that can re-calibrate their analyte readings in real time based on other chemical and physical marker concentrations (**Fig. 1-7c**). Although informative of various diseases, fluctuations in skin temperature have been demonstrated to cause substantial overestimation of sweat metabolites. To resolve this, a fully integrated wearable metabolite, electrolyte and skin temperature sensing array was created to accurately measure sweat glucose and lactate levels by calibrating sensor values based on skin temperature^{3, 144}. In parallel, multiplexed sensors have also used measurements of sweat pH and NH_4^+ as calibration to normalize enzyme activity and improve the detection accuracy of sweat urea and glucose concentrations, reducing miscalculation of the actual analyte levels¹²⁵.

Moving forward, skin-interfaced wearables should continue to expand their detection repertoire of target analytes and integrate continuous chemical sensing with biophysical markers (that is, blood pressure, heart rate). There are great opportunities for new categories of multimodal wearables; for example, more research is expected in combining brain activity monitoring (electroencephalogram) sensors with chemical sensors, which can open

invaluable possibilities, including early detection of epileptic seizures, stress, fatigue and depression¹⁴⁵. These smart multiplexed, multimodal chemical sensors will become more reliable and create personalized data for more accurate prediction of an individual's physiological response to specific health conditions. At the same time, compilation of these extended data will yield new complex information about known diseases and medications, which will consequently lead to the discovery of new effective biomarkers that can augment the selectivity and robustness of disease detection and prevention (**Fig. 1-7d**). By using various data analysis tools, the association between the molecular signatures and health conditions can be decoded and the resulted pattern regression could enable disease prediction, forecasting and diagnosis (**Fig. 1-7e**).

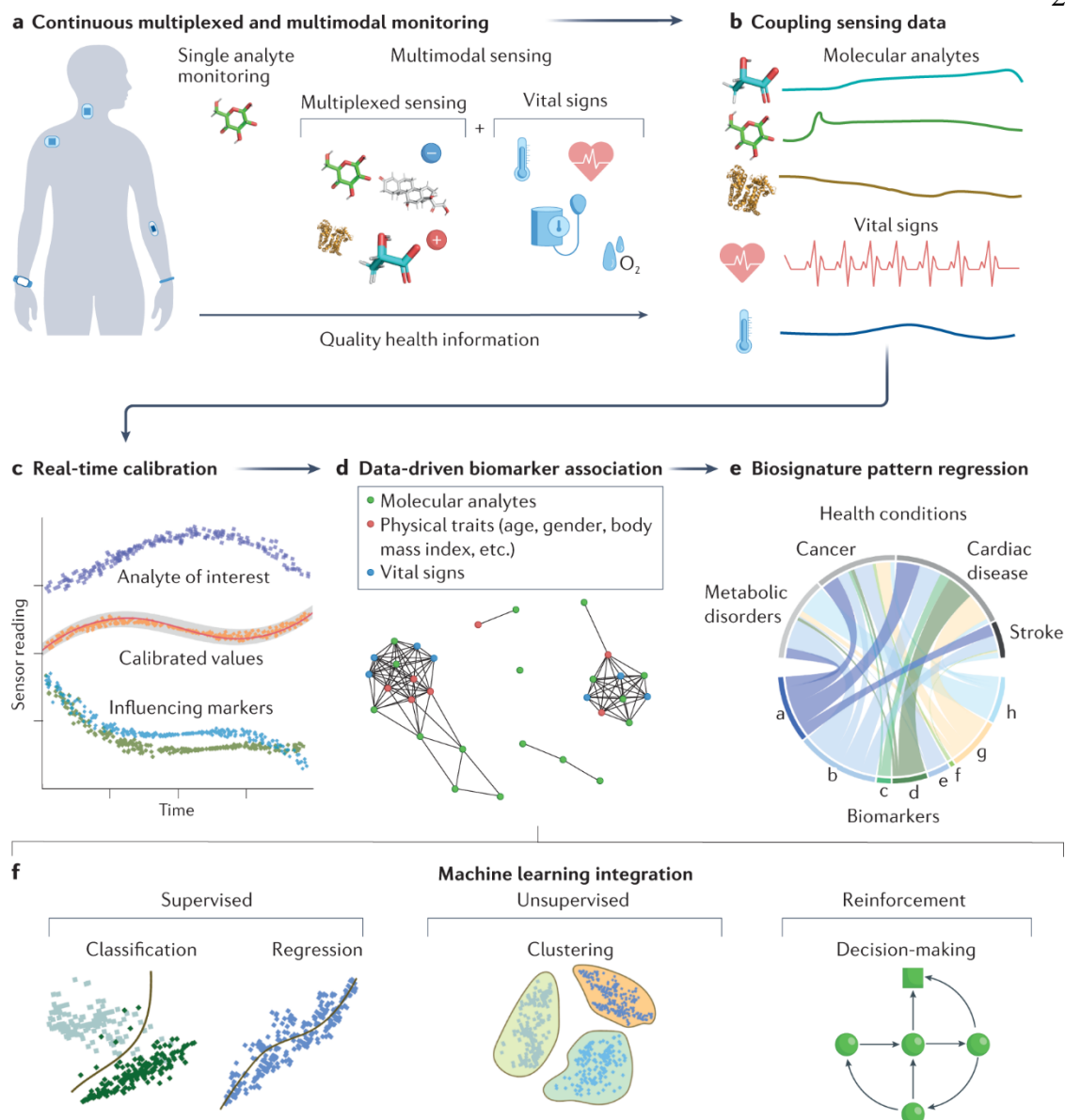


Figure 1-7. Multiplexed multimodal wearable sensing and machine learning for biomarker discovery. **a**, Multiplexed and multimodal wearable sensors continuously collect chemical and physical signatures from people's daily activities. **b**, Simultaneous collection and coupling of sensing data from multiplexed and multimodal sensors is imperative for robust post-processing. **c**, With coupled sensor information, real-time calibrated values of chemical analytes can be generated. **d**, After obtaining calibrated sensor data, multiplexed associations between other physical traits (age, gender, body mass index and so on) and markers (vital signs) can be used for improved diagnostics. **e**,

From multiplexed biomarker associations, molecular signatures corresponding to a biomarker's significance for determination of various diseases can be discovered. **f**, General classification of machine learning algorithms and models that can be implemented for biomarker discovery using wearable chemical sensors.

1.5.2 Machine learning-driven biomarker discovery

Technological innovations in the wearable multimodal and multiplexed sensors have allowed significant leaps in the continuous acquisition of high-resolution multi-omics data related to patient health from the same biofluid at the same time. These complex data are composed of multivariable, non-linear physiological patterns that cannot be readily identified by conventional processing methods. In the medical field, machine learning has already been proven an effective tool for interpreting large volumes of intricate data sets. For example, for insulin dosage recommendations for adults with type 1 diabetes, continuous glucose monitor sensors could predict the insulin bolus and perform self-calibration and correction. A similar approach was presented for other feedback sensors such as the levodopa closed loop system for Parkinson disease¹⁴⁶, feedback systems for cancer diagnosis and systems for the detection of cardiovascular diseases, pulmonary diseases and conditions related to patterns of movement, gait and posture¹⁴⁷⁻¹⁵⁴. As many possible variations, such as ionic strength, temperature, pH, humidity or body locations, could affect the readings of wearable biosensors during real-life on-body usage, machine learning can be applied for multiplexed data processing towards more accurate prediction of target analyte levels.

In generalized terms, the umbrella of machine learning algorithms can be divided into three classes: supervised, unsupervised and reinforced learning algorithms (**Fig. 1-7f**). Within each algorithm class, specific models (regression and classification models from supervised learning algorithms, clustering models from unsupervised learning algorithms and decision-making models from reinforced learning models) can be implemented based on the behaviour of the data set and the information or conclusion to be recovered from the study. Each specific machine learning model has a distinctive approach of interpreting and

associating features from a data set and the adequate selection of them has been comprehensively reviewed in the literature^{1, 133}. A number of these machine learning models, such as the support vector machine (SVM), k-nearest neighbours, neural networks, k-means clustering and the Markov decision process, can be applied for supervised and unsupervised data mining and decision-making based on big data collected by wearable biosensors (**Fig. 1-7f**).

Enhanced target sensitivity and selectivity through advances in chemical sensing have enabled new understanding of the correlations between chemical biomarkers and specific diseases^{155, 156}. Out of the three general categorizations of machine learning algorithms, supervised learning models have been used to establish input–output correlations based on molecular analytes and vital signs. Focusing on classification models, an SVM algorithm can be applied to establish relationships between volatile organic compound footprints from breath and blood glucose levels¹⁵⁷. Using published diabetic breath analyses as training data, the SVM algorithm categorized artificial breath samples to corresponding blood glucose levels with 97.1% accuracy. With increased focus on generating more durable wearable chemical sensors, implementing such SVM models will uncover new real-time correlations between validated biomarkers and the analyte monitored through non-invasively accessible bodily fluids. Furthermore, through cytokine and chemokine profiling using a single plasma sample, other machine learning classification and regression models (for example, naïve Bayes and decision tree algorithms) helped predict the sepsis host-immune response with high accuracy up to 96.64%¹⁵⁸. Using machine learning, wearable chemical sensing systems could continuously monitor various target candidates, and an individual's chemical profile of ISF, sweat or saliva will serve as feedback information on the patient's metabolic or even immune response. Additionally, multiplexed analysis of biomarkers in different body fluids will also yield connections to clinically relevant disorders. Recent studies demonstrated this by sampling armpit sweat and salivary cortisol levels during exercise; integrating a k-nearest neighbours algorithm facilitated estimation of the score on the Kessler 10 Psychological Distress Scale (a globally recognized questionnaire for determination of psychological distress with the scores range from 10 to 50) with an error rate of 3.6 (ref.¹⁸).

Looking at regression models, long short-term memory (a neural network algorithm capable of learning sequenced dependencies) was used to predict future blood glucose levels based on continuous monitoring data¹⁵⁹. Biophysical markers recorded from wearable sensors (heart rate, blood pressure), physical characteristics (weight, age, gender) and blood glucose data measured from a glucometer over 6 h were combined to predict blood glucose concentration with a 15-min horizon (predicting the concentration 15 min ahead of the actual measurement) and an average error of 15.43 mg dl⁻¹ (ref.¹⁶⁰). Using this regression model and establishing associations between blood glucose and the partitioned alternatives measured with wearable sensors, personalized ISF, sweat and saliva glucose–insulin dynamics could become a standardized method for managing individuals with diabetes. In another application, by analysing neuropsychiatric signatures in blood coupled with mental health questionnaires as training information, researchers developed a decision tree algorithm to reduce the misdiagnosis of bipolar disorder from major depressive disorder¹⁶¹. Supplementing these studies with chemical signatures provided by the wearable sensors could enhance the accuracy and efficiency of those diagnostic pathways. However, this will predominantly be achieved by generating machine learning models that can produce associations between multiple biomarker candidates in different body fluids. This was recently demonstrated by applying linear regression and neural network algorithms for learning from clinical data obtained from serum levels of creatinine in patients with renal disease, which helped correlate the serum creatinine concentrations with tear creatinine levels monitored by a wearable chemical sensor¹⁶².

As wearable chemical sensors become more robust, machine learning algorithms are more able to classify chemical and biophysical data corresponding to unique health patterns and provide accurate predictions of medical outcomes based on molecular signatures for different health conditions¹⁶³. Machine learning can be applied not only for the prediction of event but also to recognize when one has already happened; such a system can be applied in the detection of convulsion for patients with epilepsy, where galvanic skin response data, heart rate, temperature and movement sensors can detect when an event is happening and send an alert to the caregiver¹⁶⁴. Besides supervised regression and classification models,

unsupervised machine learning algorithms can be implemented to identify key unrecognized characteristics¹⁶⁵ from unlabeled molecular sensing data. This can be further used for dimensionality-reduction representations of the sensor information¹⁶⁶ and detection of anomalies within clusters of data (through models such as k-means clustering) (**Fig. 1-7f**). After comprehensive processing of the wearable biosensing data, reinforced learning algorithms can be integrated for assisted medical recommendations and timely interventions¹⁶⁷ (**Fig. 1-7f**).

Overall, the addition of multiplexed, multimodal real-time chemical sensors to the big data industry will open the gate to new correlations between existing biomarkers and new chemical signatures that can be monitored continuously through easily accessible bodily fluids. Combining these big data analytics, streaming from wearable sensing devices that monitor chemical and biophysical markers, machine learning techniques should have a profound impact upon the discovery of biomarkers and upon the healthcare field in general¹⁶⁸. A platform that could integrate chemical and biophysical data from different wearables is imperative to test and validate newly discovered biomarkers and their relationship with relevant diseases¹⁶⁹. Ultimately, integrating new chemical data from alternative body fluids provided by wearable chemical sensors will considerably facilitate the diagnostic and prevention of numerous diseases.

1.6 Outlook

In the era of omics and digital medicine, wearable chemical sensing technology is emerging as a powerful tool to aid the biomarker discovery process. Biomarker discovery can be facilitated by employing wearable multiplexed sensing systems that can perform continuous monitoring of potential molecular signatures. The data collected by these wearables can be correlated with the studied physiological and biological conditions using suitable data analytic tools. Compared with traditional laboratory tests based on blood draws and tissue biopsy, which are invasive, costly, require physical clinic visits and provide only discrete analyte information, the wearable sensor approach could substantially increase the efficiency and lower the costs of the process. Wearable chemical sensors may open a new era of medical

tools by offering means of remote, continuous and non-invasive real-time monitoring of potential multi-omics biomarkers during people's daily activities.

Despite great promise, the scope of molecular analytes able to be continuously screened on the body is currently limited to a small number of metabolites and electrolytes. There is an urgent need to develop next-generation wearable chemical sensors that can monitor a broader spectrum of biomarker candidates. In this regard, new bioaffinity sensors based on molecular switches coupled with suitable signal tags and in situ regeneration strategies are highly desired. Another critical limitation is that the catalogue of accessible and detectable analytes is limited to small molecules that can easily partition from blood or be secreted by exocrine/endocrine glands. Moreover, the time lag related to the molecular secretion into alternative body fluids from blood vessels needs to be carefully considered. Compared with gene sequencing or metabolomics analysis that can quantify thousands of molecules at a time, the number of biosensors that can be integrated into a single miniaturized wearable system is limited as each sensor requires the dimensions to reach desired selectivity, stability and sensitivity. Furthermore, the successful realization of wearable sensors with suitable sensitivity, selectivity and stability requires the careful selection of electrode materials, bioreceptor immobilization strategies and seamless wearable system integration that allows efficient fluid extraction and reliable, robust in situ data collection. Moreover, more studies are needed to verify the accuracy, precision and repeatability of the wearable sensors in participants. Such validation studies should be realized using existing gold standard tests to consolidate wearable biosensors in the omics era towards the widespread acceptance and credibility of these new non-invasive monitoring platforms¹⁷⁰.

Overall, wearable sensor-based biomarker discovery is a highly interdisciplinary field. Chemists, biologists, engineers and physicians need to work closely together to develop high-performance integrated multiplexed wearable chemical sensor systems with the power to predict and prevent the inevitable. We envisage that such evolution of wearable sensing technologies would lead to continuous real-time analyses of metabolomics, proteomics, genomics and other omics. The tremendous volume of data collected continuously from

large-scale human studies, coupled with efficient data-fusion and data-mining methods, would enable early disease prediction, diagnosis and timely interventions.

1.7 Bibliography of Chapter 1

1. Frank, R. & Hargreaves, R. Clinical biomarkers in drug discovery and development. *Nat. Rev. Drug Discov.* 2, 566–580 (2003).
2. Firestein, G. S. A biomarker by any other name. *Nat. Clin. Pract. Rheumatol.* 2, 635–635 (2006).
3. Gao, W. et al. Fully integrated wearable sensor arrays for multiplexed in situ perspiration analysis. *Nature* 529, 509–514 (2016).
4. Kim, J., Campbell, A. S., de Ávila, B. E.-F. & Wang, J. Wearable biosensors for healthcare monitoring. *Nat. Biotechnol.* 37, 389–406 (2019).
5. Zhao, J., Guo, H., Li, J., Bandodkar, A. J. & Rogers, J. A. Body-interfaced chemical sensors for noninvasive monitoring and analysis of biofluids. *Trends Chem.* 1, 559–571 (2019).
6. Yang, Y. & Gao, W. Wearable and flexible electronics for continuous molecular monitoring. *Chem. Soc. Rev.* 48, 1465–1491 (2019).
7. Broza, Y. Y. et al. Disease detection with molecular biomarkers: from chemistry of body fluids to nature-inspired chemical sensors. *Chem. Rev.* 119, 11761–11817 (2019).
8. Heikenfeld, J. et al. Accessing analytes in biofluids for peripheral biochemical monitoring. *Nat. Biotechnol.* 37, 407–419 (2019).
9. Ray, T. R. et al. Bio-integrated wearable systems: a comprehensive review. *Chem. Rev.* 119, 5461–5533 (2019).
10. Bariya, M., Nyein, H. Y. Y. & Javey, A. Wearable sweat sensors. *Nat. Electron.* 1, 160–171 (2018).
11. Picard, M., Scott-Boyer, M.-P., Bodein, A., Périn, O. & Droit, A. Integration strategies of multi-omics data for machine learning analysis. *Comput. Struct. Biotechnol. J.* 19, 3735–3746 (2021).

12. Butterfield, D. A. & Halliwell, B. Oxidative stress, dysfunctional glucose metabolism and Alzheimer disease. *Nat. Rev. Neurosci.* 20, 148–160 (2019).
13. Nahkuri, S., Becker, T., Schueller, V., Massberg, S. & Bauer-Mehren, A. Prior fluid and electrolyte imbalance is associated with COVID-19 mortality. *Commun. Med.* 1, 51 (2021).
14. Marcantonio, E. R. et al. Serum biomarkers for delirium. *J. Gerontol. A Biol. Sci. Med. Sci.* 61, 1281–1286 (2006).
15. Ernst, J. et al. Increased pregenual anterior cingulate glucose and lactate concentrations in major depressive disorder. *Mol. Psychiatry* 22, 113–119 (2017).
16. Torrente-Rodríguez et al. Investigation of cortisol dynamics in human sweat using a graphene-based wireless mHealth system. *Matter* 2, 921–937 (2020).
17. Pearlmutter, P. et al. Sweat and saliva cortisol response to stress and nutrition factors. *Sci. Rep.* 10, 19050 (2020).
18. Kallapur, B. et al. Quantitative estimation of sodium, potassium and total protein in saliva of diabetic smokers and nonsmokers: a novel study. *J. Nat. Sci. Biol. Med.* 4, 341–345 (2013).
19. Mohan, A. M. V., Rajendran, V., Mishra, R. K. & Jayaraman, M. Recent advances and perspectives in sweat based wearable electrochemical sensors. *TrAC Trends Anal. Chem.* 131, 116024 (2020).
20. Sonner, Z. et al. The microfluidics of the eccrine sweat gland, including biomarker partitioning, transport, and biosensing implications. *Biomicrofluidics* 9, 031301 (2015).
21. Katchman, B. A., Zhu, M., Blain Christen, J. & Anderson, K. S. Eccrine sweat as a biofluid for profiling immune biomarkers. *Proteom. Clin. Appl.* 12, 1800010 (2018).
22. Harvey, C. J., LeBouf, R. F. & Stefaniak, A. B. Formulation and stability of a novel artificial human sweat under conditions of storage and use. *Toxicol. Vitro* 24, 1790–1796 (2010).
23. Lin, S. et al. Noninvasive wearable electroactive pharmaceutical monitoring for personalized therapeutics. *Proc. Natl Acad. Sci. USA* 117, 19017–19025 (2020).

24. Abikshyeet, P., Ramesh, V. & Oza, N. Glucose estimation in the salivary secretion of diabetes mellitus patients. *Diabetes Metab. Syndr. Obes. Targets Ther.* 5, 149–154 (2012).
25. Peng, R. et al. A new oil/membrane approach for integrated sweat sampling and sensing: sample volumes reduced from μL 's to nL's and reduction of analyte contamination from skin. *Lab Chip* 16, 4415–4423 (2016).
26. Baker, L. B. & Wolfe, A. S. Physiological mechanisms determining eccrine sweat composition. *Eur. J. Appl. Physiol.* 120, 719–752 (2020).
27. Wang, B. et al. Wearable aptamer-field-effect transistor sensing system for noninvasive cortisol monitoring. *Sci. Adv.* 8, eabk0967 (2022).
28. Bandodkar, A. J., Jeerapan, I. & Wang, J. Wearable chemical sensors: present challenges and future prospects. *ACS Sens.* 1, 464–482 (2016).
29. Sempionatto, J. R., Jeerapan, I., Krishnan, S. & Wang, J. Wearable chemical sensors: emerging systems for on-body. *Anal. Chem.* 92, 378–396 (2019).
30. Xu, C., Yang, Y. & Gao, W. Skin-interfaced sensors in digital medicine: from materials to applications. *Matter* 2, 1414–1445 (2020).
31. Bandodkar, A. J. & Wang, J. Non-invasive wearable electrochemical sensors: a review. *Trends Biotechnol.* 32, 363–371 (2014).
32. Liu, H. & Zhao, C. Wearable electrochemical sensors for noninvasive monitoring of health — a perspective. *Curr. Opin. Electrochem.* 23, 42–46 (2020).
33. Min, J., Sempionatto, J. R., Teymourian, H., Wang, J. & Gao, W. Wearable electrochemical biosensors in North America. *Biosens. Bioelectron.* 172, 112750 (2021).
34. Xiao, J. et al. Microfluidic chip-based wearable colorimetric sensor for simple and facile detection of sweat glucose. *Anal. Chem.* 91, 14803–14807 (2019).
35. Choe, A. et al. Stretchable and wearable colorimetric patches based on thermoresponsive plasmonic microgels embedded in a hydrogel film. *NPG Asia Mater.* 10, 912–922 (2018).
36. Koh, A. et al. A soft, wearable microfluidic device for the capture, storage, and colorimetric sensing of sweat. *Sci. Transl. Med.* 8, 366ra165 (2016).

37. Ghaffari, R. et al. Soft wearable systems for colorimetric and electrochemical analysis of biofluids. *Adv. Funct. Mater.* 30, 1907269 (2019).
38. Kim, J., Campbell, A. S. & Wang, J. Wearable non-invasive epidermal glucose sensors: a review. *Talanta* 177, 163–170 (2018).
39. Lee, H., Hong, Y. J., Baik, S., Hyeon, T. & Kim, D. H. Enzyme-based glucose sensor: from invasive to wearable device. *Adv. Healthc. Mater.* 7, 1701150 (2018).
40. Wolkowicz, K. L. et al. A review of biomarkers in the context of type 1 diabetes: biological sensing for enhanced glucose control. *Bioeng. Transl. Med.* 6, e10201 (2021).
41. Teymourian, H., Barfidokht, A. & Wang, J. Electrochemical glucose sensors in diabetes management: an updated review (2010–2020). *Chem. Soc. Rev.* 49, 7671–7709 (2020).
42. Parrilla, M., Cuartero, M. & Crespo, G. A. Wearable potentiometric ion sensors. *TrAC Trends Anal. Chem.* 110, 303–320 (2019).
43. Yang, Y. et al. A laser-engraved wearable sensor for sensitive detection of uric acid and tyrosine in sweat. *Nat. Biotechnol.* 38, 217–224 (2020).
44. Zhao, J. et al. A wearable nutrition tracker. *Adv. Mater.* 33, 2006444 (2021).
45. Ariño, C. et al. Electrochemical stripping analysis. *Nat. Rev. Methods Prim.* 2, 62 (2022).
46. Gao, W. et al. Wearable microsensor array for multiplexed heavy metal monitoring of body fluids. *ACS Sens.* 1, 866–874 (2016).
47. Kim, J. et al. Wearable temporary tattoo sensor for real-time trace metal monitoring in human sweat. *Electrochem. Commun.* 51, 41–45 (2015).
48. Takaloo, S. & Moghimi Zand, M. Wearable electrochemical flexible biosensors: with the focus on affinity biosensors. *Sens. Bio-Sens. Res.* 32, 100403 (2021).
49. Elsherif, M., Hassan, M. U., Yetisen, A. K. & Butt, H. Wearable contact lens biosensors for continuous glucose monitoring using smartphones. *ACS Nano* 12, 5452–5462 (2018).
50. Deng, M. et al. Wearable fluorescent contact lenses for monitoring glucose via a smartphone. *Sens. Actuator B Chem.* 352, 131067 (2022).

51. Sekine, Y. et al. A fluorometric skin-interfaced microfluidic device and smartphone imaging module for in situ quantitative analysis of sweat chemistry. *Lab Chip* 18, 2178–2186 (2018).
52. Brinkmann, B. H. et al. Seizure diaries and forecasting with wearables: epilepsy monitoring outside the clinic. *Front. Neurol.* 12, 1128 (2021).
53. Jia, X. et al. High-sensitivity troponin I and incident coronary events, stroke, heart failure hospitalization, and mortality in the ARIC study. *Circulation* 139, 2642–2653 (2019).
54. Tu, J., Torrente-Rodríguez, R. M., Wang, M. & Gao, W. The era of digital health: a review of portable and wearable affinity biosensors. *Adv. Funct. Mater.* 29, 1906713 (2019).
55. Plaxco, K. W. & Soh, H. T. Switch-based biosensors: a new approach towards real-time, in vivo molecular detection. *Trends Biotechnol.* 29, 1–5 (2011).
56. Quijano-Rubio, A. et al. De novo design of modular and tunable protein biosensors. *Nature* 591, 482–487 (2021).
57. Modi, S. et al. A DNA nanomachine that maps spatial and temporal pH changes inside living cells. *Nat. Nanotechnol.* 4, 325–330 (2009).
58. Liu, S. et al. DNA nanotweezers for biosensing applications: recent advances and future prospects. *ACS Sens.* 7, 3–20 (2022).
59. Ahmad, O. S., Bedwell, T. S., Esen, C., Garcia-Cruz, A. & Piletsky, S. A. Molecularly imprinted polymers in electrochemical and optical sensors. *Trends Biotechnol.* 37, 294–309 (2019).
60. Subrahmanyam, S. et al. ‘Bite-and-switch’ approach using computationally designed molecularly imprinted polymers for sensing of creatinine. *Biosens. Bioelectron.* 16, 631–637 (2001).
61. Wang, M. et al. A wearable electrochemical biosensor for the monitoring of metabolites and nutrients. *Nat. Biomed. Eng.* <https://doi.org/10.1038/s41551-022-00916-z> (2022).
62. Vallée-Bélisle, A. & Plaxco, K. W. Structure-switching biosensors: inspired by nature. *Curr. Opin. Struct. Biol.* 20, 518–526 (2010).

63. Gotrik, M. R., Feagin, T. A., Csordas, A. T., Nakamoto, M. A. & Soh, H. T. Advancements in aptamer discovery technologies. *Acc. Chem. Res.* 49, 1903–1910 (2016).
64. Ahmad, K. M. et al. Probing the limits of aptamer affinity with a microfluidic SELEX platform. *PLoS ONE* 6, e27051 (2011).
65. Xiao, Y., Lai, R. Y. & Plaxco, K. W. Preparation of electrode-immobilized, redox-modified oligonucleotides for electrochemical DNA and aptamer-based sensing. *Nat. Protoc.* 2, 2875–2880 (2007).
66. Dauphin-Ducharme, P., Yang, K. & Plaxco, K. W. Electrochemical aptamer-based sensors for improved therapeutic drug monitoring and high-precision, feedback-controlled drug delivery. *ACS Sens.* 4, 2832–2837 (2019).
67. White, R. J., Phares, N., Lubin, A. A., Xiao, Y. & Plaxco, K. W. Optimization of electrochemical aptamer-based sensors via optimization of probe packing density and surface chemistry. *Langmuir* 24, 10513–10518 (2008).
68. Xiao, Y. et al. Label-free electronic detection of thrombin in blood serum by using an aptamer-based sensor. *Angew. Chem. Int. Ed.* 117, 5592–5595 (2005).
69. Chamorro-Garcia, A. et al. Switching the aptamer attachment geometry can dramatically alter the signalling and performance of electrochemical aptamer-based sensors. *Chem. Commun.* 57, 11693–11696 (2021).
70. Leung, K. K., Downs, A. M., Ortega, G., Kurnik, M. & Plaxco, K. W. Elucidating the mechanisms underlying the signal drift of electrochemical aptamer-based sensors in whole blood. *ACS Sens.* 6, 3340–3347 (2021).
71. Röthlisberger, P. & Hollenstein, M. Aptamer chemistry. *Adv. Drug Deliv. Rev.* 134, 3–21 (2018).
72. Ferguson, B. S. et al. Real-time, aptamer-based tracking of circulating therapeutic agents in living animals. *Sci. Transl. Med.* 5, 213ra165 (2013).
73. Poudineh, M. et al. A fluorescence sandwich immunoassay for the real-time continuous detection of glucose and insulin in live animals. *Nat. Biomed. Eng.* 5, 53–63 (2021).

74. Wilson, B. D., Hariri, A. A., Thompson, I. A. P., Eisenstein, M. & Soh, H. T. Independent control of the thermodynamic and kinetic properties of aptamer switches. *Nat. Commun.* 10, 5079 (2019).
75. Das, J. et al. Reagentless biomolecular analysis using a molecular pendulum. *Nat. Chem.* 13, 428–434 (2021).
76. Nguyen, P. Q. et al. Wearable materials with embedded synthetic biology sensors for biomolecule detection. *Nat. Biotechnol.* 39, 1366–1374 (2021).
77. Morales, M. A. & Halpern, J. M. Guide to selecting a biorecognition element for biosensors. *Bioconjug. Chem.* 29, 3231–3239 (2018).
78. Satishkumar, B. C. et al. Reversible fluorescence quenching in carbon nanotubes for biomolecular sensing. *Nat. Nanotechnol.* 2, 560–564 (2007).
79. Aleman, J., Kilic, T., Mille, L. S., Shin, S. R. & Zhang, Y. S. Microfluidic integration of regeneratable electrochemical affinity-based biosensors for continual monitoring of organ-on-a-chip devices. *Nat. Protoc.* 16, 2564–2593 (2021).
80. Ambaye, A. D., Kefeni, K. K., Mishra, S. B., Nxumalo, E. N. & Ntsendwana, B. Recent developments in nanotechnology-based printing electrode systems for electrochemical sensors. *Talanta* 225, 121951 (2021).
81. Mamun, M. A. Al & Yuce, M. R. Recent progress in nanomaterial enabled chemical sensors for wearable environmental monitoring applications. *Adv. Funct. Mater.* 30, 2005703 (2020).
82. Ulman, A. Formation and structure of self-assembled monolayers. *Chem. Rev.* 96, 1533–1554 (1996).
83. Joshi, P., Mishra, R. & Narayan, R. J. Biosensing applications of carbon-based materials. *Curr. Opin. Biomed. Eng.* 18, 100274 (2021).
84. Wang, M., Yang, Y. & Gao, W. Laser-engraved graphene for flexible and wearable electronics. *Trends Chem.* 3, 969–981 (2021).
85. Basu, J. & RoyChaudhuri, C. Graphene nanoporous FET biosensor: influence of pore dimension on sensing performance in complex analyte. *IEEE Sens. J.* 18, 5627–5634 (2018).

86. He, W. et al. Integrated textile sensor patch for real-time and multiplex sweat analysis. *Sci. Adv.* 5, eaax0649 (2019).
87. Downs, A. M. et al. Nanoporous gold for the miniaturization of in vivo electrochemical aptamer-based sensors. *ACS Sens.* 6, 2299–2306 (2021).
88. Bandodkar, A. J. et al. Battery-free, skin-interfaced microfluidic/electronic systems for simultaneous electrochemical, colorimetric, and volumetric analysis of sweat. *Sci. Adv.* 5, eaav3294 (2019).
89. Imani, S. et al. A wearable chemical–electrophysiological hybrid biosensing system for real-time health and fitness monitoring. *Nat. Commun.* 7, 11650 (2016).
90. Bariya, M. et al. Glove-based sensors for multimodal monitoring of natural sweat. *Sci. Adv.* 6, 8308–8336 (2020).
91. Sabaté del Río, J., Henry, O. Y. F., Jolly, P. & Ingber, D. E. An antifouling coating that enables affinity-based electrochemical biosensing in complex biological fluids. *Nat. Nanotechnol.* 14, 1143–1149 (2019).
92. Xu, K., Lu, Y. & Takei, K. Multifunctional skin-inspired flexible sensor systems for wearable electronics. *Adv. Mater. Technol.* 4, 1800628 (2019).
93. Zhang, S., Wright, G. & Yang, Y. Materials and techniques for electrochemical biosensor design and construction. *Biosens. Bioelectron.* 15, 273–282 (2000).
94. Feron, K. et al. Organic bioelectronics: materials and biocompatibility. *Int. J. Mol. Sci.* 19, 2382 (2018).
95. Kwon, Y. T. et al. All-printed nanomembrane wireless bioelectronics using a biocompatible solderable graphene for multimodal human–machine interfaces. *Nat. Commun.* 11, 3450 (2020).
96. Cui, C. et al. Recent progress in natural biopolymers conductive hydrogels for flexible wearable sensors and energy devices: materials, structures, and performance. *ACS Appl. Bio Mater.* 4, 85–121 (2021).
97. Kim, Y. & Prausnitz, M. R. Sensitive sensing of biomarkers in interstitial fluid. *Nat. Biomed. Eng.* 5, 3–5 (2021).
98. Shi, T. et al. Modeling and measurement of correlation between blood and interstitial glucose changes. *J. Diabetes Res.* 2016, 4596316 (2016).

99. Lee, H.-R., Kim, C.-C. & Sun, J.-Y. Stretchable ionics — a promising candidate for upcoming wearable devices. *Adv. Mater.* 30, 1704403 (2018).
100. Wang, Z. et al. Microneedle patch for the ultrasensitive quantification of protein biomarkers in interstitial fluid. *Nat. Biomed. Eng.* 5, 64–76 (2021).
101. Samant, P. P. & Prausnitz, M. R. Mechanisms of sampling interstitial fluid from skin using a microneedle patch. *Proc. Natl Acad. Sci. USA* 115, 4583–4588 (2018).
102. Lipani, L. et al. Non-invasive, transdermal, path-selective and specific glucose monitoring via a graphene-based platform. *Nat. Nanotechnol.* 13, 504–511 (2018).
103. Chen, Y. et al. Skin-like biosensor system via electrochemical channels for noninvasive blood glucose monitoring. *Sci. Adv.* 3, e1701629 (2017).
104. Pu, Z. et al. A thermal activated and differential self-calibrated flexible epidermal biomicrofluidic device for wearable accurate blood glucose monitoring. *Sci. Adv.* 7, 199–226 (2021).
105. Kim, J. et al. Simultaneous monitoring of sweat and interstitial fluid using a single wearable biosensor platform. *Adv. Sci.* 5, 1800880 (2018).
106. Emaminejad, S. et al. Autonomous sweat extraction and analysis applied to cystic fibrosis and glucose monitoring using a fully integrated wearable platform. *Proc. Natl Acad. Sci. USA* 114, 4625–4630 (2017).
107. Ray, T. R. et al. Soft, skin-interfaced sweat stickers for cystic fibrosis diagnosis and management. *Sci. Transl. Med.* 13, eabd8109 (2021).
108. Hauke, A. et al. Complete validation of a continuous and blood-correlated sweat biosensing device with integrated sweat stimulation. *Lab Chip* 18, 3750–3759 (2018).
109. Karpova, E. V. et al. Noninvasive diabetes monitoring through continuous analysis of sweat using flow-through glucose biosensor. *Anal. Chem.* 91, 3778–3783 (2019).
110. Paul, B., Demuru, S., Lafaye, C., Saubade, M. & Briand, D. Printed iontophoretic-integrated wearable microfluidic sweat-sensing patch for on-demand point-of-care sweat analysis. *Adv. Mater. Technol.* 6, 2000910 (2021).
111. Bolat, G. et al. Wearable soft electrochemical microfluidic device integrated with iontophoresis for sweat biosensing. *Anal. Bioanal. Chem.* 414, 5411–5421 (2022).

112. Nyein, H. Y. Y. et al. A wearable patch for continuous analysis of thermoregulatory sweat at rest. *Nat. Commun.* 12, 1823 (2021).
113. Bandodkar, A. J. et al. Sweat-activated biocompatible batteries for epidermal electronic and microfluidic systems. *Nat. Electron.* 3, 554–562 (2020).
114. Liu, C., Xu, T., Wang, D. & Zhang, X. The role of sampling in wearable sweat sensors. *Talanta* 212, 120801 (2020).
115. Nyein, H. Y. Y. et al. Regional and correlative sweat analysis using high-throughput microfluidic sensing patches toward decoding sweat. *Sci. Adv.* 5, eaaw9906 (2019).
116. Sempionatto, J. R. et al. An epidermal patch for the simultaneous monitoring of haemodynamic and metabolic biomarkers. *Nat. Biomed. Eng.* 5, 737–748 (2021).
117. Tai, L.-C. et al. Methylxanthine drug monitoring with wearable sweat sensors. *Adv. Mater.* 30, 1707442 (2018).
118. Salvisberg, C. et al. Exploring the human tear fluid: discovery of new biomarkers in multiple sclerosis. *Proteom. Clin. Appl.* 8, 185–194 (2014).
119. Bachhuber, F., Huss, A., Senel, M. & Tumani, H. Diagnostic biomarkers in tear fluid: from sampling to preanalytical processing. *Sci. Rep.* 11, 10064 (2021).
120. Yoshizawa, J. M. et al. Salivary biomarkers: toward future clinical and diagnostic utilities. *Clin. Microbiol. Rev.* 26, 781–791 (2013).
121. Iyengar, A. et al. Detection and potential utility of C-reactive protein in saliva of neonates. *Front. Pediatr.* 2, 131 (2014).
122. Mannoer, M. S. et al. Graphene-based wireless bacteria detection on tooth enamel. *Nat. Commun.* 3, 763 (2012).
123. Kim, J. et al. Wearable salivary uric acid mouthguard biosensor with integrated wireless electronics. *Biosens. Bioelectron.* 74, 1061–1068 (2015).
124. Tehrani, F. et al. An integrated wearable microneedle array for the continuous monitoring of multiple biomarkers in interstitial fluid. *Nat. Biomed. Eng.* <https://doi.org/10.1038/s41551-022-00887-1> (2022).
125. Yu, Y. et al. Biofuel-powered soft electronic skin with multiplexed and wireless sensing for human–machine interfaces. *Sci. Robot.* 5, eaaz7946 (2020).

126. Song, Y. et al. Wireless battery-free wearable sweat sensor powered by human motion. *Sci. Adv.* 6, eaay9842 (2020).
127. Song, Y., Mukasa, D., Zhang, H. & Gao, W. Self-powered wearable biosensors. *Acc. Mater. Res.* 2, 184–197 (2021).
128. Yin, L. et al. A passive perspiration biofuel cell: high energy return on investment. *Joule* 5, 1888–1904 (2021).
129. Yin, L. et al. A self-sustainable wearable multi-modular e-textile bioenergy microgrid system. *Nat. Commun.* 12, 1542 (2021).
130. Zhao, J. et al. A fully integrated and self-powered smartwatch for continuous sweat glucose monitoring. *ACS Sens.* 4, 1925–1933 (2019).
131. Terse-Thakoor, T. et al. Thread-based multiplexed sensor patch for real-time sweat monitoring. *npj Flex. Electron.* 4, 18 (2020).
132. Torrente-Rodríguez, R. M. et al. SARS-CoV-2 RapidPlex: a graphene-based multiplexed telemedicine platform for rapid and low-cost COVID-19 diagnosis and monitoring. *Matter* 6, 1981–1998 (2020).
133. Yu, K.-H., Beam, A. L. & Kohane, I. S. Artificial intelligence in healthcare. *Nat. Biomed. Eng.* 2, 719–731 (2018).
134. Yokus, M. A., Songkakul, T., Pozdin, V. A., Bozkurt, A. & Daniele, M. A. Wearable multiplexed biosensor system toward continuous monitoring of metabolites. *Biosens. Bioelectron.* 153, 112038 (2020).
135. Trejo Banos, D. et al. Bayesian reassessment of the epigenetic architecture of complex traits. *Nat. Commun.* 11, 2865 (2020).
136. Conesa, A. & Beck, S. Making multi-omics data accessible to researchers. *Sci. Data* 6, 251 (2019).
137. Eren, A. M. et al. Community-led, integrated, reproducible multi-omics with anvi'o. *Nat. Microbiol.* 6, 3–6 (2021).
138. Heifler, O. et al. Clinic-on-a-needle array toward future minimally invasive wearable artificial pancreas applications. *ACS Nano* 15, 12019–12033 (2021).
139. Teymourian, H., Tehrani, F., Mahato, K. & Wang, J. Lab under the skin: microneedle based wearable devices. *Adv. Healthc. Mater.* 17, 2002255 (2021).

140. Teymourian, H. et al. Microneedle-based detection of ketone bodies along with glucose and lactate: toward real-time continuous interstitial fluid monitoring of diabetic ketosis and ketoacidosis. *Anal. Chem.* 92, 2291–2300 (2020).
141. Jeong, H., Rogers, J. A. & Xu, S. Continuous on-body sensing for the COVID-19 pandemic: gaps and opportunities. *Sci. Adv.* 6, eabd4794 (2020).
142. Chung, H. U. et al. Skin-interfaced biosensors for advanced wireless physiological monitoring in neonatal and pediatric intensive-care units. *Nat. Med.* 26, 418–429 (2020).
143. Reddy, B. et al. Point-of-care sensors for the management of sepsis. *Nat. Biomed. Eng.* 2, 640–648 (2018).
144. Lee, H. et al. A graphene-based electrochemical device with thermoresponsive microneedles for diabetes monitoring and therapy. *Nat. Nanotechnol.* 11, 566–572 (2016).
145. Iqbal, S. M. A., Mahgoub, I., Du, E., Leavitt, M. A. & Asghar, W. Advances in healthcare wearable devices. *npj Flex. Electron.* 5, 9 (2021).
146. Teymourian, H. et al. Closing the loop for patients with Parkinson disease: where are we? *Nat. Rev. Neurol.* 18, 497–507 (2022).
147. Huang, L. et al. Machine learning of serum metabolic patterns encodes early-stage lung adenocarcinoma. *Nat. Commun.* 11, 3556 (2020).
148. Krittanawong, C. et al. Integration of novel monitoring devices with machine learning technology for scalable cardiovascular management. *Nat. Rev. Cardiol.* 18, 75–91 (2021).
149. Odish, O. F. F., Johnsen, K., van Someren, P., Roos, R. A. C. & van Dijk, J. G. EEG may serve as a biomarker in Huntington's disease using machine learning automatic classification. *Sci. Rep.* 8, 16090 (2018).
150. Green, E. M. et al. Machine learning detection of obstructive hypertrophic cardiomyopathy using a wearable biosensor. *npj Digit. Med.* 2, 57 (2019).
151. Kehoe, E. R. et al. Biomarker selection and a prospective metabolite-based machine learning diagnostic for Lyme disease. *Sci. Rep.* 12, 1478 (2022).

152. Islam, M. T. & Xing, L. A data-driven dimensionality-reduction algorithm for the exploration of patterns in biomedical data. *Nat. Biomed. Eng.* 5, 624–635 (2021).
153. Tyler, N. S. et al. An artificial intelligence decision support system for the management of type 1 diabetes. *Nat. Metab.* 2, 612–619 (2020).
154. Strain, T. et al. Wearable-device-measured physical activity and future health risk. *Nat. Med.* 26, 1385–1391 (2020).
155. Jin, X., Liu, C., Xu, T., Su, L. & Zhang, X. Artificial intelligence biosensors: challenges and prospects. *Biosens. Bioelectron.* 165, 112412 (2020).
156. Bashir, A. et al. Machine learning guided aptamer refinement and discovery. *Nat. Commun.* 12, 2366 (2021).
157. Boubin, M. & Shrestha, S. Microcontroller implementation of support vector machine for detecting blood glucose levels using breath volatile organic compounds. *Sensors* 19, 2283 (2019).
158. Sardesai, A. U. et al. An approach to rapidly assess sepsis through multi-biomarker host response using machine learning algorithm. *Sci. Rep.* 11, 16905 (2021).
159. van Doorn, W. P. T. M. et al. Machine learning-based glucose prediction with use of continuous glucose and physical activity monitoring data: the Maastricht study. *PLoS ONE* 16, e0253125 (2021).
160. Rodríguez-Rodríguez, I. et al. Utility of big data in predicting short-term blood glucose levels in type 1 diabetes mellitus through machine learning techniques. *Sensors* 19, 4482 (2019).
161. Tomasik, J. et al. A machine learning algorithm to differentiate bipolar disorder from major depressive disorder using an online mental health questionnaire and blood biomarker data. *Transl. Psychiatry* 11, 41 (2021).
162. Kalasin, S., Sangnuang, P. & Surareungchai, W. Lab-on-eyeglasses to monitor kidneys and strengthen vulnerable populations in pandemics: machine learning in predicting serum creatinine using tear creatinine. *Anal. Chem.* 93, 10661–10671 (2021).
163. Cui, F., Yue, Y., Zhang, Y., Zhang, Z. & Zhou, H. S. Advancing biosensors with machine learning. *ACS Sens.* 5, 3346–3364 (2020).

164. Bruno, E., Viana, P. F., Sperling, M. R. & Richardson, M. P. Seizure detection at home: do devices on the market match the needs of people living with epilepsy and their caregivers? *Epilepsia* 61, S11–S24 (2020).
165. Waldstein, S. M. et al. Unbiased identification of novel subclinical imaging biomarkers using unsupervised deep learning. *Sci. Rep.* 10, 12954 (2020).
166. Fortino, V. et al. Machine-learning-driven biomarker discovery for the discrimination between allergic and irritant contact dermatitis. *Proc. Natl Acad. Sci. USA* 117, 33474–33485 (2020).
167. Oh, S. H., Lee, S. J., Noh, J. & Mo, J. Optimal treatment recommendations for diabetes patients using the Markov decision process along with the South Korean electronic health records. *Sci. Rep.* 11, 6920 (2021).
168. Dunn, J. et al. Wearable sensors enable personalized predictions of clinical laboratory measurements. *Nat. Med.* 27, 1105–1112 (2021).
169. Quer, G. et al. Wearable sensor data and self-reported symptoms for COVID-19 detection. *Nat. Med.* 27, 73–77 (2021).
170. Tu, J. & Gao, W. Ethical considerations of wearable technologies in human research. *Adv. Healthc. Mater.* 10, 2100127 (2021).
171. Harris, M. I., Hadden, W. C., Knowler, W. C. & Bennett, P. H. International criteria for the diagnosis of diabetes and impaired glucose tolerance. *Diabetes Care* 8, 562–567 (1985).
172. Inzucchi, S. E. Clinical practice. Diagnosis of diabetes. *N. Engl. J. Med.* 367, 542–550 (2012).
173. Tonelli, M. & Manns, B. Supplementing creatinine-based estimates of risk in chronic kidney disease: is it time? *JAMA* 305, 1593–1595 (2011).
174. FDA-NIH Biomarker Working Group. BEST (Biomarkers, Endpoints, and Other Tools) Resource (FDA, 2016).
175. Miksenas, H., Januzzi, J. L. & Natarajan, P. Lipoprotein(a) and cardiovascular diseases. *JAMA* 326, 352–353 (2021).
176. Minton, K. Maternal sensitization to neonatal allergy. *Nat. Rev. Immunol.* 21, 2–3 (2020).

177. Valenta, R. The future of antigen-specific immunotherapy of allergy. *Nat. Rev. Immunol.* 2, 446–453 (2002).
178. Levey, A. S., Inker, L. A. & Coresh, J. Chronic kidney disease in older people. *JAMA* 314, 557–558 (2015).
179. Rubin, R. COVID-19 vaccines vs variants-determining how much immunity is enough. *JAMA* 325, 1241–1243 (2021).
180. Dieterle, F. et al. Urinary clusterin, cystatin C, β 2-microglobulin and total protein as markers to detect drug-induced kidney injury. *Nat. Biotechnol.* 28, 463–469 (2010).
181. Chertow, G. M. et al. Guided medication dosing for inpatients with renal insufficiency. *JAMA* 286, 2839–2844 (2001).

WEARABLE ELECTROCHEMICAL LIPID BIOSENSING

2.1 Introduction

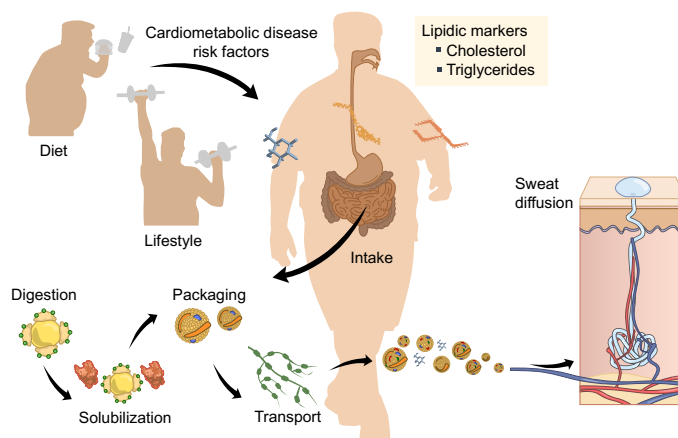


Figure 2-1. Epidermal lipid profiling. a, Overview of key contributors to cardiometabolic diseases, including diet and lifestyle, that modulate systemic lipid metabolism and lead to dysregulation of cholesterol (CH) and triglycerides (TG).

Lipid profiling is fundamental to the evaluation and management of metabolic syndrome, diabetes, obesity, and cardiovascular diseases¹. Dysregulated lipid levels are key risk factors for atherosclerosis, heart disease, and stroke, which remain leading causes of mortality worldwide². In the United States, cardiovascular disease affects 48.6% of adults, and the prevalence of obesity reached 41.8% between 2017 and 2020³. These statistics underscore the clinical importance of lipid monitoring and motivate the development of more accessible tools for tracking lipid metabolism. Because lifestyle factors such as diet, physical activity, and smoking strongly influence lipid homeostasis, more frequent and personalized lipid assessment could improve disease prevention and management (**Fig. 2-1**)⁴.

The current clinical standard, periodic blood lipid panels obtained through venipuncture, is invasive, inconvenient, and provides only a single-point measurement of systemic lipid

status. These constraints can limit patient compliance and obscure dynamic lipid fluctuations related to diet, activity, and individual metabolic state. Alternative microsampling and simplified sensing methods have reduced some barriers to lipid testing, but they still rely on blood extraction and therefore do not support fully noninvasive or continuous lipid tracking^{5,6}. As a result, real-time, noninvasive lipid profiling remains an unmet need.

Sweat offers a promising alternative biofluid because it is readily accessible at the skin surface and contains molecular information related to nutritional and metabolic status⁷⁻¹³.

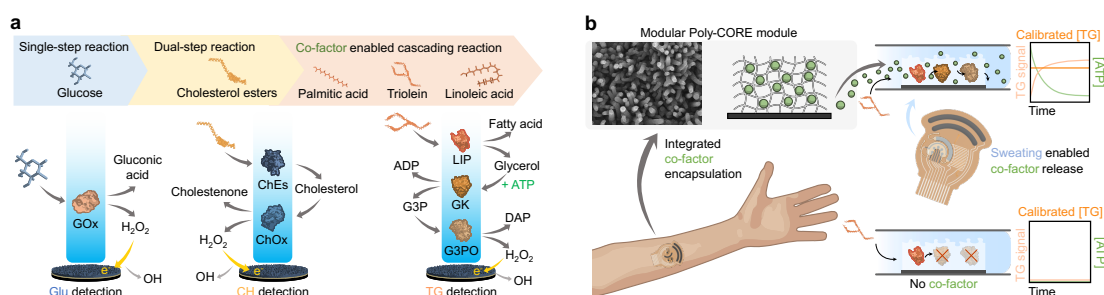


Figure 2-2. Enzymatic sensing strategy for continuous epidermal lipid profiling. a, Schematic of single-step, dual-step and co-factor enabled cascading enzymatic reactions leveraged to detect glucose, cholesterol, and triglycerides, respectively. Glu, glucose; GOx, glucose oxidase; ChEs, cholesterol esterase; ChOx, cholesterol oxidase; LIP, lipase; GK, glycerol kinase; G3PO, glycerol-3-phosphate oxidase; ATP, adenosine triphosphate; ADP, adenosine diphosphate; G3P, glycerol-3-phosphate; DAP, dihydroxyacetone phosphate. **b,** Illustration of the modular polymeric cofactor-releasing (Poly-CORE) system based on ATP-encapsulating polypyrrole (PPy/ATP) nanostructures, which enable sustained sweat-triggered ATP diffusion into the sensing chamber for long-term TG monitoring.

Transitioning from invasive blood analyses to wearable sweat-based platforms could substantially expand the amount of molecular health information acquired over time, with potential implications for earlier risk assessment and personalized interventions. Recent advances in wearable electrochemical sensors have enabled continuous tracking of diverse analytes, including metabolites, electrolytes, amino acids, and vitamins, through soft, skin-

interfaced devices^{5–12}. Among available biorecognition strategies, enzymatic reactions remain particularly attractive for continuous sensing because of their molecular specificity and catalytic signal amplification. However, continuous lipid monitoring poses unique challenges because lipid quantification often relies on multistep enzymatic cascades and non-regenerable cofactors such as adenosine triphosphate (ATP)^{13,14}. Although cholesterol (CH) and triglycerides (TG) have been detected in sweat¹⁵, existing wearable lipid-sensing systems remain limited in their ability to sustain the sequential enzymatic pathways and cofactor availability required for long-term lipid detection.

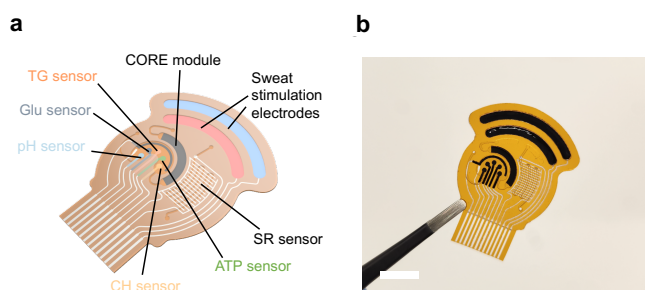


Figure 2-3. Wearable patch design for continuous epidermal lipid profiling. a, Schematic of the wearable patch design illustrating the bioelectronic components and capabilities. SR, sweat rate. **b,** Optical image of a wearable sensor patch for epidermal lipid monitoring. Scale bar, 10 mm.

To address these challenges, we developed a wearable epidermal lipid profiler for continuous, noninvasive monitoring of cardiometabolic biomarkers through sweat lipid profiling (**Fig. A-1**). This platform tracks CH and TG through two distinct enzymatic pathways: a dual-step reaction for CH and an ATP-dependent cascading reaction for TG (**Fig. 2-2a**). To maintain cofactor availability, we engineered a polymeric cofactor-releasing unit, termed Poly-CORE, composed of ATP-encapsulating polypyrrole (PPy/ATP) nanostructures that provide sweat-activated and sustained ATP release within the sensing chamber (**Fig. 2-2b**). For real-time calibration, a multi-walled carbon nanotube (MWCNT)-based ATP sensor enables voltammetric monitoring of cofactor dynamics. Complementary glucose (Glu), pH, and sweat-rate sensors provide metabolic context and environmental normalization, while a miniaturized iontophoresis (IP) module enables localized sweat

secretion on demand (**Fig. 2-3a**). The standalone patch is fabricated using scalable processes, including inkjet printing of sensor components and laser engraving of microfluidic layers (**Fig. 2-3b** and **Figs. A-2 and A-3**).

We demonstrate the device's ability to continuously track lipid dynamics during dietary intake and habitual daily activities. Across controlled dietary challenges, the platform captures postprandial changes in CH and TG that vary with meal composition, revealing temporal lipid patterns that are missed by single-point measurements. Because these responses are further shaped by individual factors such as metabolic rate, body composition, and prior intake, continuous monitoring exposes personalized trends that static measurements can obscure. Together, these results establish wearable epidermal lipid profiling as a technical foundation for individualized cardiometabolic health assessment.

2.2 Biosensor characterization

Three principal amperometric biosensors were developed on this platform to quantify Glu, CH, and TG, which are key biomarkers of cardiometabolic health. Total CH reflects systemic lipid transport and serves as a critical indicator of lipid dysregulation²⁹, while TG reflects lipid clearance and energy storage³⁰. Elevated levels of CH and TG are strongly associated with an increased risk of atherosclerosis and metabolic syndrome³¹. Glucose, closely coupled to lipid metabolism, contributes to TG accumulation and dyslipidemia when chronically elevated³². The integration of these three enzymatic sensors thus enables comprehensive, multiplexed profiling of cardiometabolic health.

Glucose biosensing was achieved through encapsulating glucose oxidase (GOx) within a bovine serum albumin–glutaraldehyde (BSA–GT) matrix (**Fig. 2-4a**), which provides a robust microenvironment for enzyme immobilization while maintaining high enzymatic activity³³. The sensing electrode was fabricated by sequentially modifying an inkjet-printed carbon electrode with nanostructured gold (Au), Prussian blue (PB) and nickel hexacyanoferrate (NiHCF) layers to enhance electroactive surface area³⁴, electrocatalytic efficiency, and long-term stability (**Fig. 2-4a**). PB served as an effective redox mediator at

low operating potentials around 0 V^{35,36}, while NiHCF acted as a PB stabilizer for extended sensing performance³⁷. The same electrode modification and encapsulation protocol was applied across all enzymatic sensors (**Fig. A-4**). The Glu sensor exhibited high sensitivity ($66.5 \pm 1.3 \text{ nA cm}^{-2} \mu\text{M}^{-1}$, $n = 5$) and linearity within the physiological sweat concentration range (0 to 200 μM) (**Fig. 2-4b**).

CH detection was enabled through a dual-step enzymatic reaction combining cholesterol esterase (ChEs) and cholesterol oxidase (ChOx) (**Fig. 2-4c**). Because most epidermal cholesterol exists in esterified form, ChEs catalyzes the hydrolysis of cholesteryl esters into free cholesterol and fatty acids, followed by oxidation of cholesterol by ChOx to generate cholest-4-en-3-one and hydrogen peroxide^{7,23}. The sensor demonstrated excellent linearity in the 0–3 μM range, consistent with expected sweat CH concentrations (**Fig. 2-4d and Fig. A-6**), and exhibited a sensitivity of $52.7 \pm 3.7 \text{ nA cm}^{-2} \mu\text{M}^{-1}$ ($n = 5$). This performance represents a substantial advance, as most reported CH sensors target millimolar concentrations typical of serum²¹ rather than the low-micromolar regime found in sweat.

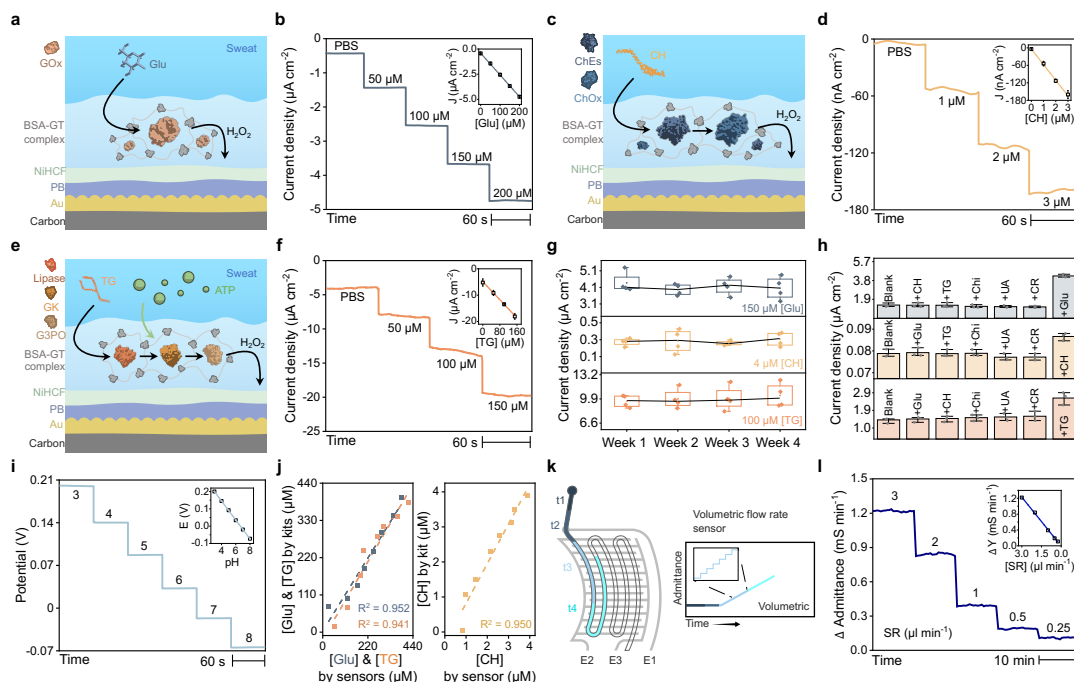


Figure 2-4. Biosensor characterization for continuous lipid monitoring. a, Glucose biosensor configuration. BSA, bovine serum albumin; GT, glutaraldehyde; NiHCF, nickel

hexacyanoferrate; PB, Prussian blue; Au, gold. **b**, Amperometric response and linear calibration for glucose detection. **c**, CH biosensor configuration. **d**, Amperometric response and linear calibration for CH detection. **e**, TG biosensor modification layout. **f**, Amperometric response and linear calibration for TG detection. Error bars in **b,d,f** represent s.d. from the mean of 5 sensors. **g**, Four-week shelf stability of Glu, CH, and TG sensors. Error bars represent s.d. from the mean from 4 sensors. **h**, Selectivity of glucose, CH, and TG sensors against common sweat interferents. UA, uric acid; CHI, choline chloride; CR, creatine. [Glu] = 100 μM ; [CH] = 1 μM ; [TG] = 50 μM ; [UA] = 40 μM ; [CHI] = 50 μM ; [CR] = 15 μM . Error bars represent s.d. from the mean from 3 sensors. **i**, Potentiometric response and calibration curve of the integrated pH sensor. **j**, Validation of glucose, CH, and TG sensors against commercial assay kits for analyzing sweat samples ($n = 8$). **k**, Schematic of the three-electrode SR sensor. **l**, Admittance variation with flow rate and the corresponding calibration plot. Error bars represent s.d. from the mean from 5 sensors. Y, admittance.

TG biosensing relied on a three-enzyme cascading reaction involving lipase (LIP), glycerol kinase (GK), and glycerol-3-phosphate oxidase (G3PO) (**Fig. 2-4e**). LIP hydrolyzes TG into glycerol and fatty acids; GK then phosphorylates glycerol to glycerol-3-phosphate (G3P) in the presence of ATP; finally, G3PO oxidizes G3P to dihydroxyacetone phosphate and hydrogen peroxide (**Supplementary Note 1**). Notably, the enzymatic cascade responds to glycerol; thus, endogenous glycerol present in sweat may contribute to the measured signal in addition to glycerol generated from triglyceride hydrolysis. As a result, the sensor does not provide a strictly selective measurement of absolute triglyceride concentration. Given that glycerol is a direct product of triglyceride metabolism (lipolysis), the sensor quantifies glycerol generated from lipid metabolism, including contributions from triglyceride hydrolysis, and thus provides a proxy for lipolytic activity. The TG sensor achieved a sensitivity of $100.4 \pm 12.9 \text{ nA cm}^{-2} \mu\text{M}^{-1}$ ($n = 5$) over the 0–150 μM range (**Fig. 2-4f** and **Fig. A-6**). As ATP is consumed during the reaction, maintaining signal stability requires continuous ATP replenishment, which underscores the need for a controllable cofactor-release module for wearable sensing application.

All sensors demonstrated good reproducibility, and excellent shelf stability, maintaining consistent signals over a 4-week period (**Fig. 2-4g** and **Figs. A-7 and A-8**). Greater sensor-to-sensor variability observed for the CH and TG sensors likely arises from the increased sensitivity of multi-enzyme cascades to small variations in fabrication and enzyme loading. All sensors exhibited strong selectivity against common sweat constituents such as uric acid, creatine, and choline (**Fig. 2-4h** and **Fig. A-8**). Sensor outputs exhibited pH-dependent baseline shifts under physiological variations induced by iontophoresis³⁸, with no substantial changes in sensitivity across the tested pH range (**Fig. A-9**). The magnitude of the pH effect was more pronounced for the CH sensor due to its lower operating concentration range. Importantly the pH-dependent response remained consistent across devices within each sensor type, enabling systematic correction using an integrated potentiometric pH sensor (**Fig. 2-4i**). The influence of physiologically relevant temperature variations on enzymatic sensor response (**Fig. A-10**), as well as their operational stability (**Fig. A-11**), was also evaluated. A potentiometric pH sensor was thus incorporated for in situ calibration (**Fig. 2-4i**). Comparison against commercial assay kits confirmed the high accuracy of the fabricated sensors for Glu, CH, and TG quantification in sweat (**Fig. 2-4j**).

To complement biomarker analysis, an impedimetric sweat rate (SR) sensor was integrated to assess dynamic sweat generation and analyte dilution. The SR module consists of three electrodes (**Fig. 2-4k**): the impedance between E1 and E2 reflects ionic strength, while the E2–E3 interdigitated electrodes capture volumetric admittance changes proportional to sweat accumulation and, consequently, sweat rate. The instantaneous SR is determined from the sweat volume accumulated in the microfluidic channel over time (**Fig. 2-4l** and **Supplementary Note 2**). In the ionic sensor pair (E1–E2), sequential introduction of two solutions with differing concentrations produces characteristic impedance shifts, enabling ionic strength quantification independent of ion identity (**Figs. A-12 and A-13**). Combining these ionic-strength measurements with volumetric impedance readings from E2–E3 allows real-time estimation of sweat rate³⁹ (**Fig. A-14**). Measurements of sweat impedance as a function of temperature and pH further improved accuracy across physiological conditions (**Figs. A-15 and A-16**). The SR sensor geometry can be tailored to specific sweat volumes or experiment durations by adjusting the microchannel dimensions (**Fig. A-17**). The accuracy

of the sensor configuration was validated in on-body tests using colorimetric flow tracking with a pre-filled dye near the microfluidic inlet (**Fig. A-18**). Together, these capabilities enable continuous, high-fidelity sweat-rate monitoring, providing essential normalization data for quantitative biomarker analysis.

2.3 Sweating-enabled Poly-CORE module

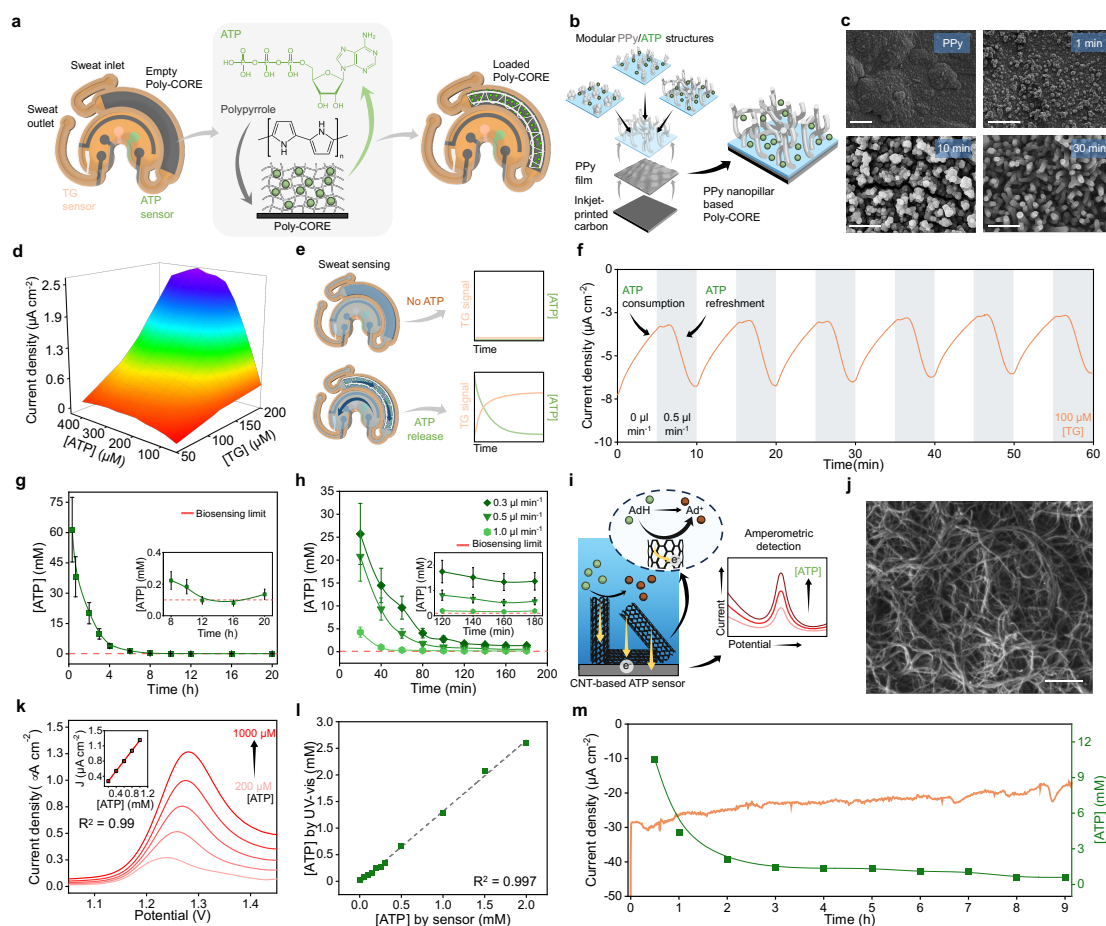


Figure 2-5. Characterization of the Poly-CORE module. **a**, General schematic illustrating the integration of PPy/ATP nanostructures within the Poly-CORE. **b**, Fabrication workflow showing electrodeposition of PPy/ATP nanopillars on inkjet-printed electrodes. **c**, Scanning electron microscopy (SEM) images of PPy/ATP nanopillars synthesized for different deposition durations at a constant current density. Scale bar, 30 μm . **d**, Three-dimensional surface plot showcasing the relationship between ATP

concentration and the amperometric TG sensor response. **e**, Microfluidic layout of the integrated Poly-CORE module showing sweating-activated ATP diffusion into the TG sensing chamber. **f**, Dynamic amperometric measurement demonstrating periodic ATP consumption and signal recovery under continuous flow conditions. **g**, Long-term ATP release profile of PPy/ATP structure (synthesized at 0.15 mA cm^{-2} for 1 h) over 20 hours. **h**, ATP release profile of PPy/ATP nanostructures (synthesized at 0.15 mA cm^{-2} for 10 min) under different flow rates mimicking physiological sweat rates. Error bars represent s.d. of the mean from 3 sensors. **i**, Schematic of multi-walled carbon-nanotube (MWCNT)-based sensor for direct cofactor quantification. AdH and Ad⁺ represent the reduced and oxidized forms of the adenosine moiety. **j**, SEM image of MWCNT electrode surface. Scale bar, 400 nm. **k**, Differential pulse voltammetry (DPV) curves for ATP detection (200–1000 μM). **l**, Validation of ATP measurements against UV–vis spectroscopy at 260 nm. **m**, Representative time-course of in vitro TG and ATP detection demonstrating real-time cofactor-refreshing biosensing.

Detection of TG has not previously been demonstrated in a wearable platform because of its cascading enzymatic reaction requires a continuous supply of a non-regenerable cofactor. Continuous TG biosensing demands prolonged access to ATP to enable phosphorylation of glycerol to glycerol-3-phosphate and adenosine diphosphate in the secondary enzymatic step (**Fig. A-19**)²². To address this challenge, we engineered a Poly-CORE module that integrates PPy/ATP nanostructures synthesized via electrodeposition (**Fig. 2-5a**). PPy was selected because its redox-active backbone enables electrostatic incorporation of anionic species such as ATP during electropolymerization, forming a conductive and porous reservoir that supports sustained cofactor release through ion exchange and diffusion processes. Under matched fabrication conditions, PPy exhibited a more sustained and gradual ATP release profile following the initial burst phase compared to other tested polymers (**Fig. A-20**). Further comparisons with representative polymer entrapment strategies confirmed that dopant-mediated incorporation in PPy provided the most stable ATP release above the biosensing threshold (**Fig. A-21**).

A thin PPy film was first electrodeposited on an inkjet-printed carbon electrode to promote uniform growth of the PPy/ATP network⁴⁰. By modulating current density and deposition time, distinct PPy/ATP architectures were synthesized with tunable ATP releasing profiles (**Fig. 2-5b**). Electrochemical impedance spectroscopy confirmed increased surface impedance after polymerization, suggesting successful formation of the co-factor releasing layer (**Fig. A-22**). Scanning electron microscopy (SEM) revealed uniform PPy films (top left) and distinct PPy/ATP morphologies generated by electrodeposition at 0.15 mA cm⁻² for 1, 10, and 30 minutes (**Fig. 2-5c**).

To optimize the Poly-CORE structures for continuous TG detection, we first determined the limiting ATP concentration for enzymatic activity. Amperometric analysis showed that TG sensors produced distinct signals in the presence of physiologically relevant TG levels at ATP concentrations ≥ 100 μ M (**Fig. 2-5d**), and this threshold was further confirmed under skin-temperature conditions (**Fig. A-23**). Thus, the Poly-CORE module was designed to sustain ATP levels above 100 μ M to ensure reliable TG detection.

For continuous operation, the Poly-CORE module was incorporated upstream of the biosensing chamber within the microfluidic platform. As sweat passes through the PPy/ATP nanostructures, ATP diffuses passively into the fluid (**Fig. 2-5e**), maintaining cofactor availability. In static conditions, ATP depletion leads to rapid signal decay, confirming that periodic ATP refreshment is essential for continuous enzymatic function; dynamic in vitro measurements demonstrated that replenishing ATP restored the TG signal following depletion (**Fig. 2-5f**). We next characterized the long-term ATP release behavior of the Poly-CORE module (**Fig. 2-5g**). The optimized PPy/ATP structures sustained ATP concentrations above the biosensing limit for more than 20 hours, supporting prolonged operation. This defines the effective operational lifetime of the system under continuous exposure conditions, with the actual duration dependent on sweat-rate-driven depletion kinetics. By fixing electrodeposition time to 10 minutes, we evaluated structures synthesized under different current densities (0.075, 0.15, 0.3 and 1.0 mA cm⁻²), which exhibited distinct release profiles (**Figs. A-24 and A-25**). Moreover, modifying electrodeposition solution

compositions further tuned cofactor-release kinetics (**Fig. A-25**), highlighting the versatility of this approach for other cofactor-dependent enzymatic reactions.

Based on the TG sensor's dynamic range, PPy/ATP structures prepared at current densities $\geq 0.15 \text{ mA cm}^{-2}$ provided optimal performance. Flow-dependent studies confirmed that higher sweating rates led to reduced ATP concentrations in the sensing chamber due to accelerated mass transfer (**Fig. 2-5h**). Nonetheless, the mean ATP concentration remained above the biosensing limit ($1,494 \pm 163 \text{ } \mu\text{M}$, $646 \pm 111 \text{ } \mu\text{M}$, and $180 \pm 14 \text{ } \mu\text{M}$ at flow rates of 0.3, 0.5, and $1.0 \text{ } \mu\text{L min}^{-1}$, respectively), sufficient for continuous TG detection. We further evaluated the effects of varying sweat rate cycles, mimicking physiological fluctuations in sweat production, on the ATP release kinetics of the Poly-CORE structure (**Fig. A-26**). In addition, ATP release was evaluated under repeated hydration–dehydration cycles to mimic on-body use conditions, demonstrating consistent release behavior across cycles (**Fig. A-27**). These findings define key design constraints for reliable operation, namely maintaining ATP concentrations above the enzymatic threshold through controlled loading density and release kinetics that accommodate physiological variations in sweat rate. In addition, because ATP acts as a dopant within the PPy matrix, its release can be electrochemically stimulated to modulate release kinetics. Application of a -0.8 V potential for 15 min following 1 h of passive release increased the ATP concentration from $\sim 450 \text{ } \mu\text{M}$ to $\sim 1 \text{ mM}$ (**Fig. A-28**), demonstrating that electrochemical stimulation can dynamically regulate cofactor availability and enable adaptive control under varying physiological conditions.

To enable real-time calibration, we developed an MWCNT–based ATP sensor that detects ATP oxidation via differential pulse voltammetry (DPV) (**Fig. 2-5i**). Incorporating MWCNT onto the inkjet-printed carbon electrodes enhanced electron transfer (**Fig. 2-5j** and **Fig. A-29**), while a Nafion coating improved analyte preconcentration and sensitivity. The sensor exhibited a clear oxidation peak near 1.25 V and a sensitivity of $1.29 \text{ } \mu\text{A cm}^{-2} \text{ } \mu\text{M}^{-1}$ (**Fig. 2-5k** and **Fig. A-30**) and maintained high accuracy across varying pH values (**Fig. A-31**). The selectivity of the ATP sensor was further evaluated both in vitro and in human sweat, demonstrating minimal interference from common sweat constituents at the ATP oxidation

potential (**Fig. A-32**). Validation against UV–vis spectrophotometry confirmed excellent agreement between methods (**Fig. 2-5l**).

Combining the TG enzymatic sensor, Poly-CORE ATP release system, and MWCNT ATP sensor enabled continuous TG detection with real-time cofactor calibration (**Fig. 2-5m**). Because ATP concentration is directly measured by the integrated ATP sensor, cofactor availability can be continuously monitored, providing real-time feedback on system state and enabling identification of conditions where ATP depletion may affect sensing performance. Correlating ATP concentrations with TG sensor output allowed generation of an interpolation-based calibration model that compensates for temporal ATP variation (**Fig. A-33**, **Supplementary Note 3**, and **Table A-1**). This calibration model was constructed over the operational ATP range produced by the optimized Poly-CORE protocol used for continuous TG sensing, whereas higher ATP concentrations observed during upper-limit release-capacity studies were used to characterize the reservoir limits rather than define the TG calibration domain; the TG sensor response at elevated ATP concentrations was evaluated separately (**Fig. A-34**). Additional validation of integrated dynamic TG sensing at skin temperature is provided in **Fig. A-35**. This integrated system demonstrates the feasibility of continuous cofactor-refreshing biosensing, establishing a generalizable strategy for long-term monitoring of complex enzymatic reactions in wearable bioelectronics.

2.4 Continuous epidermal lipid profiling

The wearable device enables continuous detection of glucose, CH, and TG for comprehensive assessment of cardiometabolic health (**Fig. 2-6a** and **Figs. A-36–A-38**). To demonstrate its practical capability, we evaluated the device’s response to various dietary conditions by monitoring dynamic changes in epidermal lipid levels (**Fig. 2-6b**). Sweat was locally induced using a miniaturized IP module, which applies a low-intensity current across two hydrogel electrodes in contact with the skin. The current drives diffusion of the cholinergic agent carbachol into the dermis, activating sweat glands via the cholinergic pathway (**Fig. 2-6c**). As described previously⁴¹, this process yields continuous sweat flow that refreshes both the sensor chamber and SR microchannels (**Fig. 2-6d**).

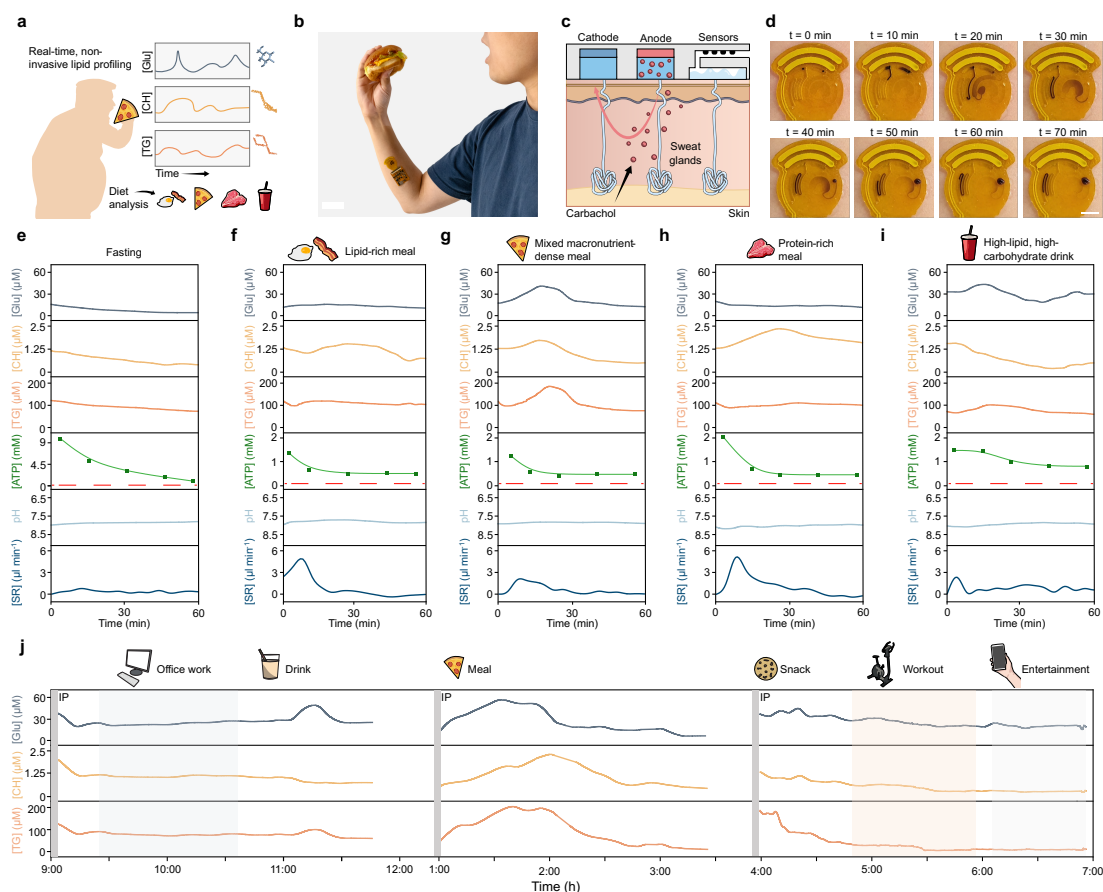


Figure 2-6. Continuous epidermal lipid profiling. **a**, Schematic overview of on-body lipid profiling during different dietary challenges. **b**, Optical image of integrated wearable patch attached to the forearm of a participant. **c**, Conceptual illustration of the iontophoresis (IP) module within the microfluidic system for localized sweat induction and collection. **d**, Sequential optical images showing sweat filling and refreshment in the designed microfluidic sensor chamber and SR channels following IP. Scale bar, 3 mm. **e–i**, Continuous epidermal profiling of glucose, CH, and TG under different dietary conditions: fasting (**e**), lipid-rich meal (**f**), mixed macronutrient-dense meal (**g**), protein-rich meal (**h**), and high-lipid, high-carbohydrate drink (**i**). **j**, Long-term on-body monitoring of glucose, CH, and TG during daily habitual activities.

Cardiometabolic health is strongly influenced by factors such as genetics, metabolic rate, insulin sensitivity, and lifestyle, with dietary composition playing a central role in lipid regulation⁴². Nutritional balance directly affects blood sugar and lipid metabolism, impacting

both disease prevention and management^{4,43}. To evaluate diet-dependent metabolic dynamics, we continuously profiled biomarker levels during and after consumption of four meal types, with the fasting state serving as a control (**Fig. 2-6e**): a lipid-rich meal (**Fig. 2-6f**), a mixed macronutrient meal (pizza; **Fig. 2-6g**), a protein-dense meal (**Fig. 2-6h**), and a high-lipid, high-carbohydrate liquid drink (milkshake; **Fig. 2-6i**). Notably, sweat rate during the fasting condition was lower than in postprandial states, likely reflecting reduced hydration status associated with fasting. Paired fasting and postprandial measurements of CH and TG in sweat and blood were performed for a representative condition to support the observed trends, showing consistent directional changes between the two matrices (**Fig. A-39**). Each meal was consumed ~30 minutes before sensor placement.

The epidermal sensors captured distinct postprandial trends. It is important to note that the temporal profiles of sweat CH and TG are not governed solely by passive diffusion from blood. Accordingly, similar peak timing relative to glucose does not imply comparable transport kinetics, but rather reflects the combined effects of transport, secretion, and partitioning processes within the skin and sweat matrix. Glucose levels rose sharply following the mixed macronutrient meal and high-lipid, high-carbohydrate drink, consistent with their higher glycemic index and rapid absorption of simple sugars. In contrast, meals enriched in fats, cholesterol, and protein elicited minimal changes in sweat glucose due to their lower carbohydrate content.

Interestingly, both CH and TG levels demonstrated pronounced variability across meal types. During fasting, CH and TG levels gradually decreased, suggesting localized lipid depletion within the sweat glands and epidermis due to slow diffusion from deeper tissue. In contrast, postprandial lipid-rich, mixed, protein-rich, and liquid meals induced substantial elevations in both biomarkers. These dynamic responses likely result from a combination of (i) lipid diffusion from circulation, (ii) lipoprotein remodeling and efflux, and (iii) enhanced sebaceous lipid secretion that mixes with eccrine sweat^{7,44,45}.

The temporal patterns of CH and TG also reflected the macronutrient composition of each meal. High-fat meals such as bacon and eggs (**Fig. 2-6f**) or ground beef (**Fig. 2-6h**) produced sustained increases in CH and TG. In contrast, the high-fat, high-carbohydrate drink (**Fig. 2-**

6i) induced a faster, transient response due to rapid gastrointestinal absorption, such that the measurements capture a later stage of the postprandial profile in which CH and TG levels begin to decline. The pizza meal (**Fig. 2-6g**), rich in both carbohydrates and fats, elicited the largest combined rise in epidermal CH and TG (**Fig. A-40**), likely driven by hepatic lipoprotein production following digestion.

It is important to note that individual physiological factors such as age, body composition, fitness, and prior meals also contributed to inter-subject variability^{46,47}. Such personalized metabolic signatures highlight the value of continuous monitoring. By capturing real-time fluctuations in lipid and glucose biomarkers, this wearable platform provides individualized insights into diet-induced metabolic responses and enables personalized cardiometabolic health assessment (**Fig. 2-6j**).

2.5 Conclusions

This study demonstrates a wearable epidermal platform for continuous, non-invasive monitoring of glucose, cholesterol, and triglycerides, metabolic markers relevant to cardiometabolic health. Glucose, cholesterol, and triglyceride sensing were achieved through single-step, dual-step, and cascading enzymatic reactions, respectively. Unlike glucose and cholesterol detection, triglyceride sensing requires ATP as an external cofactor, creating a major barrier for continuous wearable operation because ATP is consumed during the reaction and cannot be regenerated electrochemically at the sensing interface.

To address this limitation, we developed and integrated a polymeric cofactor-releasing module, termed Poly-CORE, within the microfluidic sensing system. The module uses an electrodeposited PPy/ATP reservoir to continuously replenish ATP in the sensing chamber, while a co-located MWCNT-based ATP sensor enables real-time tracking of cofactor availability. This configuration sustained ATP concentrations above the enzymatic threshold required for triglyceride detection and enabled long-term stable operation of the multistep enzymatic cascade. Because ATP release can be tuned through electrodeposition conditions and electrochemical stimulation, the Poly-CORE architecture provides a modular route for controlling cofactor delivery in wearable enzymatic sensors.

The platform further integrates carbachol-based iontophoresis for autonomous sweat induction, along with pH and sweat-rate sensors for physiological calibration. In on-body meal and activity studies, the wearable system captured dynamic changes in sweat glucose, cholesterol, and triglycerides in response to dietary intake and habitual activity. These results highlight the value of real-time biochemical monitoring for resolving metabolic dynamics that are inaccessible through isolated blood measurements alone.

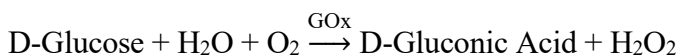
Beyond lipid sensing, this work addresses a broader limitation in wearable biochemical monitoring: the continuous operation of multistep enzymatic cascades that rely on sacrificial cofactors. By coupling controlled ATP release with real-time cofactor sensing, the Poly-CORE strategy expands wearable biosensing beyond the oxidase-based chemistries that currently dominate the field and establishes a generalizable framework for continuous monitoring of analytes that require non-regenerable cofactors.

Appendix

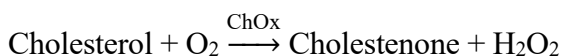
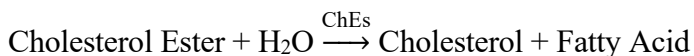
Supplementary Note 1 | Enzymatic reaction mechanisms for lipid profiling

Determination of glucose, cholesterol (CH), and triglycerides (TG) was achieved through a series of enzymatic reaction as integrated with a Prussian blue–nickel hexacyanoferrate (PB/NiHCF)–based working electrode. The specific enzymatic pathways are described below.

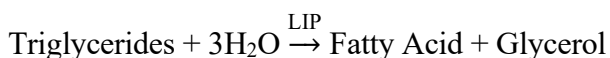
Glucose detection was accomplished using glucose oxidase (GOx), which catalyzes the following reaction:



Cholesterol esters were enzymatically hydrolyzed and oxidized through a dual-step reaction involving cholesterol esterase (ChEs) and cholesterol oxidase (ChOx):



Triglycerides were detected via a three-enzyme cascading reaction consisting of lipase (LIP), glycerol kinase (GK), and glycerol-3-phosphate oxidase (G3PO):



Hydrogen peroxide generated in each pathway was subsequently detected via the PB/NiHCF electrode transducer.

Since Prussian blue (PB) exhibits instability under alkaline conditions—similar to the mildly basic pH found in sweat after iontophoresis (IP)—nickel hexacyanoferrate (NiHCF) was incorporated as a catalytic stabilizer to enhance electrochemical durability and maintain efficient H_2O_2 transduction. Optimization of the PB/NiHCF hybrid transducer was extensively characterized in previous studies^{1,2}.

Supplementary Note 2 | SR real-time calculation using impedance-based three electrodes

The sweat rate was determined using a dual-pair electrode configuration composed of three electrodes (E1, E2, and E3). E1 and E2 served as the ionic strength sensing pair, while E2 and E3 formed an interdigitated structure for sweat volume sensing. As sweat filled the microfluidic channel, the impedance between E2 and E3 decreased due to the increased conductive volume, whereas its magnitude was also affected by the ionic concentration of the sweat. Therefore, the E2–E3 admittance alone could not independently represent the sweat volume without correcting for the effect of ionic strength.

To address this coupling, the ionic strength was first quantified from the impedance between E1 and E2, which primarily reflects the solution ionic strength near the channel entrance. A calibration map was constructed to correlate the E2–E3 admittance with both the ionic strength (from E1–E2) and the filled sweat volume (**Fig. A-11c**). During real-time measurements, the instantaneous ionic strength value obtained from E1–E2 was used to correct the E2–E3 admittance signal, allowing extraction of the volume-dependent component. The corresponding sweat volume was estimated from the established calibration surface, and the sweat rate was calculated as the temporal derivative of the volume ($\Delta V / \Delta t$), where V and t denote the volume and time, respectively. This dual-sensing scheme

effectively decouples the effects of ionic strength and volume, enabling accurate, real-time determination of sweat rate across varying electrolyte concentrations.

Supplementary Note 3 | Determination of triglyceride concentrations from sensor data

Accurate determination of TG concentrations is challenging because the cascading enzymatic reaction exhibits a dynamic dependence on the availability of the external cofactor adenosine triphosphate (ATP). To achieve continuous and reliable TG quantification, a real-time calibration framework was developed to integrate the electrochemical TG sensor signal with instantaneous ATP concentration measurements from the cofactor-releasing (Poly-CORE) module (**Fig. A-23**).

During calibration, phosphate-buffered saline (PBS) samples were collected from the outlet of the microfluidic channel at various time points, and their ATP concentrations were quantified. The corresponding TG sensor current responses were recorded simultaneously. These paired datasets were then used to construct a calibration matrix and table (**Table A-1**) that relates TG sensor current to ATP concentration across a range of TG levels.

MATLAB was used to calibrate TG concentrations by combining real-time signals from the TG enzymatic sensor and the ATP cofactor sensor. The program referenced an interpolation matrix derived from experimental calibration data that relates sensor current to ATP concentration at different TG levels. For each time point, the measured ATP concentration was compared with reference values in the matrix to locate the nearest cofactor range, and the corresponding sensor current interval was identified. When both parameters matched reference points, the corresponding TG concentration was directly assigned; otherwise, bilinear interpolation between neighboring data points was performed to estimate the TG level. This approach dynamically compensated for fluctuations in ATP availability, enabling accurate and continuous TG quantification during on-body measurements (**Fig. A-24**).



Fig. A-1. Images of the wearable cardiometabolic tracking patch. Photographs showing the fabricated epidermal lipid-profiling patch used for continuous cardiometabolic monitoring. Scale bars, 10 mm (top row), 100 mm (bottom row).

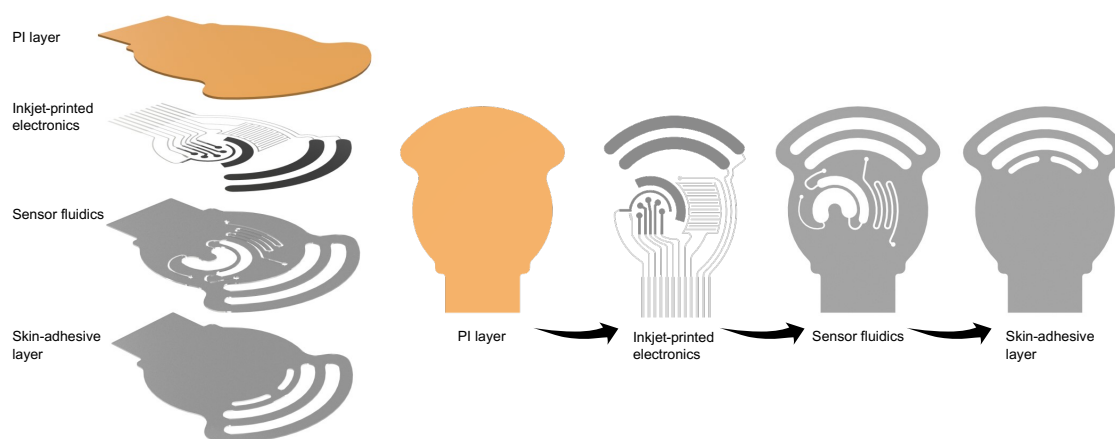


Fig. A-2. Structural layout of the wearable sweat-sensing patch. Schematic illustration of the multilayer configuration of the wearable patch, comprising a skin-adhesive layer, a

microfluidic channel layer, and a polyimide (PI) substrate with inkjet-printed electronic components.

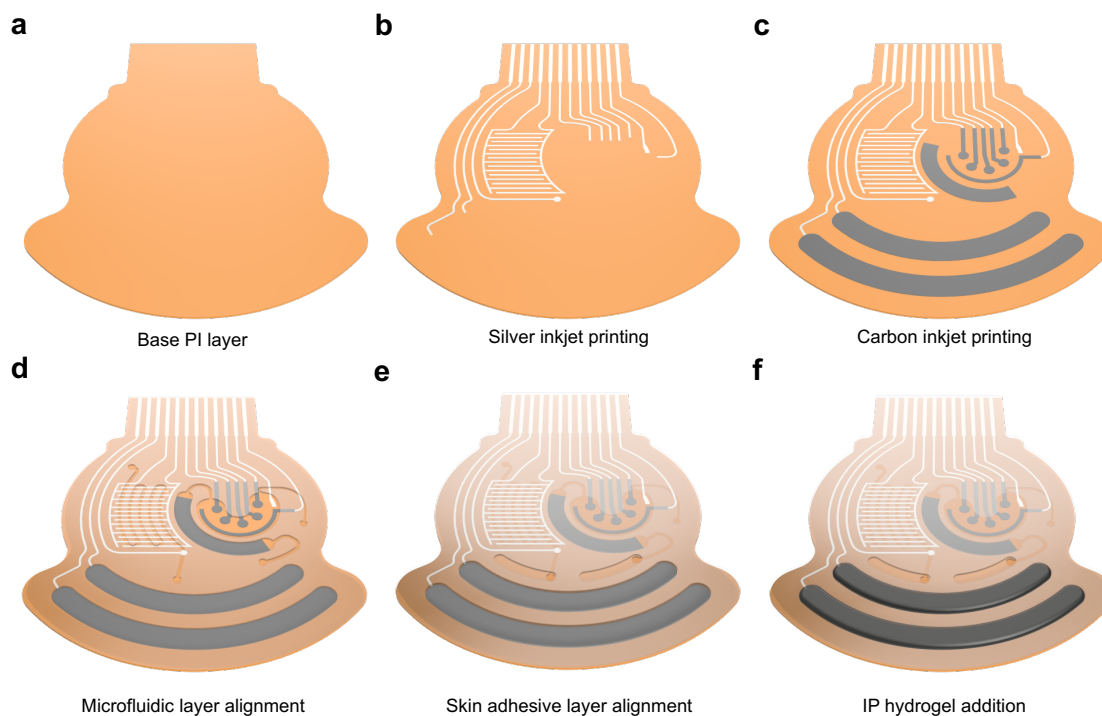


Fig. A-3. Fabrication process of the wearable sweat-sensing patch. Stepwise illustration of the fabrication sequence for the wearable patch. **a**, Cleaning of the polyimide (PI) substrate. **b**, Inkjet printing of the silver interconnects. **c**, Inkjet printing of the carbon working electrodes. **d**, Alignment and attachment of the microfluidic channel layer. **e**, Alignment and attachment of the skin-adhesive layer. **f**, Placement of iontophoresis (IP) hydrogels onto the IP electrodes.

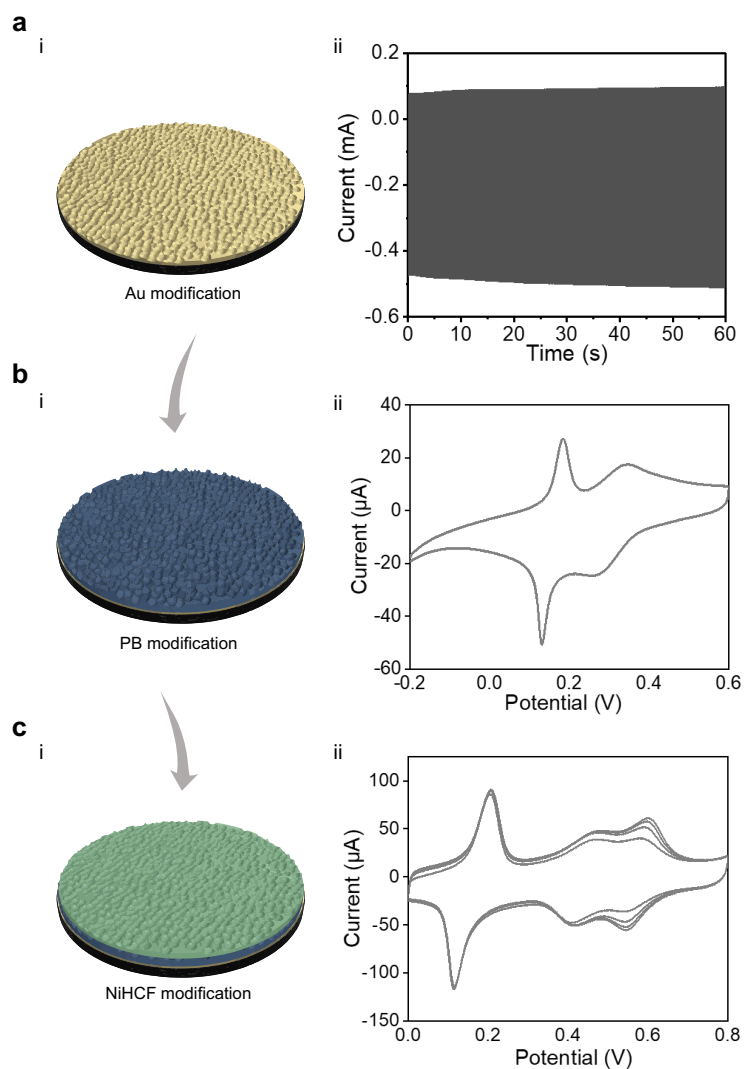


Fig. A-4. Stepwise electrode surface modification. **a**, Deposition of a gold (Au) layer on the carbon electrode and the corresponding multi-potential response. **b**, Electrodeposition of a PB layer onto the Au surface and the associated cyclic voltammetry (CV) curve. **c**, Deposition of a NiHCF layer onto the PB-modified electrode and its corresponding CV response.

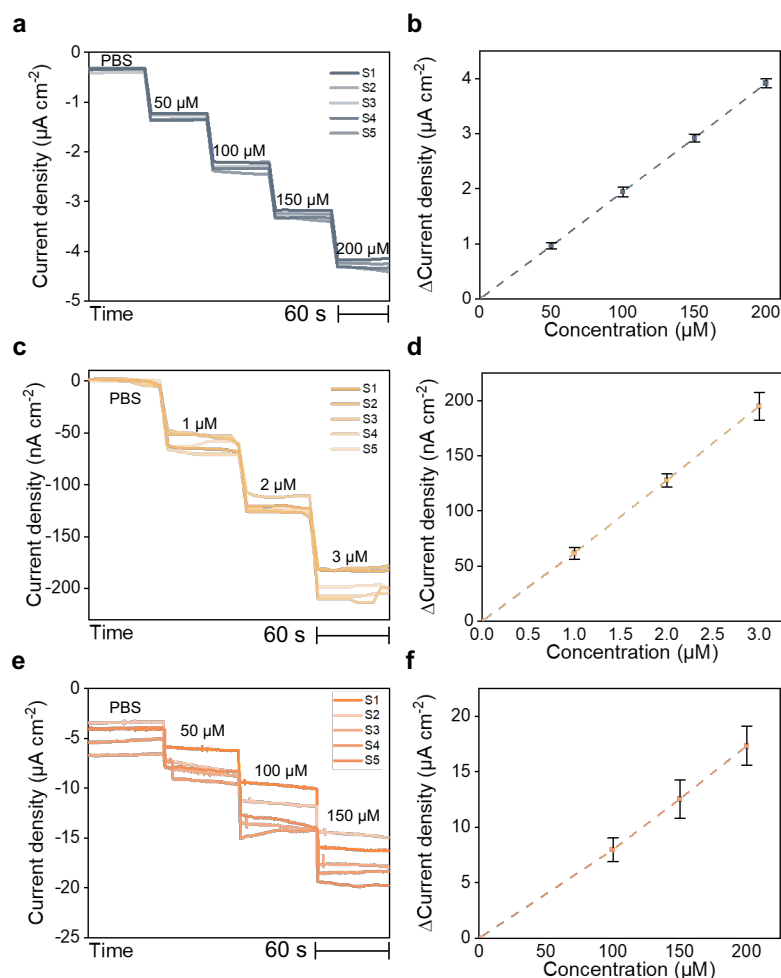


Fig. A-5. Calibration and reproducibility of enzymatic biosensors. **a**, Amperometric responses of five glucose sensors to increasing glucose concentrations. **b**, Corresponding linear calibration curve showing normalized current density versus glucose concentration. **c**, Amperometric responses of five CH sensors to increasing cholesterol concentrations. **d**, Normalized linear calibration of CH sensor current density. **e**, Amperometric responses of five TG sensors to varying triglyceride concentrations. **f**, Corresponding normalized calibration curve for TG detection. Error bars in **b,d,f** represent s.d. from the mean from 5 sensors.

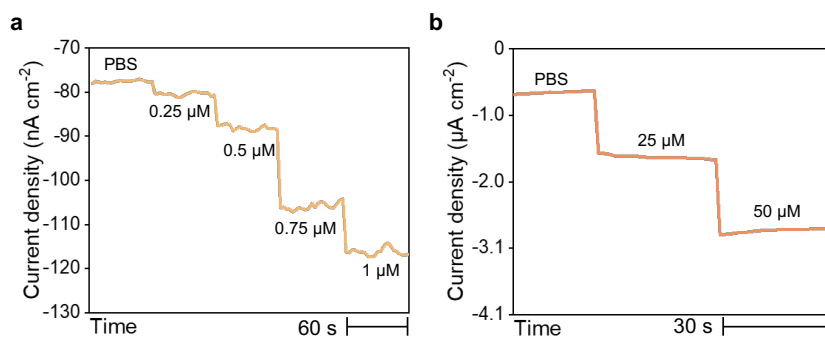


Fig. A-6. Low-concentration sensitivity of CH and TG sensors. **a**, Amperometric response of the CH sensor at concentrations below 1 μM . **b**, Amperometric response of the TG sensor at concentrations below 50 μM .

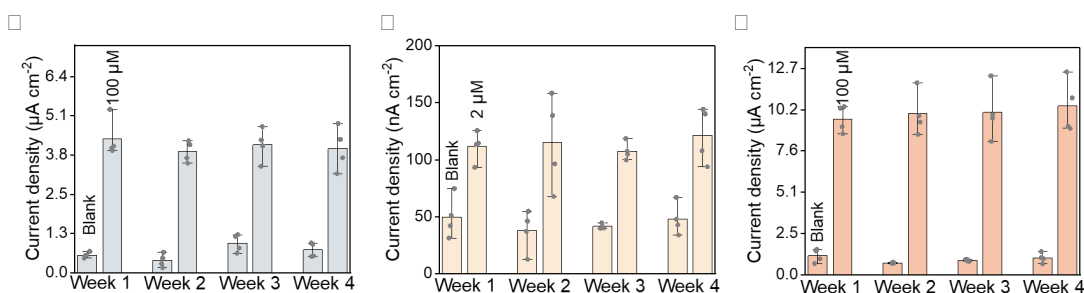


Fig. A-7. Shelf stability of enzymatic biosensors. **a–c**, Four-week stability analysis of the glucose, CH, and TG sensors stored under 4 °C. Error bars represent s.d. from the mean from 4 sensors.

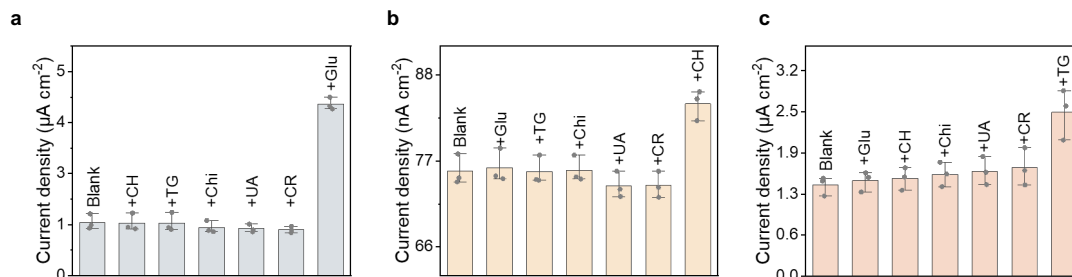


Fig. A-8. Selectivity of electrochemical sensors in the absence of target analytes. **a**, Glucose sensor response to common sweat interferents without glucose present and after addition of 100 μM glucose. **b**, CH sensor selectivity sensor response to common sweat interferents without CH present and after addition of 1 μM CH. **c**, TG sensor selectivity sensor response to common sweat interferents without CH present and after addition of 50 μM TG. Tested interferents include choline (Chi) (50 μM), glucose (50 μM for CH and 200 μM for TG), uric acid (UA) (40 μM), TG (50 μM), CH (3 μM), and creatine (CR) (15 μM). Error bars represent s.d. of the mean from 3 sensors.

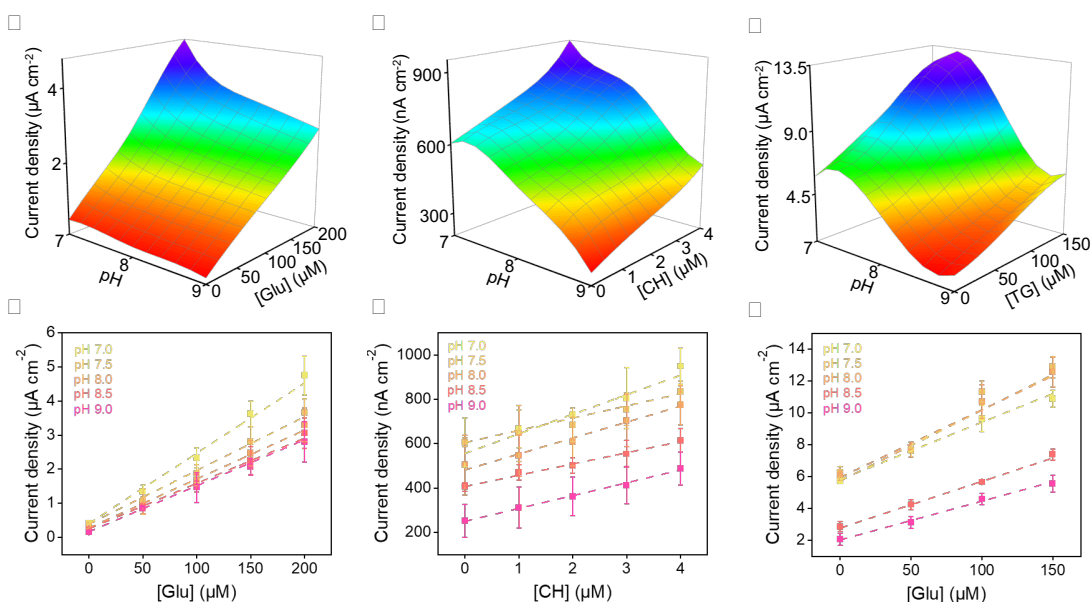


Fig. A-9. pH dependency of electrochemical biosensor performance. **a–c**, Three-dimensional plots showing the effect of pH on the electrochemical responses of glucose (a), CH (b), and TG (c) sensors. **d–f**, Corresponding linear pH-dependent responses of glucose (d), CH (e), and TG (f) sensors. Error bars represent s.d. of the mean from 4 sensors.

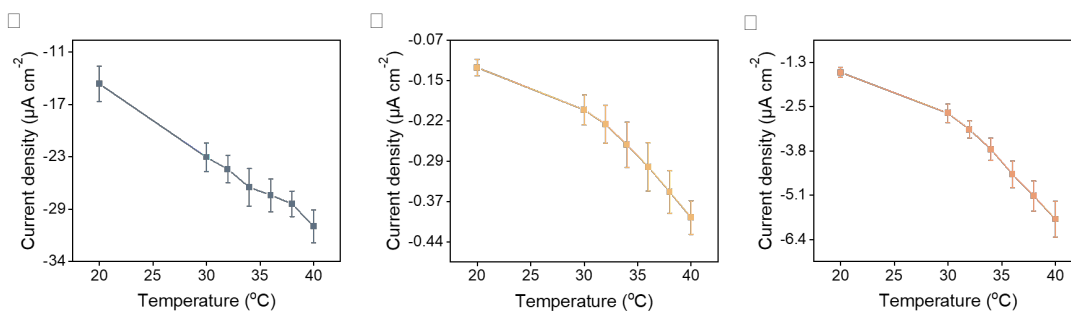


Fig. A-10. Temperature dependence of enzymatic sensors. **a**, Temperature-dependent response of the glucose sensor in 150 μM glucose. **b**, Temperature-dependent response of the CH sensor in 3 μM CH. **c**, Temperature-dependent response of the TG sensor in 50 μM TG. Error bars represent s.d. of the mean from 3 sensors.

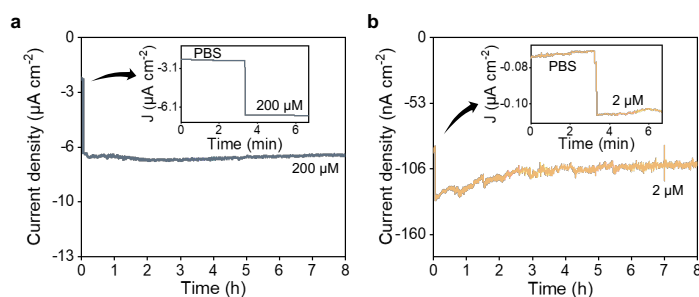


Fig. A-11. Operational stability of glucose and CH sensors over 8 h. **a**, Continuous amperometric response of the glucose sensor demonstrating stable signal in 200 μM glucose. **b**, Continuous amperometric response of the CH sensor demonstrating stable signal in 2 μM CH.

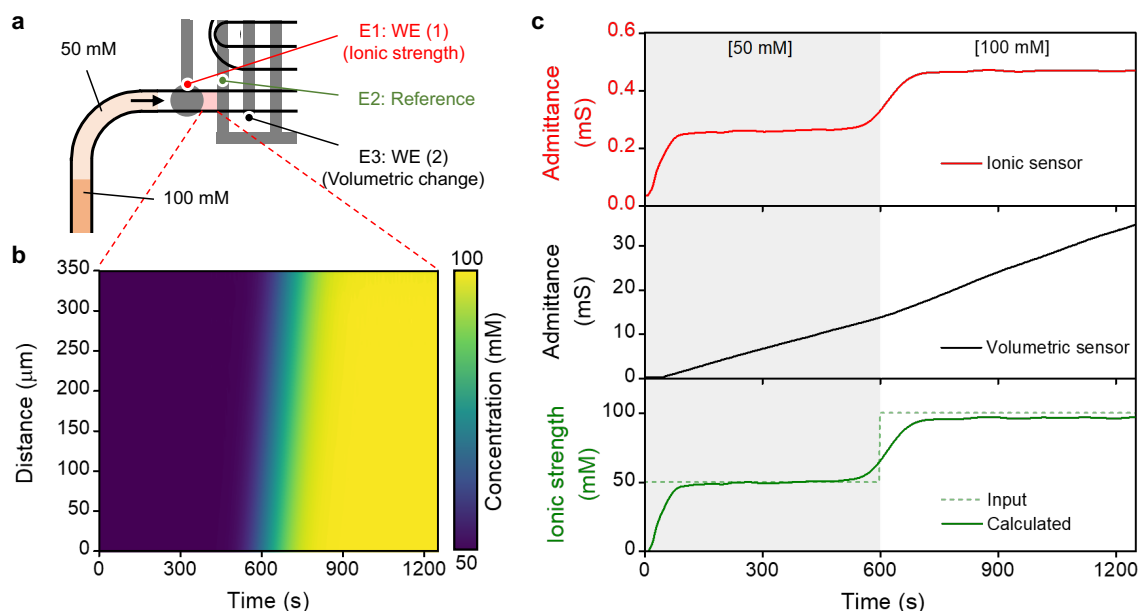


Fig. A-12. Effect of ionic diffusion and drift in the sweat rate sensor. **a**, Schematic of the two-channel sweat-rate sensor showing the positions of the working electrodes (WEs) when NaCl solutions of different concentrations (50 and 100 mM) were introduced. **b**, Simulated concentration distribution between the electrodes in an ionic strength sensor as a mixed NaCl solution fills the channel. **c**, Experimental response of sweat rate sensor, showing admittance from WE (1), admittance from WE (2), and comparison between the input solution concentration and the calculated ionic strength (pH 7.4, at 25 °C).

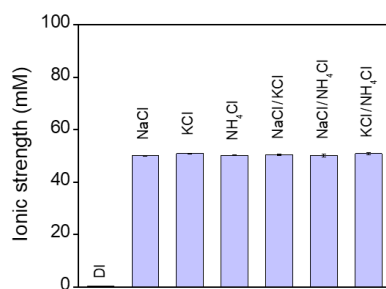


Fig. A-13. Selectivity of the ionic strength sensor. Response of the ionic-strength module within the sweat-rate sensor to different ionic species (NaCl, KCl, CaCl_2 , NaH_2PO_4 , and Na_2HPO_4) at identical concentrations (50 mM) (pH 7.4, at 25 °C).

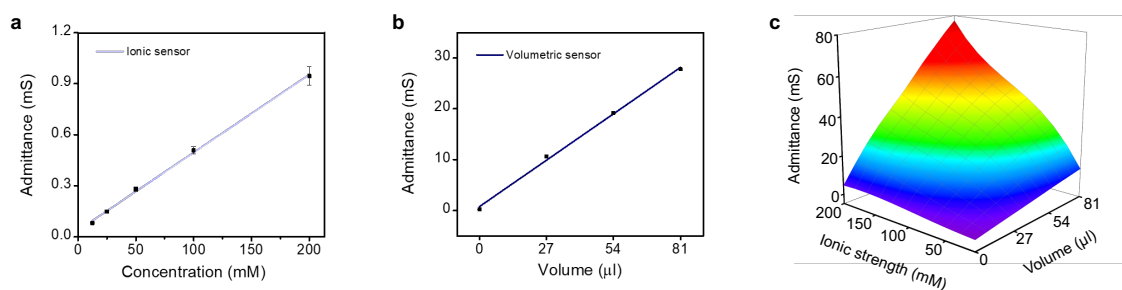


Fig. A-14. Calibration of sweat rate sensor. a–c, Calibration plots showing the relationship between admittance and ionic strength measured by the ionic sensor ($n = 5$) (a), sweat volume measured by the volumetric sensor (b), and combined three-dimensional plot illustrating admittance as a function of both ionic strength and sweat volume (c).

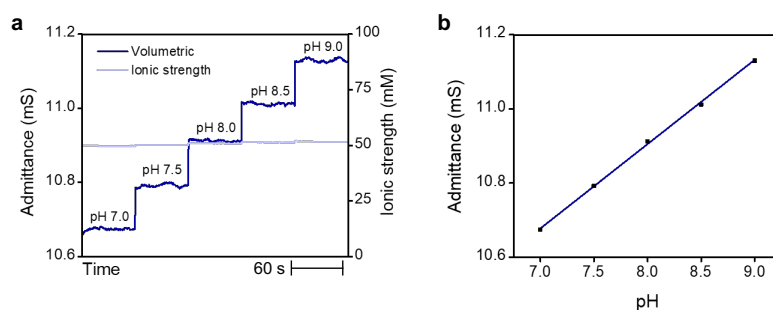


Fig. A-15. pH dependence of the sweat rate sensor. a, Admittance response of ionic strength and volumetric change in sweat rate sensor as 50 mM NaCl with varying pH levels. b, Calibration curve showing the relationship between volumetric admittance and pH.

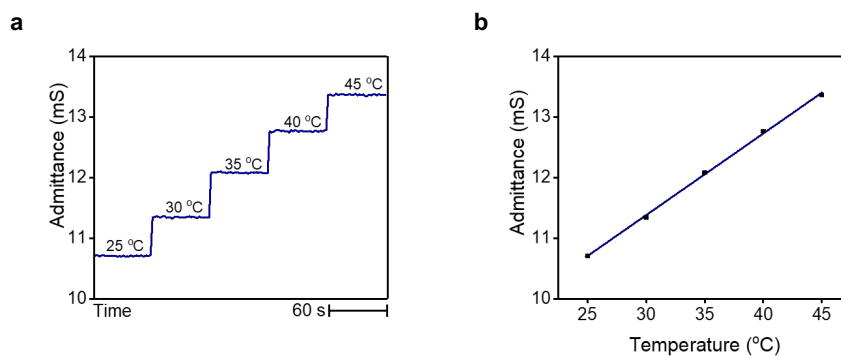


Fig. A-16. Temperature dependence of the sweat-rate sensor. **a**, Admittance response of the sweat-rate sensor under varying temperatures (25–45 °C). **b**, Corresponding calibration plot showing a linear relationship between admittance and temperature.

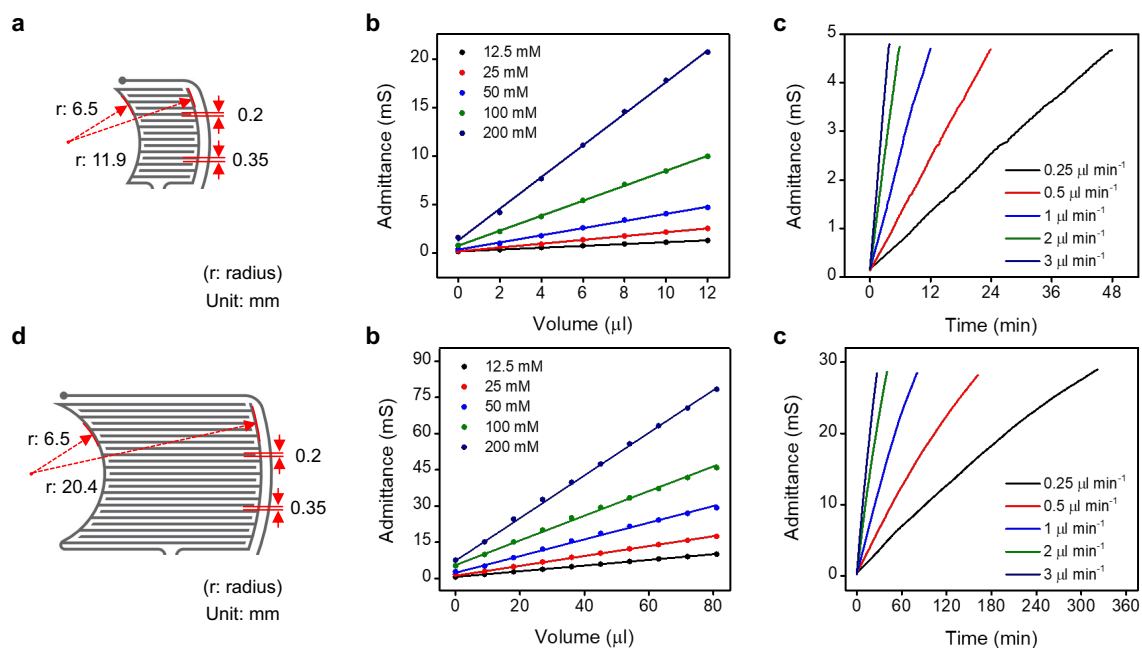


Fig. A-17. Characterization of the sweat rate sensor. **a**, Schematic of the three-electrode sweat-rate sensor with a 12 μl volumetric sweat reservoir. **b**, Admittance responses at different NaCl concentrations (12.5–200 mM). **c**, Sensor response under varying flow rates (0.25–3 $\mu\text{l min}^{-1}$) while the channel was filled with 50 mM NaCl. **d–f**, Repeated characterization of a sensor design incorporating an 81 μl volumetric sweat reservoir, showing consistent admittance trends across ionic strengths and flow rates.

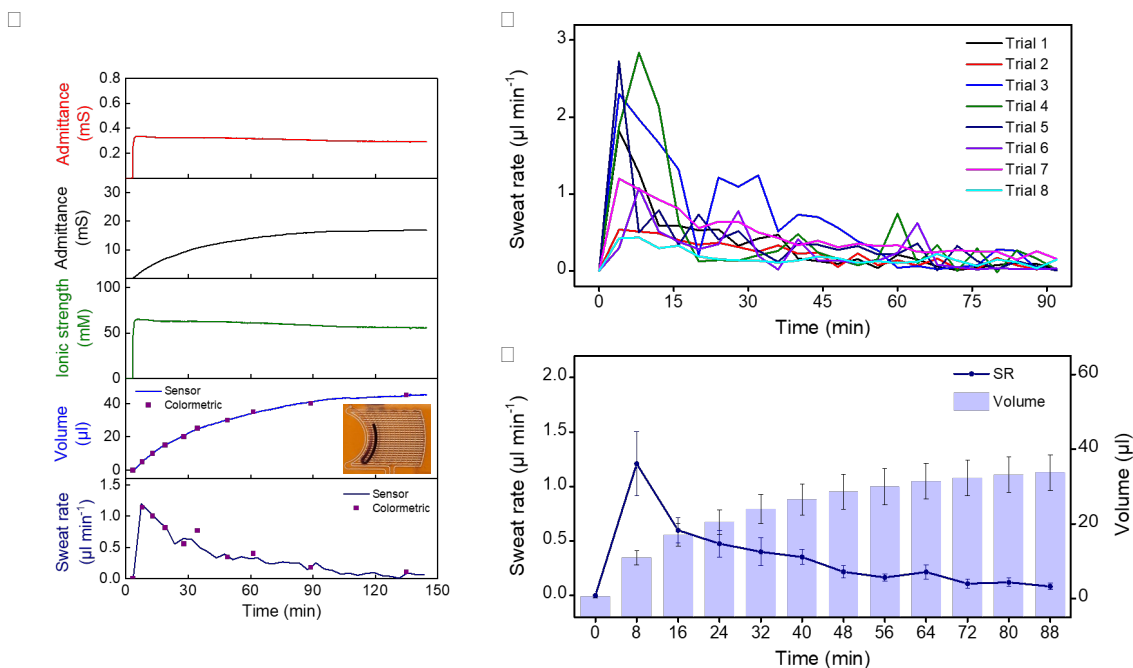


Fig. A-18. On-body evaluations of the sweat rate sensor. a, Representative on-body measurement from a single subject showing admittance, ionic strength, volume, and calculated sweat rate during rest. **b,** Sweat rate profiles from multiple subjects ($n = 8$) demonstrating inter-individual variability in perspiration dynamics. **c,** Analysis of cumulative sweat volume and corresponding sweat rate.

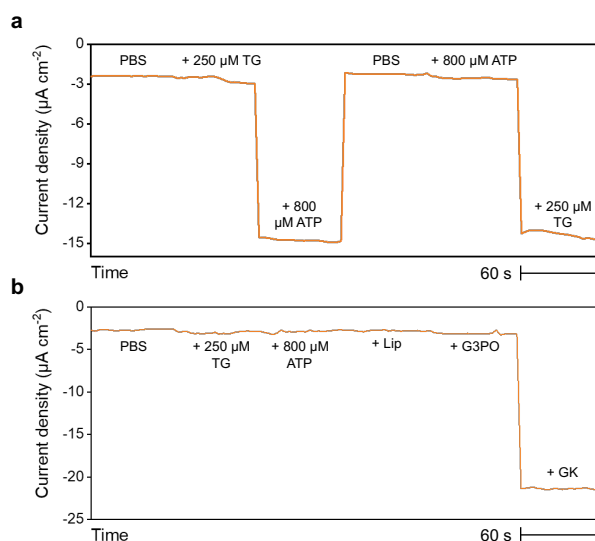


Fig. A-19. Validation of the TG enzymatic sensing mechanism. **a**, Amperometric responses showing sequential addition of ATP to a solution containing TG and, inversely, TG to a solution containing ATP, confirming the cofactor-dependent nature of the TG enzymatic reaction ($[TG] = 250 \mu M$; $[ATP] = 800 \mu M$). **b**, Amperometric response of a bare electrode following stepwise addition of TG detection enzymes—lipase (LIP), glycerol kinase (GK), and glycerol-3-phosphate oxidase (G3PO)—in a mixed TG/ATP solution, demonstrating the necessity of the complete enzymatic cascade for signal generation ($[TG] = 250 \mu M$; $[ATP] = 800 \mu M$).

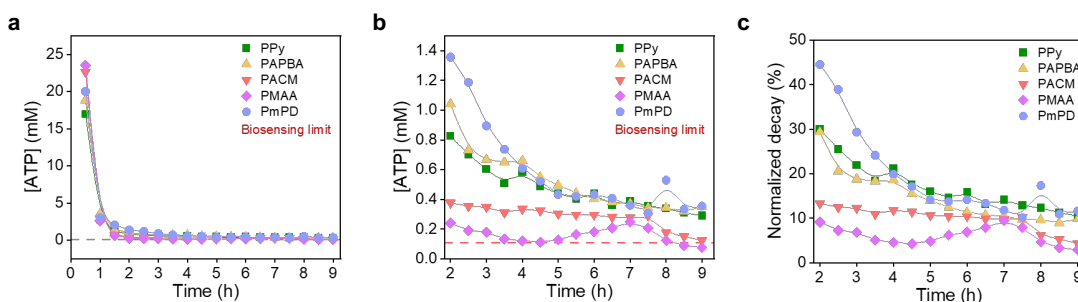


Fig. A-20. ATP release kinetics from polymer matrices under matched electropolymerization conditions. Comparison of ATP release profiles from electrodeposited polymer films including PPy, poly(3-aminophenylboronic acid) (PAPBA), polyacrylamide (PAM), polymethacrylic acid (PMAA), and poly(m-phenylenediamine) (PmPD). All films were electrodeposited galvanostatically in the presence of ATP to enable incorporation during film formation. Deposition was performed under identical conditions (0.14 M monomer, 0.07 M LiClO₄, 0.1 M ATP; 30 min) to allow direct comparison of ATP encapsulation and release behavior. ATP release was measured under passive conditions in PBS. **a,b**, Raw release profiles. **c**, ATP concentrations normalized to the 60 min time point, highlighting differences in initial loading and subsequent decay behavior.

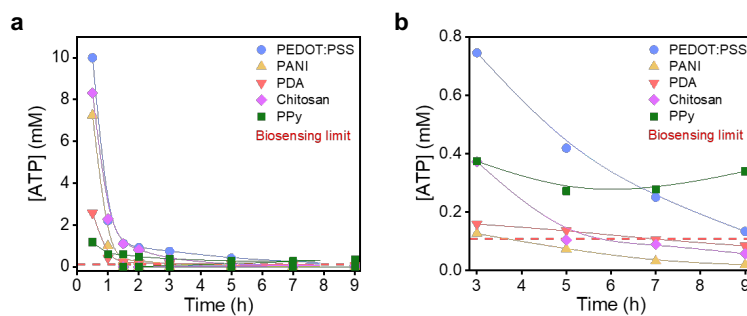


Fig. A-21. ATP release kinetics from representative polymer entrapment matrices. a,b, Comparison of ATP release profiles from polymer coatings including poly(3,4-ethylenedioxythiophene):poly(styrenesulfonate) (PEDOT:PSS), polyaniline (PANI), polydopamine (PDA), chitosan, and PPy. All ATP solutions were prepared at 20 mM. PPy films were electrodeposited galvanostatically in the presence of ATP to enable dopant-mediated incorporation. Chitosan films were electrodeposited potentiostatically (-1.2 V, 120 s) from ATP containing solutions. PEDOT:PSS films were formed by drop-casting with ATP incorporated prior to deposition, while PANI and PDA films were first electrodeposited and subsequently loaded with ATP via post-deposition soaking. ATP release was measured under passive conditions in PBS.

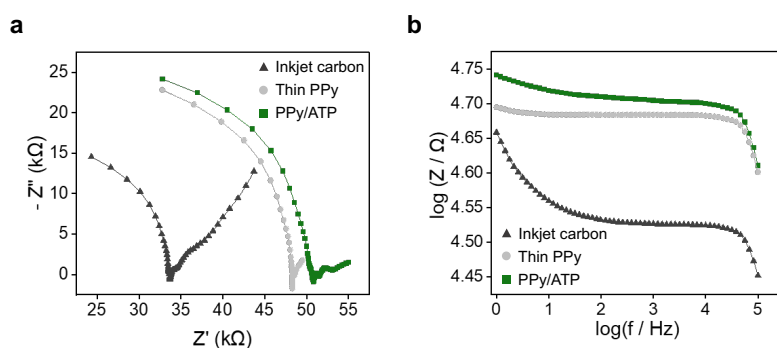


Fig. A-22. Electrochemical impedance characterization of ATP-releasing electrodes. a, Nyquist plots comparing the impedance spectra of the bare inkjet-printed carbon electrode, thin PPy layer, and PPy/ATP nanostructure. **b,** Bode plot of as-prepared ink-jet carbon electrode and after deposition of thin PPy layer and the PPy/ATP nanostructure.

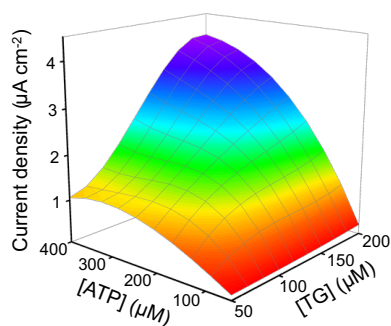


Fig. A-23. ATP-dependent TG sensor response under skin-temperature conditions.

Three-dimensional surface plot showing the relationship between ATP concentration, TG concentration, and amperometric TG sensor response at skin temperature.

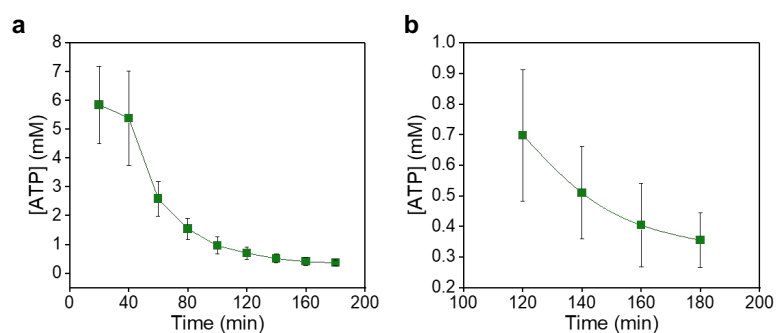


Fig. A-24. Reproducibility of ATP release from PPy/ATP electrodes. **a**, ATP release profiles from PPy/ATP nanostructures. **b**, Expanded view of the release profile between 120 and 180 min. Error bars represent s.d. of the mean from 5 electrodes.

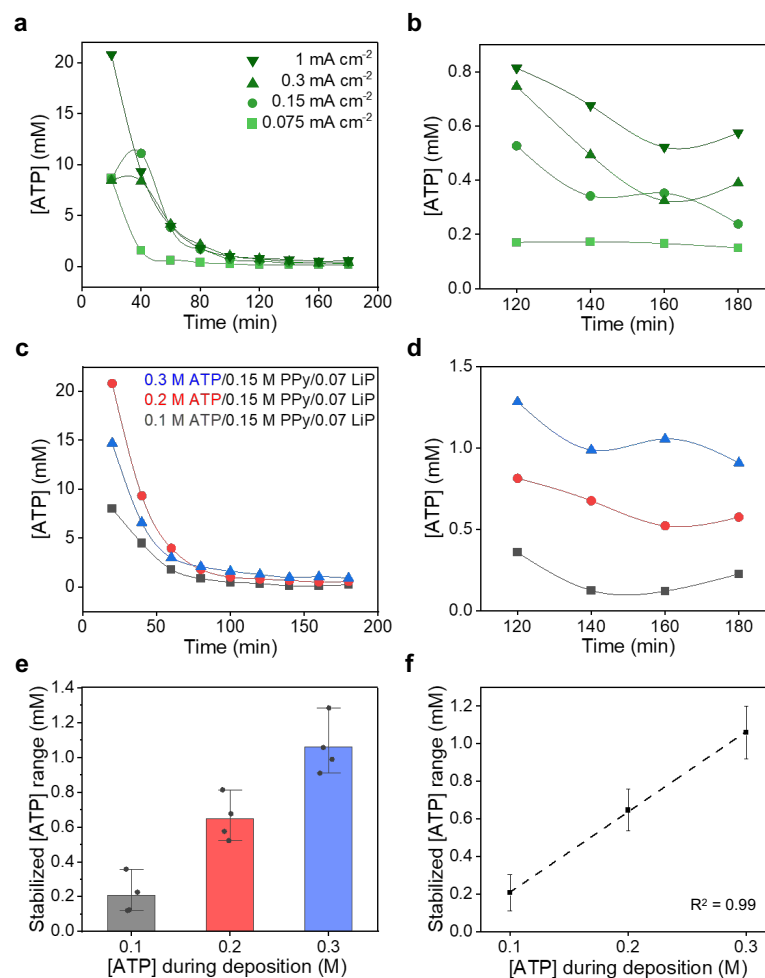


Fig. A-25. Influence of electrodeposition parameters on ATP release from Poly-CORE electrodes. **a,b**, ATP release profile of PPY/ATP structures synthesized at different current densities (**a**) and the corresponding expanded view between 120 and 180 min (**b**). **c,d**, ATP release profiles of PPY/ATP structures synthesized using different ATP concentrations in the electrodeposition solution (0.1, 0.2, and 0.3 M) (**c**) and the corresponding stabilization profiles between 120 and 180 min (**d**). **e**, Averaged stabilized ATP concentrations (120–180 min) plotted as a function of ATP concentration in the electrodeposition bath. **f**, Linear correlation between stabilized ATP concentration and ATP concentration during electrodeposition.

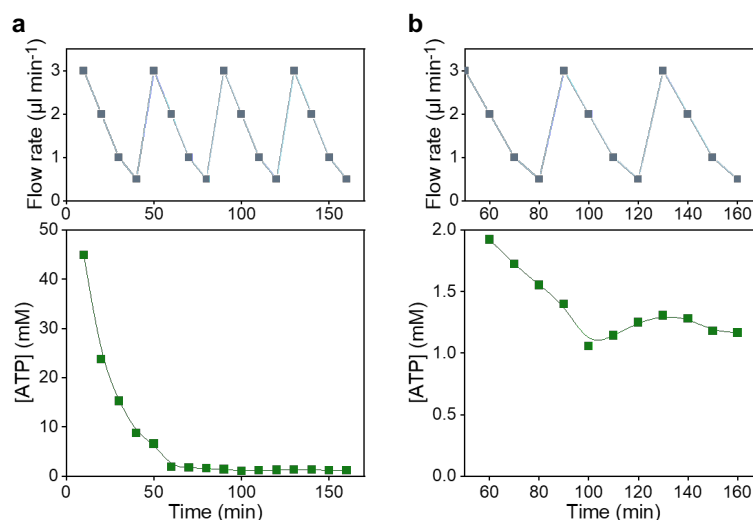


Fig. A-26. Effect of flow-rate variations on ATP release from the Poly-CORE module. ATP releasing profile of the Poly-CORE module under dynamically varying flow conditions. The flow rate was cycled between $3 \mu\text{l min}^{-1}$ and $0.5 \mu\text{l min}^{-1}$ every 40 min to simulate physiological fluctuations in sweat production.

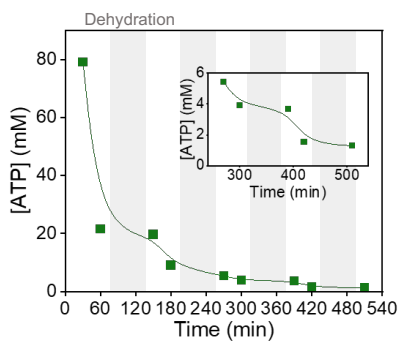


Fig. A-27. ATP release kinetics of the Poly-CORE module under repeated hydration–dehydration cycles. ATP release was measured for 1 h at a flow rate of $0.3 \mu\text{l min}^{-1}$, followed by a 1 h drying period. This cycle was repeated four times to simulate repeated on-body use conditions.

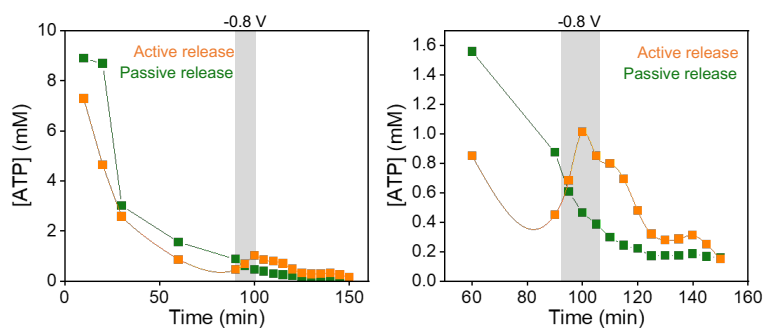


Fig. A-28. Passive and electrochemically stimulated ATP release from PPy/ATP structures. ATP release kinetics from the PPy/ATP module under passive conditions and upon application of a -0.8 V potential for 15 min following 1 h of passive release.

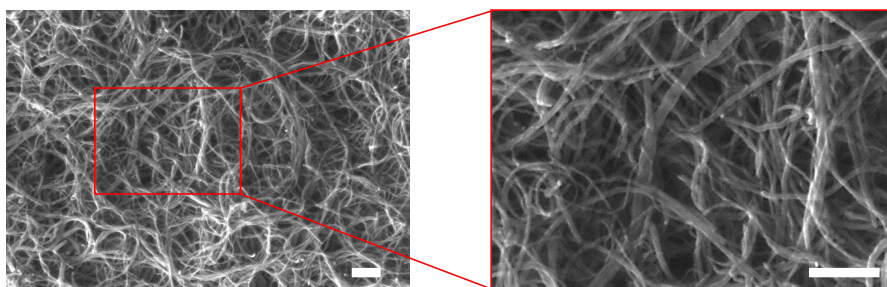


Fig. A-29. SEM imaging of the ATP sensor surface. SEM images of the ATP sensor showing the MWCNT/Nafion composite layer deposited on the inkjet-printed carbon electrode. Scale bars, 200 nm.

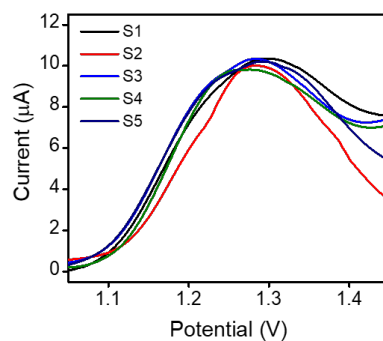


Fig. A-30. Reproducibility of the ATP sensor response. Differential pulse voltammetry (DPV) curves showing reproducible current responses of the ATP sensor measured in 1 mM ATP solution ($n = 5$).

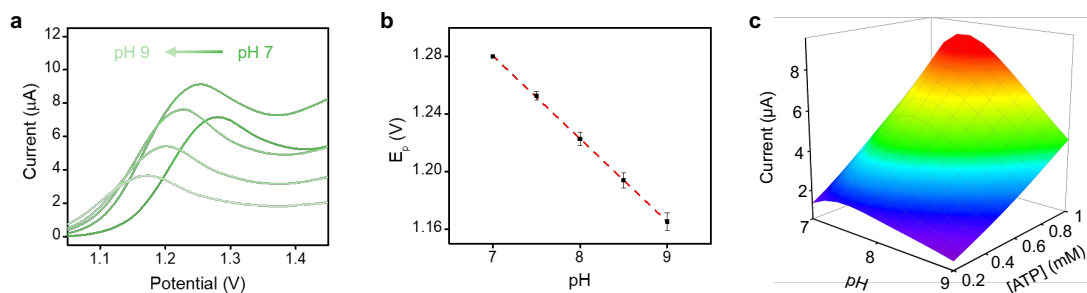


Fig. A-31. pH dependence of the ATP sensor. (a) DPV responses of the ATP sensor in 1 mM ATP at varying pH values. (b) Calibration plot showing the linear relationship between oxidation peak potential (E_p) and pH ($n = 5$). (c) Three-dimensional calibration surface illustrating the variation of peak current as a function of both pH and ATP concentration.

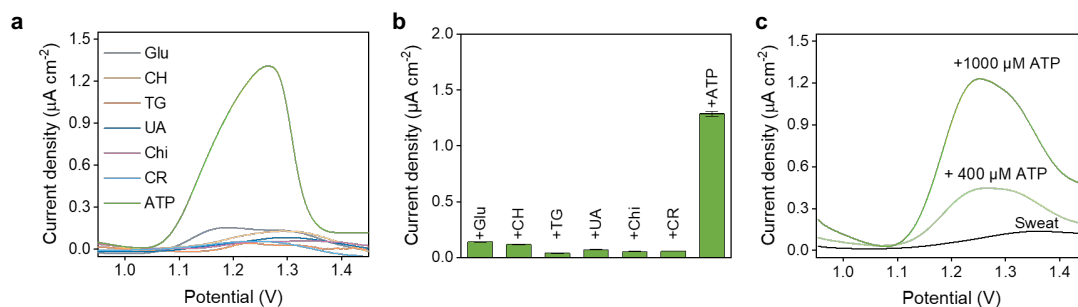


Fig. A-32. Selectivity of the ATP sensor in the presence of sweat interferences. **a**, DPV responses of the ATP sensor to representative sweat constituents. Tested species include ATP (1 mM), glucose (200 μM), Chi (50 μM), CH (3 μM), UA (40 μM), TG (50 μM), and creatine (CR) (15 μM). **b**, Summarized signal contributions from individual interferences. Error bars represent s.d. of the mean from 5 sensors. **c**, ATP sensor response in untreated human sweat and after addition of ATP (400 and 1000 μM).

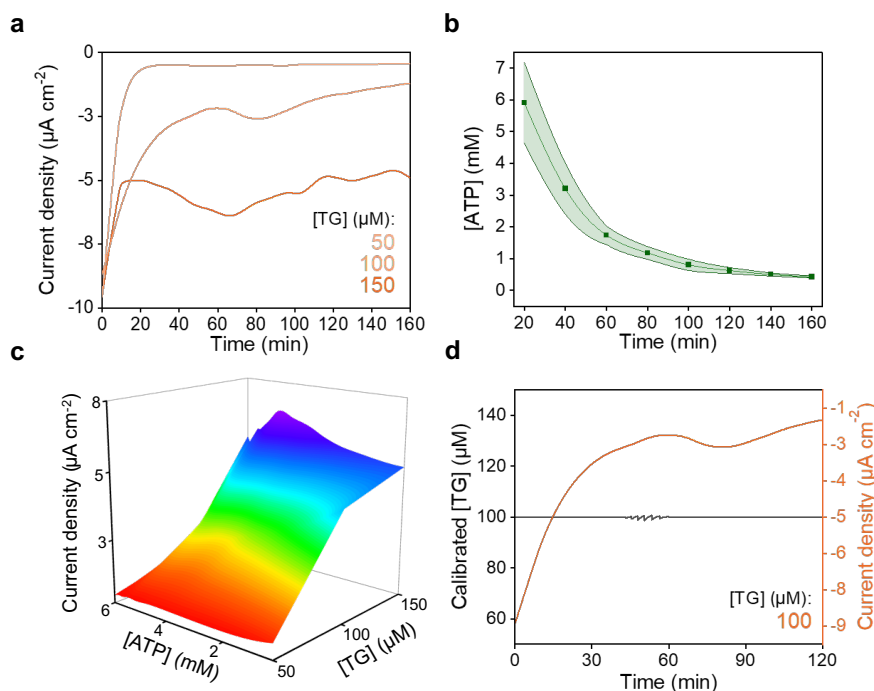


Fig. A-33. Calibration of TG sensing based on ATP concentration. **a**, In vitro dynamic TG sensing using the integrated sweating-enabled wearable analytical reaction module (Poly-CORE) for solutions with TG concentrations of 50, 100, and 150 μM . **b**, Average ATP concentrations measured from the Poly-CORE during in vitro testing. **c**, Three-dimensional calibration matrix plot showing the compiled TG sensor current-density responses as a function of both TG and ATP concentrations. **d**, Calibrated TG concentrations derived from sensor responses after interpolation using the TG-ATP calibration algorithm.

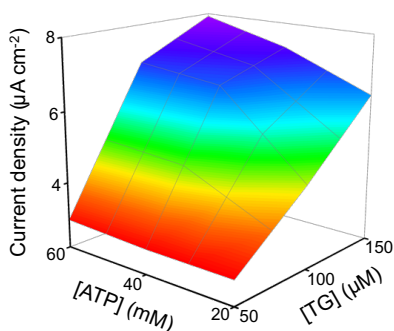


Fig. A-34. TG sensor response at elevated ATP concentrations. Amperometric response of the TG sensor measured at elevated ATP concentrations spanning 20–60 mM. This experiment was performed to evaluate sensor behavior across the higher ATP concentrations observed during upper-limit Poly-CORE release-capacity characterization.

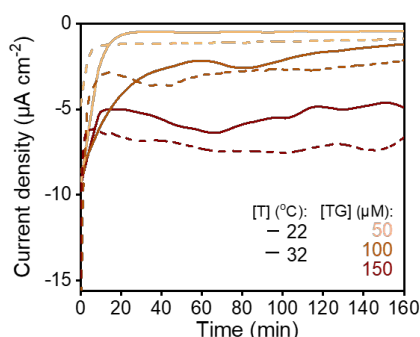


Fig. A-35. Skin-temperature validation of TG sensing based on ATP concentration. In vitro dynamic TG sensing using the integrated sweating-enabled wearable analytical reaction module (Poly-CORE) under skin-temperature conditions for solutions with TG concentrations of 50, 100, and 150 μM .

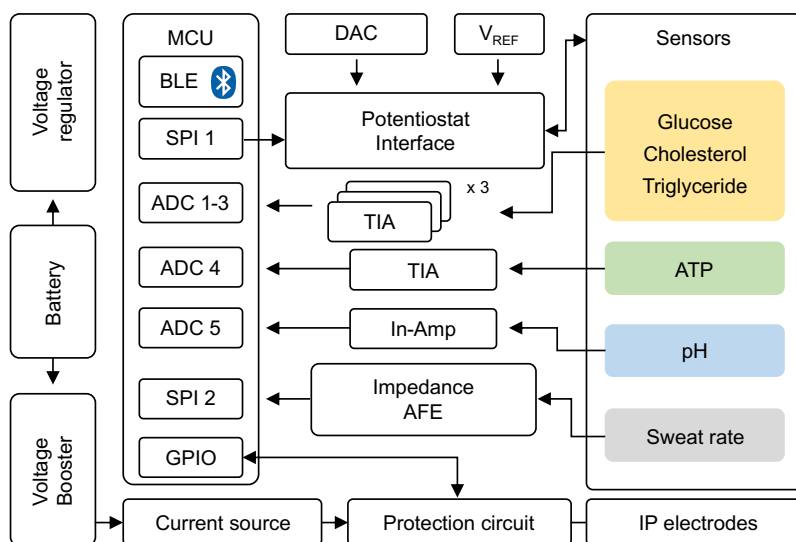


Fig. A-36. System-level block diagram of the wireless wearable patch for lipid profiling. MCU, microcontroller unit; BLE, Bluetooth Low Energy; SPI, serial peripheral

interface; ADC, analogue-to-digital converter; GPIO, general purpose input/output; DAC, digital-to-analogue converter; TIA, trans-impedance amplifier; In-Amp, instrumentation amplifier; AFE, analog front ends.

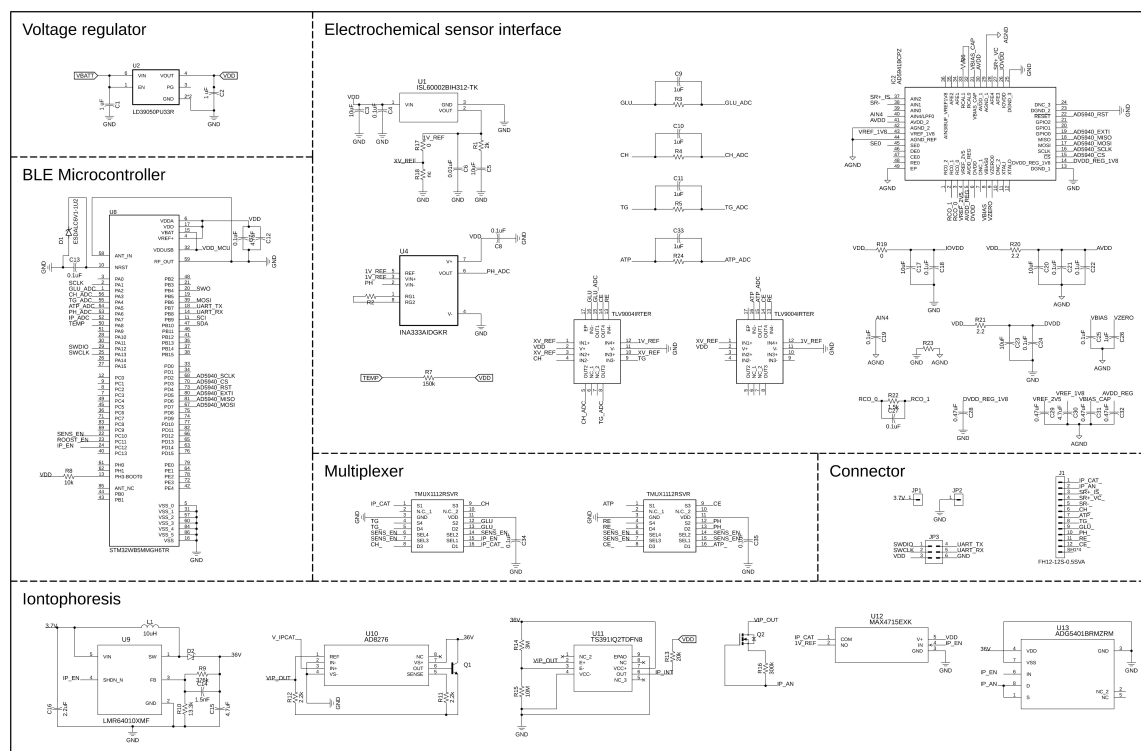


Fig. A-37. Printed circuit board (PCB) circuit design of the wearable patch for lipid profiling. Schematic of the PCB layout, including the voltage regulation module, BLE microcontroller, electrochemical sensing interface, multiplexers, iontophoresis circuitry, and connector interfaces for multimodal sensor integration.

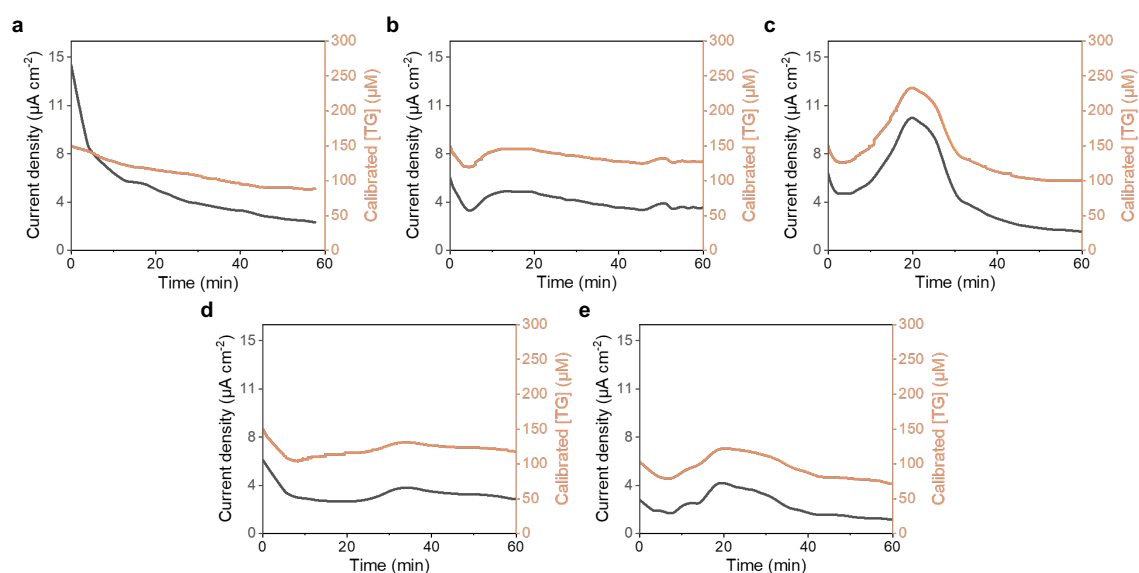


Fig. A-38. Computed TG calibrations from on-body meal studies. **a**, TG calibration from raw sensor data during fasting study. **a–e**, Computed TG concentrations derived from raw sensor data collected during different dietary conditions: fasting (a), lipid-rich meal (b), mixed macronutrient meal (c), protein-rich meal (d), and high-lipid, high-carbohydrate drink (e).

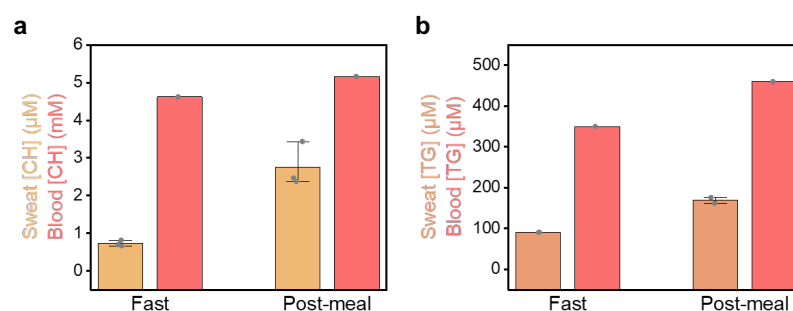


Fig. A-39. Fasting and post-meal comparison of sweat and blood lipid levels. **a**, CH levels measured in sweat and blood under fasting and post-meal conditions. **b**, TG levels measured in sweat and blood under fasting and post-meal conditions. Sweat samples were quantified using standardized assay kits and blood lipids were measured using a CardioChek lipid analyzer. Error bars represent s.d. of the mean from 3 measurements.

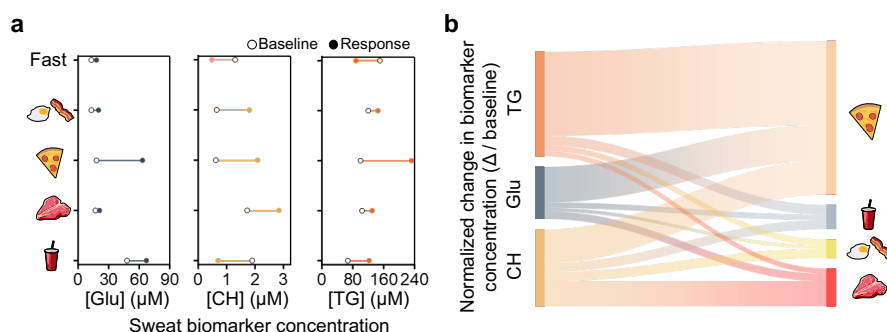


Fig. A-40. Baseline and postprandial biomarker responses following different meals.

a, Baseline and peak sweat concentrations of Glu, CH, and TG following each meal condition. White circles indicate baseline concentrations and colored circles indicate the corresponding peak response; the horizontal separation represents the magnitude and direction of the concentration change (μM). **b**, Sankey diagram illustrating the normalized biomarker responses across meal conditions. The normalized response was calculated as (peak–baseline)/baseline. Biomarkers (TG, CH, and Glu) are shown on the left and meal conditions on the right. The thickness of each connection represents the magnitude of the normalized response linking each biomarker to the corresponding meal condition. Node lengths reflect the cumulative normalized responses associated with each biomarker and meal.

Table A-1. Interpolation table for determination of triglycerides.

[ATP] (μM)	J (μA cm ⁻¹) of 150 μM [TG]	J (μA cm ⁻¹) of 100 μM [TG]	J (μA cm ⁻¹) of 50 μM [TG]
10000	8.6	8.3	8.0
9500	7.8	7.8	6.6
9000	6.9	7.3	5.1
8500	6.1	6.7	3.6
8000	5.3	6.2	2.5
7500	5.1	5.7	1.8
7000	5.1	5.2	1.3
6500	5.1	4.7	0.9

6000	5.1	4.3	0.7
5500	5.1	3.9	0.6
5000	5.2	3.6	0.5
4500	5.3	3.3	0.5
4000	5.4	3.0	0.5
3500	5.6	2.8	0.5
3000	5.9	2.6	0.5
2500	6.2	2.4	0.5
2000	6.4	2.2	0.5
1500	6.4	2.5	0.5
1000	5.7	2.5	0.5
900	5.6	2.3	0.5
800	5.6	2.1	0.5
700	5.2	1.9	0.5
600	5.0	1.7	0.5
500	5.0	1.5	0.5
400	5.2	1.2	0.4

Bibliography of Chapter 2

1. Huang, Y. *et al.* Lipid profiling identifies modifiable signatures of cardiometabolic risk in children and adolescents with obesity. *Nat. Med.* 31, 294–305 (2025).
2. Libby, P. *et al.* Atherosclerosis. *Nat. Rev. Dis. Primer* 5, 56 (2019).
3. Powell-Wiley, T. M. *et al.* Obesity and Cardiovascular Disease: A Scientific Statement From the American Heart Association. *Circulation* 143, e984–e1010 (2021).
4. O'Connor, E. A., Evans, C. V., Rushkin, M. C., Redmond, N. & Lin, J. S. Behavioral Counseling to Promote a Healthy Diet and Physical Activity for Cardiovascular Disease Prevention in Adults With Cardiovascular Risk Factors: Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. *JAMA* 324, 2076 (2020).
5. Shen, X. *et al.* Multi-omics microsampling for the profiling of lifestyle-associated changes in health. *Nat. Biomed. Eng.* 8, 11–29 (2023).

6. Pourafshar, S., Parikh, M., Abdallah, B., Al Thubian, N. & Jacobson, J. W. An Assessment of Individual Preference for a Novel Capillary Blood Collection System. *Patient Prefer. Adherence* 18, 531–541 (2024).
7. Baker, L. B. Physiology of sweat gland function: The roles of sweating and sweat composition in human health. *Temperature* 6, 211–259 (2019).
8. Brunmair, J. *et al.* Finger sweat analysis enables short interval metabolic biomonitoring in humans. *Nat. Commun.* 12, 5993 (2021).
9. Zhao, C. *et al.* Skin-like drift-free biosensors with stretchable diode-connected organic field-effect transistors. *Nat. Electron.* <https://doi.org/10.1038/s41928-025-01465-4> (2025) doi:10.1038/s41928-025-01465-4.
10. Clark, K. M. & Ray, T. R. Recent Advances in Skin-Interfaced Wearable Sweat Sensors: Opportunities for Equitable Personalized Medicine and Global Health Diagnostics. *ACS Sens.* 8, 3606–3622 (2023).
11. Davis, N., Heikenfeld, J., Milla, C. & Javey, A. The challenges and promise of sweat sensing. *Nat. Biotechnol.* 42, 860–871 (2024).
12. Min, J. *et al.* Skin-Interfaced Wearable Sweat Sensors for Precision Medicine. *Chem. Rev.* 123, 5049–5138 (2023).
13. Yang, D. S., Ghaffari, R. & Rogers, J. A. Sweat as a diagnostic biofluid. *Science* 379, 760–761 (2023).
14. Childs, A. *et al.* Diving into Sweat: Advances, Challenges, and Future Directions in Wearable Sweat Sensing. *ACS Nano* 18, 24605–24616 (2024).
15. Zhong, B. *et al.* Interindividual- and blood-correlated sweat phenylalanine multimodal analytical biochips for tracking exercise metabolism. *Nat. Commun.* 15, 624 (2024).
16. Kwon, K. *et al.* An on-skin platform for wireless monitoring of flow rate, cumulative loss and temperature of sweat in real time. *Nat. Electron.* 4, 302–312 (2021).
17. Liu, W., Cheng, H. & Wang, X. Skin-interfaced colorimetric microfluidic devices for on-demand sweat analysis. *Npj Flex. Electron.* 7, 43 (2023).
18. Sempionatto, J. R. *et al.* An epidermal patch for the simultaneous monitoring of haemodynamic and metabolic biomarkers. *Nat. Biomed. Eng.* 5, 737–748 (2021).

19. Yin, L. *et al.* A stretchable epidermal sweat sensing platform with an integrated printed battery and electrochromic display. *Nat. Electron.* 5, 694–705 (2022).
20. Lin, S. *et al.* Non-Invasive Touch-Based Lithium Monitoring Using an Organohydrogel-Based Sensing Interface. *Adv. Mater. Technol.* 8, 2202141 (2023).
21. Narwal, V. *et al.* Cholesterol biosensors: A review. *Steroids* 143, 6–17 (2019).
22. Hooda, V., Gahlaut, A., Gothwal, A. & Hooda, V. Recent trends and perspectives in enzyme based biosensor development for the screening of triglycerides: a comprehensive review. *Artif. Cells Nanomedicine Biotechnol.* 46, 626–635 (2018).
23. Kvasnička, A. *et al.* SLIDE—Novel Approach to Apocrine Sweat Sampling for Lipid Profiling in Healthy Individuals. *Int. J. Mol. Sci.* 22, 8054 (2021).
24. Sempionatto, J. R., Lasalde-Ramírez, J. A., Mahato, K., Wang, J. & Gao, W. Wearable chemical sensors for biomarker discovery in the omics era. *Nat. Rev. Chem.* 6, 899–915 (2022).
25. Smith, K. R. & Thiboutot, D. M. Thematic review series: Skin Lipids. Sebaceous gland lipids: friend or foe? *J. Lipid Res.* 49, 271–281 (2008).
26. Vietri Rudan, M. & Watt, F. M. Mammalian Epidermis: A Compendium of Lipid Functionality. *Front. Physiol.* 12, 804824 (2022).
27. Prosperi, M. *et al.* Causal inference and counterfactual prediction in machine learning for actionable healthcare. *Nat. Mach. Intell.* 2, 369–375 (2020).
28. Feuerriegel, S. *et al.* Causal machine learning for predicting treatment outcomes. *Nat. Med.* 30, 958–968 (2024).
29. Expert Panel On Detection, Evaluation, And Treatment Of High Blood Cholesterol In Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA J. Am. Med. Assoc.* 285, 2486–2497 (2001).
30. Miller, M. *et al.* Triglycerides and Cardiovascular Disease: A Scientific Statement From the American Heart Association. *Circulation* 123, 2292–2333 (2011).

31. Sattar, N., McGuire, D. K. & Gill, J. M. R. High Circulating Triglycerides Are Most Commonly a Marker of Ectopic Fat Accumulation: Connecting the Clues to Advance Lifestyle Interventions. *Circulation* 146, 77–79 (2022).
32. Parhofer, K. G. Interaction between Glucose and Lipid Metabolism: More than Diabetic Dyslipidemia. *Diabetes Metab. J.* 39, 353–362 (2015).
33. Sabaté Del Río, J., Henry, O. Y. F., Jolly, P. & Ingber, D. E. An antifouling coating that enables affinity-based electrochemical biosensing in complex biological fluids. *Nat. Nanotechnol.* 14, 1143–1149 (2019).
34. Weber, C. J., Strom, N. E. & Simoska, O. Electrochemical deposition of gold nanoparticles on carbon ultramicroelectrode arrays. *Nanoscale* 16, 16204–16217 (2024).
35. Matos-Peralta, Y. & Antuch, M. Review—Prussian Blue and Its Analogs as Appealing Materials for Electrochemical Sensing and Biosensing. *J. Electrochem. Soc.* 167, 037510 (2020).
36. Ying, S. *et al.* Synthesis and Applications of Prussian Blue and Its Analogues as Electrochemical Sensors. *ChemPlusChem* 86, 1608–1622 (2021).
37. Xu, C. *et al.* A physicochemical-sensing electronic skin for stress response monitoring. *Nat. Electron.* 7, 168–179 (2024).
38. Song, Y. *et al.* 3D-printed epifluidic electronic skin for machine learning–powered multimodal health surveillance. *Sci. Adv.* 9, eadi6492 (2023).
39. Davis, N. *et al.* Reusable, Fully Integrated Sweat Monitor Band with Peel-and-Stick- Replacement Printed Microfluidic Sensor. *Adv. Mater. Technol.* 10, 2500477 (2025).
40. Ru, X. *et al.* Synthesis of polypyrrole nanowire network with high adenosine triphosphate release efficiency. *Electrochimica Acta* 56, 9887–9892 (2011).
41. Simmers, P., Li, S. K., Kasting, G. & Heikenfeld, J. Prolonged and localized sweat stimulation by iontophoretic delivery of the slowly-metabolized cholinergic agent carbachol. *J. Dermatol. Sci.* 89, 40–51 (2018).
42. Wang, P., Gao, X., Willett, W. C. & Giovannucci, E. L. Socioeconomic Status, Diet, and Behavioral Factors and Cardiometabolic Diseases and Mortality. *JAMA Netw. Open* 7, e2451837 (2024).

43. Wu, J. H. Y., Micha, R. & Mozaffarian, D. Dietary fats and cardiometabolic disease: mechanisms and effects on risk factors and outcomes. *Nat. Rev. Cardiol.* 16, 581–601 (2019).
44. Gugliucci, A. The chylomicron saga: time to focus on postprandial metabolism. *Front. Endocrinol.* 14, 1322869 (2023).
45. Mucinski, J. M. *et al.* High-throughput LC-MS method to investigate postprandial lipemia: considerations for future precision nutrition research. *Am. J. Physiol.-Endocrinol. Metab.* 320, E702–E715 (2021).
46. Berry, S. E. *et al.* Human postprandial responses to food and potential for precision nutrition. *Nat. Med.* 26, 964–973 (2020).
47. Mendes-Soares, H. *et al.* Assessment of a Personalized Approach to Predicting Postprandial Glycemic Responses to Food Among Individuals Without Diabetes. *JAMA Netw. Open* 2, e188102 (2019).

MACHINE LEARNING-DRIVEN LIPID BIOMARKER VALIDATION

3.1 Introduction

Furthermore, translating sweat biomarkers into clinically meaningful insights requires rigorous cross-validation against blood lipid measurements²⁴. However, direct sweat–blood relationships are often weak and confounded by interindividual physiological and demographic variability, particularly for hydrophobic lipid species whose appearance in sweat is constrained by complex skin and glandular transport processes^{25,26}. Conventional correlation-based analyses and purely associative machine learning models can capture predictive patterns, but they do not explicitly account for the physiological confounders that influence both sweat composition and systemic lipid levels^{27,28}. Causal inference frameworks offer a way to incorporate these dependencies and estimate individualized, confounder-adjusted mappings between sweat and blood lipids.

In this chapter, we address this challenge using a causal machine learning framework guided by directed acyclic graphs that encode hypothesized physiological relationships among sweat biomarkers, sweat rate, body composition, and systemic lipid levels. Rather than relying on direct pairwise correlations, this approach adjusts for relevant confounders and identifies the factors that most strongly influence sweat-based estimation of blood cholesterol and triglycerides. By integrating wearable biosensing with causal modeling, we improve both the accuracy and interpretability of noninvasive lipid profiling.

In a 24-subject human study, simultaneous sweat and blood sampling revealed weak direct sweat–blood correlations but substantially improved lipid estimation after causal adjustment (**Fig. 3-1**). These results support the use of physiology-informed modeling as a critical step toward continuous, individualized cardiometabolic monitoring using wearable chemical sensors.

3.2 Lipid biomarker validation via causal machine learning

Translating non-invasive biomarkers into clinically relevant indicators requires rigorous validation against established standards. To this end, we conducted a pre-clinical study employing a causal machine learning (ML) framework to derive a more accurate mapping between sweat and blood lipid concentrations by integrating sweat rate and individual physiological factors (**Fig. 3-1a**). Direct association between sweat and blood CH and TG (**Fig. 3-1b,c**), which represent raw sweat–blood comparisons without model-based adjustment, yielded poor correlation, largely due to the limited solubility of hydrophobic lipids²⁵, barrier-limited transport across skin and glandular membranes²⁶, and local lipid secretion from sebaceous glands⁴⁸, and inter-individual physiological variability. These weak direct correlations highlight the limitation of pairwise relationships and motivate the use of a causal ML framework to estimate blood lipid levels from sweat measurements and physiological confounders. Accordingly, the objective is not to identify strong pairwise correlations, but to recover predictive relationships through joint modeling of sweat biomarkers and physiological confounders. To this end, we collected demographic and physiological data from 24 participants (**Fig. 3-1d**) to identify potential candidate covariates for causal adjustment (**Table B-1**).

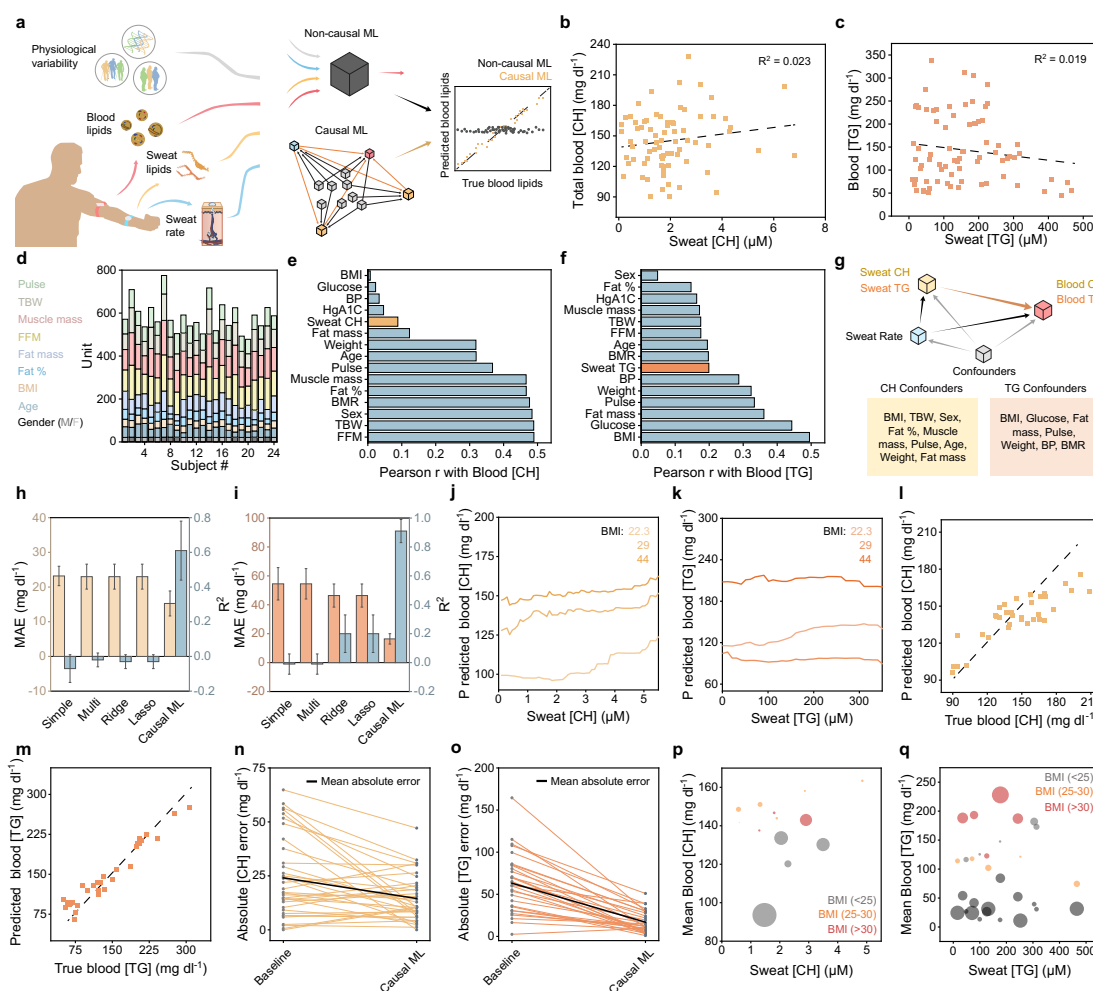


Figure 3-1. Validation of sweat lipid biomarkers using causal machine learning. **a**, Overview of the lipid validation workflow integrating sweat and blood analyses with causal machine learning (ML). **b,c**, Raw bivariate scatterplots showing direct pairwise relationships between sweat and blood CH (**b**) and TG (**c**), with linear-fit R^2 values. **d**, Demographic and physiological characteristics of the 24 participants included in the validation study. Each column represents one participant and is stacked to display multiple variables. The stacked visualization illustrates inter-subject variability across these attributes and does not represent a combined quantitative metric. BMI, body mass index; FFM, fat-free mass (lb); TBW, total body water (lb). **e,f**, Pearson correlation screening results showing marginal Pearson correlation coefficients (r) between each candidate demographic and physiological variable and blood CH (**e**) or TG (**f**), used for covariate

prioritization and interpreted separately from the model-based response curves in **j,k**. **g**, Task-specific directed acyclic graphs (DAGs) depicting the hypothesized causal relationships among sweat biomarkers, dynamic sweat-rate effects, physiological covariates, and blood lipid outcomes. **h,i**, Model performance comparisons for CH (**h**) and TG (**i**) predictions across linear regression, multivariate regression, regularized regressions (Ridge, Lasso), and causal ML models, showing improved mean absolute error (MAE) and coefficient of determination (R^2) under causal adjustment. **j,k**, Individualized model response curves for CH (**j**) and TG (**k**), showing how predicted blood lipid levels change as sweat lipid inputs vary under fixed subject-level physiological covariates. These curves represent model-predicted responses and do not represent continuously measured blood lipid trajectories. **l,m**, Agreement between model-predicted blood CH (**l**) and TG (**m**) values generated from sweat-based inputs and paired blood lipid measurements used as clinical reference labels. Blood lipid measurements were used for model training and validation, not as prediction inputs. **n,o**, Per-subject change in MAE for CH (**n**) and TG (**o**) showing improved accuracy for most participants under the causal model. **p,q**, Stratified evaluation of CH (**p**) and TG (**q**) models across BMI categories (normal, overweight, obese), with size indicating the number of subjects and color denoting BMI category. Causal modeling disentangled demographic confounding, revealing BMI-dependent bias in sweat-blood cholesterol relationships and robust, BMI-independent triglyceride coupling, underscoring the improved robustness of the causal framework across diverse populations.

We then performed Pearson correlation screening between each collected variable and the corresponding blood lipid endpoint (blood CH or blood TG). Using a loose prioritization threshold ($|r| \geq 0.2$), several physiological variables showed non-negligible association with blood lipid levels, whereas direct sweat–blood lipid associations remained comparatively weak (**Fig. 3-1e,f**). This screening analysis evaluates each variable independently and therefore does not capture how subject-level covariates may modify the relationship between sweat biomarkers and blood lipid levels. For example, although BMI showed only a modest marginal association with blood CH in the Pearson analysis, it remained informative in the

causal model by accounting for inter-individual differences in the sweat CH–blood CH mapping. These observations motivated the use of a directed acyclic graph (DAG)-based framework to account for physiological variability beyond raw sweat biomarkers.

Final task-specific DAGs were constructed by combining the Pearson screening results with physiological knowledge of sweat secretion, lipid transport, and body composition. In these DAGs, sweat rate was treated separately as a dynamic secretion-related factor, while static body-composition effects were represented parsimoniously by body mass index (BMI), with sex additionally retained for the CH task (**Fig. 3-1g**). The resulting adjustment sets satisfied the backdoor criterion, ensuring that major confounding pathways were controlled in downstream modeling.

Using these causal structures, we trained models to estimate blood CH and TG levels from sweat data. Specifically, the model inputs were sweat lipid measurements, sweat rate, and subject-level physiological covariates, whereas paired blood lipid measurements were used only as clinical reference labels for model training and validation. Blood lipid measurements were not used as prediction inputs. This distinction is important because the goal of the model is to learn a sweat-to-blood mapping, rather than to reproduce blood measurements from blood-derived inputs. The causal models achieved strong predictive performance, with coefficient of determination $R^2 = 0.912$ for TG and $R^2 = 0.610$ for CH, together with low mean absolute errors (MAEs) (16.44 and 15.27 mg dl⁻¹, respectively) (**Fig. 3-1h,i**). We compared dual-variable causal models (jointly incorporating sweat rate and sweat lipid levels) against baseline linear and regularized regressions, and observed consistently lower MAE and higher R^2 values than all baseline models. To assess whether predictive performance could be explained primarily by demographic and physiological covariates, we performed a negative-control analysis using covariates alone while excluding sweat CH and TG measurements (**Table B-2**). Removing the sweat biochemical inputs resulted in a marked reduction in performance. For TG, model performance decreased from an MAE of 16.44 and an R^2 of 0.912 in the full model to an MAE of 45.46 and an R^2 of 0.301 in the covariate-only model. For CH, performance decreased from an MAE of 15.27 and an R^2 of 0.610 to an MAE of 23.49 and an R^2 of 0.062. These results indicate that demographic and physiological

covariates alone are insufficient to recover blood lipid levels with comparable accuracy, and that inclusion of sweat lipid measurements substantially improves predictive performance. This distinction is particularly important for TG prediction: BMI primarily captures static between-subject physiological differences, whereas continuous sweat TG provides time-varying biochemical information that cannot be captured by static demographic or body-composition covariates alone. The improvement was more pronounced for CH, where unadjusted correlations were weak due to intrinsic transport and partitioning constraints of lipids, whereas triglyceride prediction benefited primarily from refinement of moderate associations (**Table B-3**).

The causal framework also highlights physiological relationships among the sweat-domain variables used in the model. For CH, the weak negative correlation between sweat CH and sweat rate ($r = -0.11$) reflected mild dilution at higher sweating rates. For TG, the inverse correlation ($r = -0.31$) suggested faster turnover and clearance, supporting the inclusion of sweat rate as an informative physiological covariate in the model.

At the individual level, causal adjustment reduced unexplained inter-subject variability and produced more consistent subject-specific response patterns (**Fig. 3-1j,k**). These panels visualize individualized model response curves showing how the trained model predicts blood lipid output as sweat lipid input changes under fixed subject-level physiological covariates, rather than directly measured continuous temporal blood trajectories. When evaluated against paired blood measurements collected at discrete clinical sampling points, the causal model demonstrated improved agreement between predicted and measured blood lipid concentrations (**Fig. 3-1l,m** and **Fig. B-1**) and reduced per-subject errors (**Fig. 3-1n,o**). These performance gains persisted across BMI-stratified subgroups, including normal-weight, overweight, and obese participants, indicating robust subgroup generalizability (**Fig. 3-1p,q**). Further bootstrap resampling ($n = 1000$) and subgroup-specific analyses confirmed the stability of these causal estimates while preserving biologically meaningful population-level heterogeneity. Sweat rate consistently emerged as a global marker of metabolic activity and dilution, while sweat CH showed stronger dependence on BMI and sex. In contrast, sweat TG correlated more directly with blood TG, supporting the notion that TG excretion

reflects systemic lipid clearance process rather than a demographically confounded phenomenon. While sweat rate was the dominant physiological variable in the present framework, additional biochemical or physiological factors, including pH, may also contribute to inter-individual variability; however, pH variation remained limited under carbachol-based iontophoresis conditions in this study and our prior reports^{49–51}.

These causal findings inform translational value of causal inference in biomarker validation. For CH estimation, adjustment for BMI and sex is essential to mitigate systematic bias, with sweat rate serving as a physiological calibrator. For TG, joint modeling of sweat TG and sweat rate yields robust, generalizable predictions across diverse populations. Overall, integrating causal structure and confounder control enables non-invasive sweat lipid measurements to more accurately reflect underlying blood lipid physiology, supporting their potential for continuous, personalized metabolic monitoring.

3.3 Conclusions

In a 24-participant preclinical study, simultaneous sweat and blood lipid measurements were used to evaluate whether wearable sweat biomarkers could be mapped to systemic lipid levels through physiology-informed causal modeling. Rather than relying on weak pairwise sweat–blood correlations, which have historically limited noninvasive lipid estimation, this chapter framed the problem as one of confounder adjustment. Task-specific directed acyclic graphs encoded hypothesized relationships among sweat biomarkers, sweat rate, and body composition, enabling adjustment sets that controlled major confounding pathways linking sweat composition to blood lipid levels.

This confounder-adjusted formulation recovered predictive relationships that direct correlations failed to capture, achieving stronger agreement with paired blood measurements than linear and regularized baseline models. The final models reached R^2 values of 0.912 for triglycerides and 0.610 for cholesterol under the evaluated modeling framework. A negative-control analysis using physiological covariates alone, with the sweat biochemical inputs removed, markedly degraded performance, supporting the conclusion that continuous sweat

lipid measurements provide information beyond static demographic and body-composition variables. Together, these results show that wearable biochemical sensing becomes substantially more informative when interpreted through a physiology-informed causal framework rather than through associative correlations alone.

These findings should be interpreted within the limits of the present study. Although the dataset included repeated longitudinal sweat–blood measurements, the number of independent participants remained modest, so the causal modeling analysis is best regarded as a preclinical proof-of-concept rather than a definitive population-scale model. The framework also depends on the validity of the hypothesized causal structure; unmeasured confounders or misspecified relationships could bias the recovered mappings, and the adjustment sets used here were deliberately parsimonious. These constraints bound the strength of the present conclusions while defining the experimental and modeling conditions required for broader validation.

Appendix

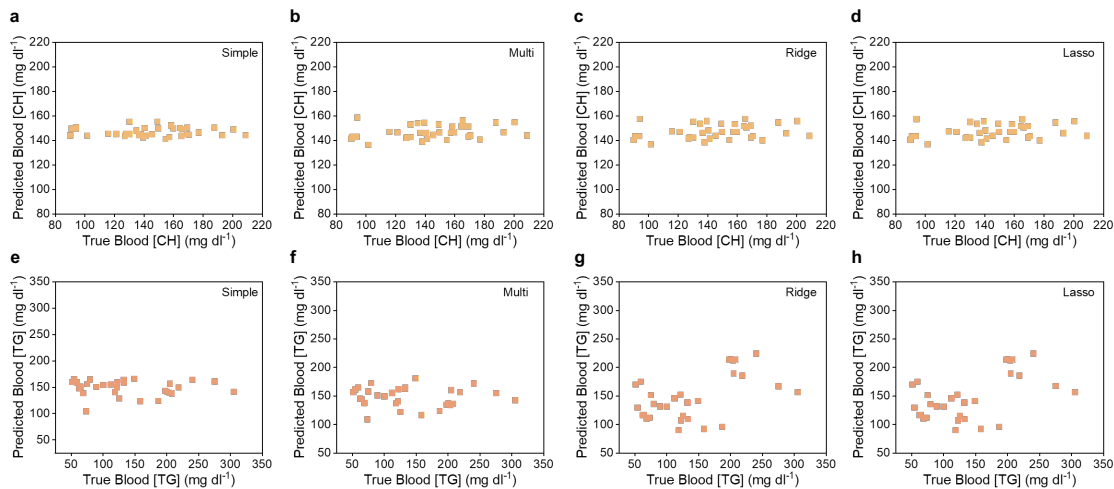


Fig. B-1. Linear regression results of non-causal ML models. a–d, Linear regression plots from non-causal models illustrating correlations between blood and sweat CH for simple (a), multivariate (b), Ridge (c), and Lasso regressions (d). e–h Linear regression

plots from non-causal models illustrating correlations between blood and sweat TG for simple (e), multivariate (f), Ridge (g), and Lasso regressions (h).

Table B-1. Patient demographics for lipid biomarker validations.

Patient	Age	Sex	BP	Weight (lb)	BMI	BMR (kcal)	Fat (%)	Fat mass (lb)	FFM (lb)	Predicted muscle mass (lb)	TB W (lb)
1	50	F	111/69	184	34.8	1413	45. 9	84.4	99.6	94.6	73
2	46	M	129/76	227.4	31.7	2146	30. 1	68.4	159	151.8	116.4
3	63	F	145/82	183	41	1613	33. 7	61.2	120. 2	114.2	88
4	65	F	162/108	161	24.8	1275	43. 6	70.2	90.8	86.2	66.4
5	37	M	119/80	190.2	27.7	1822	28. 8	54.8	135. 4	129.4	99.2
6	44	F	123/49	204.8	31.1	1638	42. 7	42.7	117. 4	111.4	86
7	45	M	134/84	263.4	38.9	2324	36. 1	95	168. 4	160.6	123.2
8	58	F	109/69	107	20.9	1086	25. 1	26.8	80.2	76.4	58.8
9	60	M	106/78	163.4	25.6	1647	22. 9	37.4	126	102.4	92.2
10	33	M	108/72	134	22.3	1413	21. 3	28.6	105. 4	100.6	77.2
11	22	F	132/81	196.8	31.8	1659	41. 4	81.4	115. 4	109.6	84.4
12	60	F	122/74	156.2	26.8	1283	41 64	64	92.2	87.4	67.6
13	44	F	127/85	171	33.4	1341	45. 1	77	94	89	68.8
14	56	F	123/77	143.2	28	1148	44. 1	63.2	80	76.2	58.6
15	32	M	148/77	232.4	32.4	2167	31. 3	72.8	159. 6	152.2	116.8
16	48	F	129/84	162	25.4	1367	39. 2	63.6	98.4	93.6	72
17	51	F	116/81	212	32.2	1747	40. 1	85	127	120.6	93
18	33	F	109/80	176.4	29.4	1444	42. 5	75	101. 4	96.2	74.2
19	24	M	105/83	204.8	29	1985	30. 8	64	143. 8	137.2	105.2

20	35	F	103/69	144	25.5	1367	30. 8	44.4	99.6	92.4	73
21	26	F	102/71	147.8	26.2	1299	39. 4	58.2	89.6	84.8	65.6
22	29	M	102/65	184.2	28.8	1878	24. 2	44.6	139. 6	133.4	102.2
23	47	F	124/84	167.6	29.7	1438	37. 5	62.8	104. 8	99.6	76.6
24	35	M	127/76	188.8	27.1	1934	23. 2	43.8	145	138.4	106.2
25	44	F	118/64	191.6	33.9	1597	39. 7	76.2	115. 4	109.6	84.6

BP, blood pressure; BMI, body mass index; BMR, basal metabolic rate; FFM, fat-free mass; TBW, total body water.

Table B-2. Causal ML performance compared with negative control (nonsensor covariates only).

Target	Model input	MAE (mg dl ⁻¹)	R ²	ΔMAE (mg dl ⁻¹)	R ²
TG	Sweat TG + nonsensor covariates	16.44	0.912	-	-
	Nonsensor covariates only	45.46	0.301	+29.02	-0.611
CH	Sweat CH + nonsensor covariates	15.27	0.61	-	-
	Nonsensor covariates only	23.49	0.062	+8.2	-0.547

MAE, mean absolute error.

Table B-3. Causal ML performance against conventional baselines in sweat-to-blood lipid inference

Model Type	[CH] MAE (mg dl ⁻¹)	R ²	[TG] MAE (mg dl ⁻¹)	R ²
Simple (Sweat only)	23.2 ± 2.8	-0.07 ± 0.08	54.5 ± 11.2	-0.01 ± 0.07
Multi (Sweat + Rate)	23.0 ± 3.6	-0.02 ± 0.04	54.5 ± 10.5	-0.04 ± 0.07
Ridge (Sweat + Rate + BMI)	23.0 ± 3.6	-0.03 ± 0.04	46.4 ± 8.0	0.20 ± 0.13
Lasso (Sweat + Rate + BMI)	23.0 ± 3.6	-0.03 ± 0.04	46.4 ± 8.0	0.20 ± 0.13
Causal ML (same inputs)	23.5 ± 3.6	0.61 ± 0.17	16.44 ± 3.6	0.91 ± 0.08

Bibliography of Chapter 3

1. Sempionatto, J. R. et al. An epidermal patch for the simultaneous monitoring of haemodynamic and metabolic biomarkers. *Nat. Biomed. Eng.* 5, 737–748 (2021).
2. Yin, L. et al. A stretchable epidermal sweat sensing platform with an integrated printed battery and electrochromic display. *Nat. Electron.* 5, 694–705 (2022).
3. Lin, S. et al. Non-Invasive Touch-Based Lithium Monitoring Using an Organohydrogel-Based Sensing Interface. *Adv. Mater. Technol.* 8, 2202141 (2023).
4. Narwal, V. et al. Cholesterol biosensors: A review. *Steroids* 143, 6–17 (2019).
5. Hooda, V., Gahlaut, A., Gothwal, A. & Hooda, V. Recent trends and perspectives in enzyme based biosensor development for the screening of triglycerides: a comprehensive review. *Artif. Cells Nanomedicine Biotechnol.* 46, 626–635 (2018).
6. Prosperi, M. et al. Causal inference and counterfactual prediction in machine learning for actionable healthcare. *Nat. Mach. Intell.* 2, 369–375 (2020).
7. Feuerriegel, S. et al. Causal machine learning for predicting treatment outcomes. *Nat. Med.* 30, 958–968 (2024).
8. Wang, P., Gao, X., Willett, W. C. & Giovannucci, E. L. Socioeconomic Status, Diet, and Behavioral Factors and Cardiometabolic Diseases and Mortality. *JAMA Netw. Open* 7, e2451837 (2024).
9. Wu, J. H. Y., Micha, R. & Mozaffarian, D. Dietary fats and cardiometabolic disease: mechanisms and effects on risk factors and outcomes. *Nat. Rev. Cardiol.* 16, 581–601 (2019).
10. Gugliucci, A. The chylomicron saga: time to focus on postprandial metabolism. *Front. Endocrinol.* 14, 1322869 (2023).
11. Mucinski, J. M. et al. High-throughput LC-MS method to investigate postprandial lipemia: considerations for future precision nutrition research. *Am. J. Physiol.-Endocrinol. Metab.* 320, E702–E715 (2021).
12. Berry, S. E. et al. Human postprandial responses to food and potential for precision nutrition. *Nat. Med.* 26, 964–973 (2020).

13. Mendes-Soares, H. et al. Assessment of a Personalized Approach to Predicting Postprandial Glycemic Responses to Food Among Individuals Without Diabetes. JAMA Netw. Open 2, e188102 (2019).

Chapter 4

SUMMARY AND OUTLOOK

4.1 Overall Summary

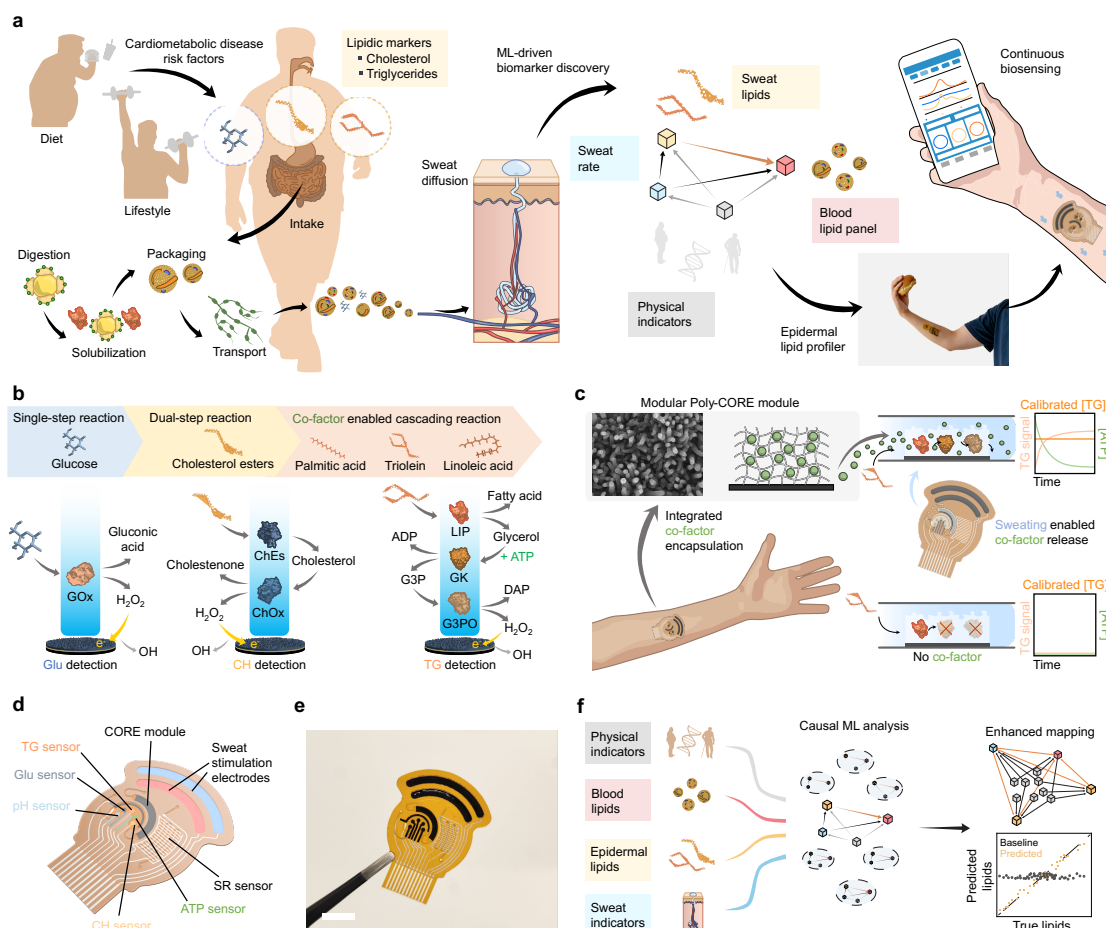


Figure 4-1. Wearable platform for continuous epidermal lipid profiling. **a**, Overview of key contributors to cardiometabolic diseases, including diet and lifestyle, that modulate systemic lipid metabolism and lead to dysregulation of cholesterol (CH) and triglycerides (TG). These lipidic biomarkers can be continuously and noninvasively monitored through sweat-based analysis. **b**, Schematic of single-step, dual-step and co-factor enabled cascading enzymatic reactions leveraged to detect glucose, cholesterol, and triglycerides,

respectively. Glu, glucose; GOx, glucose oxidase; ChEs, cholesterol esterase; ChOx, cholesterol oxidase; LIP, lipase; GK, glycerol kinase; G3PO, glycerol-3-phosphate oxidase; ATP, adenosine triphosphate; ADP, adenosine diphosphate; G3P, glycerol-3-phosphate; DAP, dihydroxyacetone phosphate. **c**, Illustration of the modular polymeric cofactor-releasing (Poly-CORE) system based on ATP-encapsulating polypyrrole (PPy/ATP) nanostructures, which enable sustained sweat-triggered ATP diffusion into the sensing chamber for long-term TG monitoring. **d**, Schematic of the wearable patch design illustrating the bioelectronic components and capabilities. SR, sweat rate. **e**, Optical image of a wearable sensor patch for epidermal lipid monitoring. Scale bar, 10 mm. **f**, Workflow of causal machine learning-based analysis integrating physical indicators, sweat biomarkers, and blood lipid data to identify physiological confounders and enhance individualized mapping between sweat and blood lipid concentrations.

This thesis demonstrates a wearable epidermal platform for continuous and non-invasive lipid profiling through the integration of enzymatic biosensing, cofactor-refreshing materials, microfluidic sampling, physiological calibration, and causal machine learning. Conventional lipid assessment relies on periodic blood collection and therefore provides only discrete measurements of systemic lipid status. Although these clinical measurements are essential for the diagnosis and management of cardiometabolic diseases, they cannot capture the dynamic lipid fluctuations associated with dietary intake, physical activity, and individual metabolic state. Wearable chemical sensors provide an attractive alternative by enabling continuous molecular monitoring through accessible biofluids. However, the detection of complex lipid biomarkers remains challenging because it requires multistep enzymatic reactions, sustained cofactor availability, and careful interpretation of molecular signals affected by sweat secretion and inter-individual physiological variability. The work presented in this thesis addresses these limitations and establishes a framework for continuous epidermal lipid monitoring (Fig. 4-1).

Chapter 1 provides an overview of wearable chemical sensors for biomarker discovery and continuous health monitoring. Wearable sensing platforms can collect molecular information from non-invasively accessible biofluids, including sweat, saliva, tears, and

interstitial fluid, while minimizing the need for repeated clinical sampling. The chapter describes electrochemical and optical biosensing strategies, emerging bioaffinity-based sensing approaches, functional materials, fluid extraction and sampling systems, and integrated wearable platforms for wireless data collection. It further highlights the importance of multiplexed and multimodal sensing for improving the accuracy of wearable biomarker measurements. Because molecular signals obtained from wearable sensors are affected by environmental, physiological, and demographic factors, the integration of machine learning and other data-processing methods is essential for identifying meaningful relationships between continuously measured molecular signatures and health conditions.

Chapter 2 presents the development of a wearable epidermal platform for continuous monitoring of glucose, cholesterol, and triglyceride-related signals in sweat. The platform leverages enzymatic reactions of increasing complexity, including a single-step reaction for glucose, a dual-step enzymatic reaction for cholesterol, and a three-enzyme cascading reaction for triglycerides. The triglyceride cascade requires adenosine triphosphate as an external cofactor for the phosphorylation of glycerol. Because adenosine triphosphate is consumed during the reaction, maintaining continuous enzymatic activity requires sustained cofactor replenishment within the sensing chamber.

To address this challenge, a polymeric cofactor-releasing module, termed Poly-CORE, was developed using adenosine triphosphate-encapsulating polypyrrole nanostructures. The Poly-CORE module enabled sweat-triggered release of adenosine triphosphate and maintained cofactor concentrations above the enzymatic threshold required for triglyceride sensing. The release profiles could be tuned by modifying the electrodeposition conditions and further regulated through electrochemical stimulation. An integrated multi-walled carbon nanotube-based adenosine triphosphate sensor enabled direct measurement of cofactor availability and provided real-time calibration of the triglyceride sensor response. Together, these components enabled continuous operation of a cofactor-dependent enzymatic cascade and established a generalizable strategy for extending wearable biosensing beyond conventional single-enzyme and oxidase-based reactions.

The wearable platform also integrated a potentiometric pH sensor and an impedimetric sweat-rate sensor to account for physiological and sampling-related variations. A miniaturized iontophoresis module enabled localized and continuous sweat stimulation, while the microfluidic system directed sweat through the cofactor-releasing and biosensing chambers. The enzymatic sensors demonstrated sensitivity within physiologically relevant concentration ranges, selectivity against common sweat interferents, and operational stability suitable for continuous measurements. During controlled dietary challenges and habitual daily activities, the integrated platform captured dynamic changes in sweat glucose, cholesterol, and triglyceride-related signals. These studies demonstrated the ability of the wearable system to resolve time-dependent metabolic responses that cannot be obtained from isolated measurements alone.

Chapter 3 evaluates the relationship between sweat and blood lipid measurements using a physiology-informed causal machine learning framework. Direct comparisons between sweat and blood cholesterol and triglycerides produced weak associations, reflecting the effects of sweat rate, body composition, demographic variability, and the complex transport and secretion behavior of lipid species. Directed acyclic graphs were therefore used to represent hypothesized relationships among sweat biomarkers, sweat rate, physiological covariates, and systemic lipid levels. These causal structures guided the selection of adjustment variables and enabled the development of individualized mappings between sweat and blood lipids.

The causal models demonstrated improved agreement with paired blood lipid measurements compared with conventional linear and regularized regression models. The final models achieved coefficients of determination of 0.610 for cholesterol and 0.912 for triglycerides under the evaluated framework. Negative-control analyses excluding the sweat biochemical measurements resulted in substantially reduced predictive performance, demonstrating that sweat lipid signals provided information beyond static demographic and physiological covariates. These findings highlight that the interpretation of wearable biochemical measurements requires models that account for the physiological

processes governing biofluid composition, rather than relying only on direct sweat-to-blood correlations.

Overall, this thesis establishes a wearable cofactor-refreshing biosensing framework for continuous epidermal lipid profiling. By integrating sustained cofactor delivery, real-time cofactor measurement, multiplexed biochemical sensing, physiological calibration, and causal machine learning, this work expands the range of enzymatic reactions that can be implemented in wearable devices. Although additional validation across larger and more diverse populations will be required before sweat lipid profiling can support clinical decision-making, the technologies developed in this thesis provide a foundation for continuous monitoring of complex metabolic processes and for the translation of multistep enzymatic assays into wearable bioelectronic platforms.

4.2 Limitations

Several considerations define the scope of these results and motivate future work. The triglyceride cascade responds to glycerol generated from lipid hydrolysis as well as endogenous sweat glycerol, so the sensor reports a proxy for lipolytic activity rather than an absolute triglyceride concentration; pairing the measurement with orthogonal markers or subject-specific calibration would sharpen its specificity. The multi-enzyme CH and TG sensors also exhibited greater sensor-to-sensor variability than the single-enzyme glucose sensor, reflecting the heightened sensitivity of cascading reactions to fabrication and enzyme-loading variations, which points to a need for tighter manufacturing control as the platform scales. Finally, although on-body studies established the feasibility of continuous lipid profiling across diverse dietary conditions, broader validation across larger and more heterogeneous cohorts will be required to translate these dynamic measurements into clinically actionable cardiometabolic indicators. Addressing these challenges would position continuous epidermal lipid profiling, and the cofactor-refreshing strategy that enables it, as a practical foundation for the next generation of noninvasive metabolic monitoring.

4.3 Future Outlook

4.3.1 MS-Wearable Convergence

In biology and medicine, access governs insight. The ability to monitor molecular processes in real time, within their native physiological context, has long remained limited. Most current molecular diagnostics rely on static snapshots, including blood draws or biopsies processed in centralized laboratories, which provide delayed interpretations of transient biological events¹. In acute settings such as pneumonia or sepsis, these delays can be life-threatening, underscoring the need for timely and reliable biomarkers that reflect dynamic physiological states.

Even the most sophisticated omics platforms, despite their breadth and sensitivity, remain constrained by invasive sampling, limited temporal resolution, and disconnection from everyday physiology. These limitations are critical in rapidly evolving conditions such as pain, inflammation, metabolic dysregulation, and neuroendocrine stress, where meaningful molecular changes often occur within minutes or hours. Capturing these dynamics requires a shift from episodic measurement toward continuous molecular sensing.

This emerging need is driving the convergence of omics-enabled mass spectrometry and wearable chemical sensing technologies in a bidirectional framework. Among omics layers, metabolomics, lipidomics, and proteomics most directly reflect phenotype, making them highly relevant for real-time, noninvasive monitoring^{2,3}. Their molecular targets, including metabolites, lipids, and proteins, act as downstream effectors responsive to physiological stimuli and are present in accessible biofluids. Unlike DNA or RNA, which primarily reflects genetic or regulatory potential, these molecular classes capture dynamic phenotypic responses and can be noninvasively sampled from sweat, saliva, tears, interstitial fluid, sebum, or cerumen. Because the identities and patterns of these molecules cannot be inferred from genomic sequence alone, mass spectrometry has an indispensable role in their empirical characterization. However, despite its diagnostic power, mass spectrometry-based insights are typically confined to laboratory settings, limiting their translation into real-time, context-aware health applications.

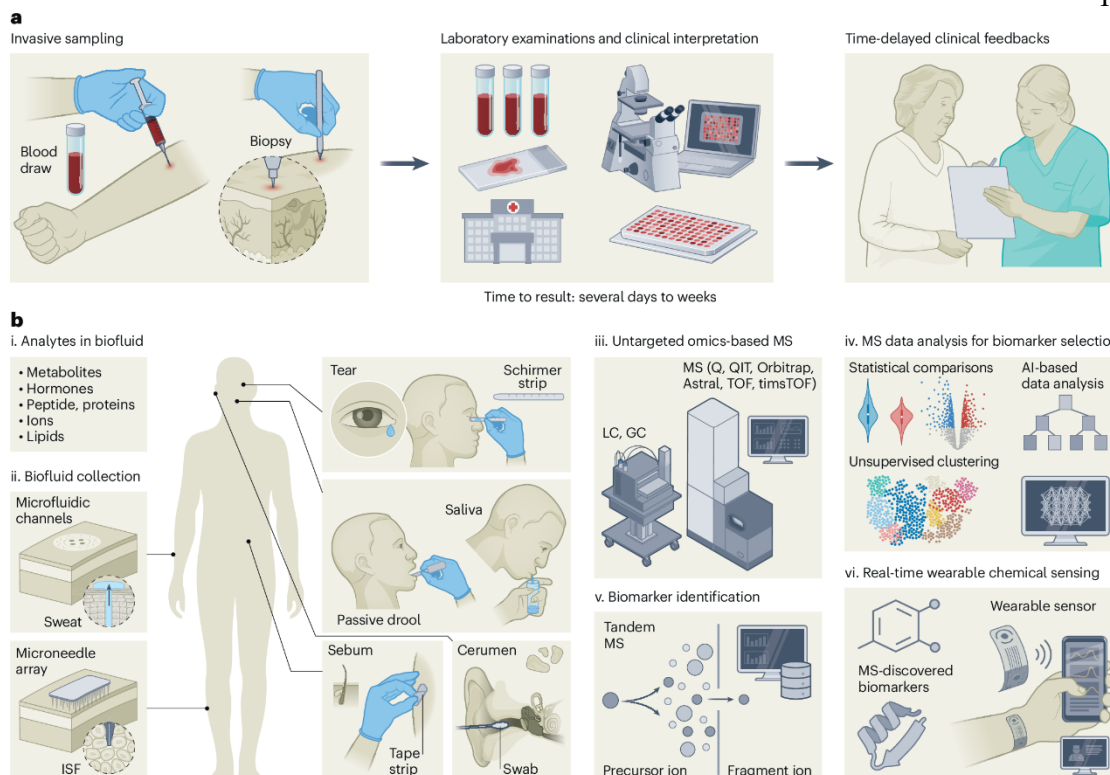


Fig. 4-2. From episodic diagnostics to continuous molecular monitoring. a, Conventional clinical diagnostics relying on invasive procedures such as blood draws or biopsies followed by centralized laboratory analysis, resulting in delayed feedback over days to weeks and limiting detection of rapid or transient physiological changes. **b,** Continuous molecular monitoring by integrating untargeted MS with wearable chemical sensors. **i,** key analyte classes in noninvasive biofluids include ions, metabolites, hormones, lipids, proteins and peptides. **ii,** Biofluids are collected through microfluidics for sweat, microneedles for ISF, passive drool for saliva, Schirmer strips for tears, tape strips for sebum and swabs for cerumen. **iii,** Untargeted MS platforms such as Q, QIT, Orbitrap and TOF enable omics-scale profiling. Advanced architectures such as TIMS (for example, timsTOF Pro 2) and Orbitrap–Astral hybrid analyzers further enhance ion use and MS/MS acquisition speed, enabling high-throughput analysis of low-volume, noninvasive biofluids. **iv,** Biomarker discovery is driven by statistical comparisons and AI-based analyses. **v,** Tandem-MS supports structural annotation. **vi,** Validated biomarkers are ultimately translated into wearable sensors for longitudinal, on-body

monitoring. Together, these steps form a closed-loop pathway from molecular discovery to continuous, real-time health sensing. **Figure** created in BioRender; Kim, M. J. <https://BioRender.com/83fkyaq> (2026). Q, quadrupole; QIT, quadrupole ion trap; TIMS, trapped-ion-mobility spectrometry.

In contrast, wearable chemical sensors offer continuous access to molecular signals in situ⁴⁻⁶. Advances in materials, microfluidics, and bioelectronics have enabled real-time tracking of electrolytes, metabolites, hormones, and certain proteins directly on the body⁷. These platforms operate outside clinical infrastructure, providing longitudinal biochemical insight during daily life. However, most current wearable devices remain limited to narrow panels of preselected analytes and generally lack the discovery capacity of untargeted omics platforms.

The integration of untargeted mass spectrometry discovery and on-body sensing therefore offers a path for translating molecular insights into real-time diagnostics. In this framework, high-dimensional mass spectrometry workflows can inform analyte selection for wearable sensing, while wearable platforms can provide longitudinal real-world data that reveal temporal dynamics, individual variability, and context-dependent physiological responses. As illustrated in **Fig. 4-2**, this convergence establishes an omics-to-wearables translation loop in which noninvasive biofluid sampling, untargeted molecular profiling, biomarker selection, and wearable sensing are connected within a closed pathway from discovery to continuous monitoring^{6,8-27}.

The wearable lipid-sensing platform developed in this thesis represents one step toward this broader vision. By enabling noninvasive monitoring of lipid-related biomarkers, this work expands wearable chemical sensing beyond traditional electrolytes and small metabolites toward molecular markers of cardiometabolic health. However, realizing the full potential of wearable lipid profiling will require advances in biomarker discovery, biofluid normalization, sensor stability, multiplexed detection, and longitudinal clinical validation.

4.3.2 Sensor-Ready Biomarkers

Untargeted mass spectrometry has emerged as a leading tool for biomarker discovery because it enables comprehensive molecular profiling without requiring predefined hypotheses. While genomics and transcriptomics define regulatory potential, metabolomics, lipidomics, and proteomics reveal dynamic functional phenotypes that respond to physiological and environmental changes^{2,31}. These molecular signatures are especially relevant for wearable sensing because they often exist in accessible biofluids and can reflect transient biochemical events over minutes to hours.

A major challenge in biomarker discovery is that individual molecules often lack diagnostic specificity. For example, elevated lactate can reflect sepsis-related anaerobic metabolism, but it can also arise from exercise or hypoxia²⁸. Similarly, inflammatory cytokines such as interleukin-6 and tumor necrosis factor can increase across multiple disease states, limiting their specificity as isolated biomarkers^{29,30}. These examples highlight the need for unbiased molecular profiling capable of identifying context-specific patterns rather than relying on single analyte thresholds.

Untargeted mass spectrometry addresses this limitation by resolving multi-analyte, pathway-aware fingerprints and time-dependent molecular trajectories. Product-to-precursor ratios can provide more interpretable information than absolute concentrations because they encode pathway-level constraints and reduce the influence of dilution and matrix effects. For example, the lactate-to-pyruvate ratio reflects cytosolic redox state and can be more informative than either analyte alone³². Similarly, tryptophan-pathway ratios have been used to reveal disease-associated metabolic alterations in high-resolution mass spectrometry studies³³. Coregulated molecular modules can also summarize coordinated analyte sets into panel-level signatures, improving discrimination when single markers overlap across conditions^{34,35}.

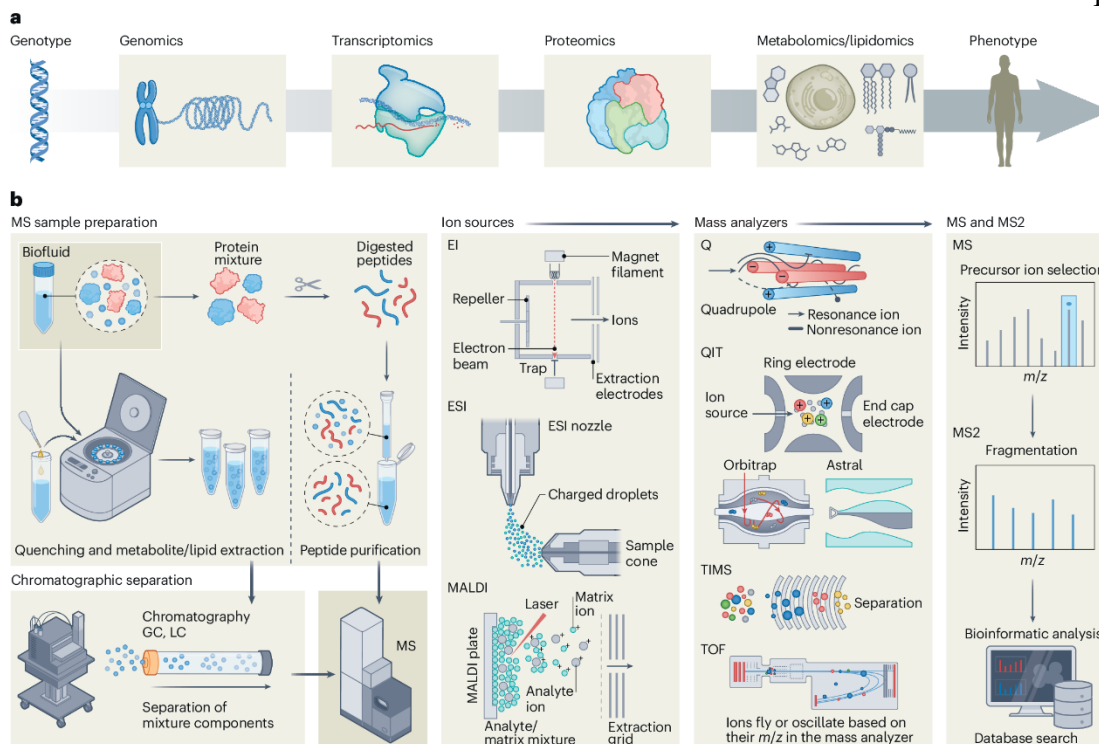


Fig. 4-3. Mass spectrometry-based untargeted omics for biomarker discovery. a, Multiscale omics cascade from genome to phenotype, showing how protein and metabolite/lipid states reflect both genetic programs and environmental cues, ultimately determining phenotypic expression. **b,** Experimental workflow of MS-based untargeted metabolomics, lipidomics and proteomics. Biofluids undergo metabolite/lipid extraction or protein digestion and purification, followed by chromatographic separation and ionization such as EI, ESI and MALDI. Ions are analyzed using various mass analyzers, including Q, QIT, Orbitrap and advanced platforms such as timsTOF and Orbitrap–Astral hybrid analyzers. By maximizing ion use and accelerating fragmentation cycles, these systems enable rapid, quantitative analysis of microscale, noninvasive biofluids. Tandem MS (MS/MS) provides precursor fragmentation and structural identification using spectral databases. **Figure** created in BioRender; Kim, M. J. <https://BioRender.com/1qraq6> (2026). EI, electron ionization; ESI, electrospray ionization.

Dynamic molecular features provide an additional layer of information. Metrics such as rise slope, decay slope, time-to-peak, recovery half-life, and short-window area under the curve

can capture biochemical kinetics under standardized perturbations. For example, metabolomic trajectories measured during oral glucose tolerance testing have been associated with insulin sensitivity, demonstrating that temporal molecular responses can provide information beyond static baseline measurements³⁶. This panel-centric and kinetics-centric view is highly relevant to wearable lipid monitoring because lipid metabolism is inherently dynamic, especially in response to meals, exercise, metabolic stress, and pharmacological interventions.

For future wearable lipid platforms, mass spectrometry can guide the transition from measuring a small number of conventional lipid markers toward identifying broader lipidomic and metabolic signatures. Rather than limiting wearable lipid sensing to total cholesterol and triglycerides, future studies could use untargeted lipidomics to identify lipid species, lipid ratios, or pathway-linked panels that better reflect cardiometabolic state, postprandial lipid handling, or metabolic risk. This would allow wearable platforms to be designed around biologically meaningful lipid signatures rather than only around historically available clinical assays.

However, not every mass spectrometry-discovered molecule is suitable for wearable translation. MS can reveal thousands of molecular features across metabolomic, lipidomic, and proteomic datasets, but only a fraction can be feasibly monitored by wearable sensors. Many candidate biomarkers exist at trace concentrations, degrade rapidly, are strongly affected by biofluid secretion variability, or lack a practical sensing mechanism. Therefore, future biomarker selection should prioritize sensor-ready targets with five key properties: detectability in microliter or submicroliter biofluid volumes, responsiveness on physiologically relevant timescales, short-term chemical stability, robustness to secretion variability, and compatibility with practical transduction chemistries^{70,71}.

This framework is particularly important for lipid biomarkers. Lipid species differ widely in abundance, polarity, stability, transport into noninvasive biofluids, and compatibility with electrochemical or optical detection. Future studies should therefore use mass spectrometry not only to identify lipid candidates, but also to define their physiological concentration

ranges, temporal dynamics, interindividual variability, and relationship to blood-based lipid measurements. These data can then guide the engineering requirements for wearable lipid sensors, including limit of detection, dynamic range, calibration strategy, normalization approach, and sampling frequency.

4.3.3 Platform Development and Validation

While untargeted mass spectrometry provides the discovery engine for identifying candidate biomarkers, wearable biosensors provide the route to real-time monitoring. Wearable biochemical systems can generate continuous data streams that capture dynamic molecular changes across the body or within localized tissues. Such platforms are increasingly being developed for accessible biofluids including sweat, interstitial fluid, saliva, tears, exhaled breath condensate, wound exudate, gastrointestinal fluids, sebum, and cerumen^{6,60–68}. Each biofluid presents distinct opportunities and technical constraints related to sampling, stability, contamination, transport, and molecular abundance.

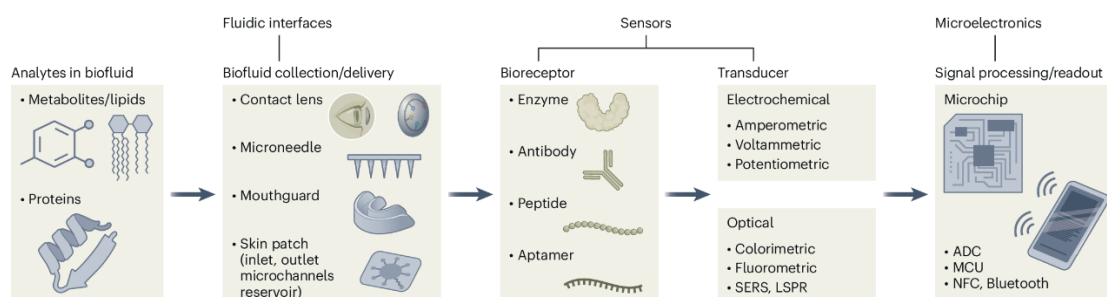


Fig. 4-4. Architecture of wearable biochemical sensors. Analytes (for example, metabolites/lipids and proteins) are accessed through biofluids collected through skin-interfaced systems such as contact lenses, microneedles, mouthguards or skin patches. Biofluids interface with bioreceptors (enzymes, antibodies, peptides or aptamers) that confer target specificity. Signals are transduced and processed by electronic modules for on-device or wireless readout. **Figure** created in BioRender; Kim, M. J. <https://BioRender.com/0zsn92d> (2026). SERS, surface-enhanced Raman scattering; LSPR, localized surface plasmon resonance; ADC, analog-to-digital converter; MCU, microcontroller unit; NFC, near-field communication.

Wearable biosensor design can be organized around three essential components: the fluidic interface, the sensing element, and the electronic readout system. Fluidic interfaces control biofluid extraction, transport, storage, and delivery to the sensing region. Recognition elements such as enzymes, antibodies, peptides, aptamers, or molecularly imprinted polymers provide target specificity. Transducers then convert molecular recognition into electrochemical, optical, or other measurable signals, which are processed through integrated electronics and transmitted wirelessly⁷. This architecture provides a useful framework for future wearable lipid sensors, which will require coordinated advances in lipid sampling, recognition chemistry, signal transduction, and integrated system design.

One of the central engineering challenges is biofluid handling. Wearable-accessible fluids are typically available in small volumes and are affected by secretion rate, evaporation, contamination, and transport delay. Sweat, for example, is secreted at rates on the order of 10 to 100 nl min⁻¹ cm⁻², meaning that only microliter or submicroliter volumes may be available over short collection periods³⁷. Conventional LC-MS and GC-MS workflows often require larger sample volumes, creating a mismatch between molecular discovery workflows and wearable-accessible sampling^{8,12}. Miniaturized MS workflows, nanoflow LC, microsampling, and nanomaterial-enhanced laser desorption or ionization approaches can help bridge this gap, but wearable translation still requires robust on-body fluidics and sampling control⁴⁵⁻⁵⁹.

Biofluid secretion variability is another major challenge. Wearable-accessible fluids exhibit time-varying secretion influenced by hydration, thermoregulation, stress, local skin physiology, anatomical site, and environmental conditions³⁸. As a result, measured analyte intensity can reflect both true molecular concentration and dilution caused by secretion dynamics. Without correction, fluctuations may be misinterpreted as biochemical changes when they actually arise from sampling variability. Sweat metabolomics studies have shown that secretion-rate effects can account for a large fraction of total variance, highlighting the need for rate-aware correction³⁹. Practical normalization strategies include co-measuring flow or volume, tracking dilution surrogates such as conductivity or osmolality, using

internal standards, and incorporating microfluidic designs that provide on-skin flow or volume readouts^{40–44}.

For wearable lipid platforms, secretion-aware normalization will be essential. Lipid-related signals in sweat or interstitial fluid should not be interpreted as isolated concentration values without accounting for sweat rate, pH, temperature, sampling location, evaporation, and device-skin interface conditions. Future devices should therefore integrate lipid sensing with contextual channels such as sweat rate, pH, temperature, conductivity, osmolality, and physical activity. These auxiliary measurements can help distinguish true lipid dynamics from dilution, transport, or device-related artifacts.

Future wearable lipid sensors will also require improvements in sensitivity, stability, multiplexing, and calibration. MS-discovered biomarkers may exist at nanomolar or picomolar concentrations, requiring high-surface-area transducers, enzyme-coupled amplification, affinity-based recognition, low-noise electronics, and environmental compensation. Signal stability must be maintained over hours to days despite motion, temperature shifts, biofouling, and changes in ionic strength. Multiplexing will be necessary because MS-derived specificity often relies on multi-analyte patterns rather than single markers. This can be achieved through parallelized electrodes, distinct recognition chemistries, microfluidic partitioning, or combined electrochemical and optical modalities^{4,69}.

Mass spectrometry can directly inform these engineering specifications. Physiological concentrations measured by MS define the calibration span and linear-response range required for wearable sensors. Variance decomposition in MS datasets can identify how much signal fluctuation arises from dilution or secretion rate, motivating the inclusion of normalization channels such as flow, conductivity, or osmolality. Perturbation-based MS time courses can define rise, decay, and recovery kinetics, which then specify the temporal sampling resolution needed for accurate monitoring. These molecular and kinetic parameters can be translated into concrete device requirements, including limit of detection, dynamic range, normalization architecture, sampling interval, and calibration strategy.

Even when lipid biomarkers and sensing mechanisms are technically feasible, staged validation is required before clinical deployment. Initial *in vitro* testing should define sensitivity, selectivity, detection limits, dynamic range, calibration linearity, and robustness against pH, temperature, and interferents. *Ex vivo* testing in authentic biofluids should then evaluate accuracy and precision in complex sample matrices. Regulatory bioanalytical validation frameworks often expect accuracy within 85 to 115% of nominal concentration and coefficient of variation below 15%⁷¹. Short-term on-body testing should compare wearable outputs against reference methods, such as mass spectrometry measurements of collected biofluids or clinical blood lipid panels. Finally, larger independent cohorts will be needed to assess generalizability across age, sex, hydration status, metabolic state, comorbidities, and environmental conditions.

For the wearable lipid sensor developed in this thesis, this validation ladder defines a clear path forward. Future studies should expand analytical validation in authentic sweat and interstitial-fluid-relevant matrices, compare wearable lipid outputs with LC-MS and standard blood lipid measurements, evaluate fasting and postprandial conditions, and test whether wearable lipid trends provide clinically meaningful information beyond conventional blood testing.

4.3.4 Dynamic Health Monitoring

The long-term opportunity for wearable lipid profiling is not simply to miniaturize blood lipid assays. Instead, the goal is to move from static concentration measurements toward dynamic, context-aware interpretation of lipid metabolism during daily life. Conventional blood lipid panels provide clinically useful snapshots of cholesterol, triglycerides, HDL, and LDL, but they do not fully capture postprandial responses, recovery kinetics, day-to-day variability, or individual responses to diet, exercise, medication, sleep, and stress.

Integrating mass spectrometry and wearable sensing could redefine biomarkers as dynamic molecular profiles rather than fixed concentration values. MS provides molecular identity, structural annotation, and pathway mapping, while wearable platforms provide continuous, context-rich temporal data. Together, these approaches can capture rates of change, recovery

kinetics, circadian or ultradian rhythms, secretion dynamics, and environmental context^{72,73}. MS-derived priors can guide wearable data analysis by identifying biologically meaningful co-fluctuations among pathway-linked molecules, while wearable data can reveal real-world temporal patterns that motivate targeted MS reassessment.

This bidirectional model is especially relevant for lipid sensing. MS-based lipidomics could first identify lipid species, lipid ratios, or metabolic signatures that are detectable in noninvasive biofluids and associated with cardiometabolic state. Wearable sensors could then monitor selected targets longitudinally under real-world conditions. Unexpected wearable trends, such as abnormal postprandial lipid excursions, delayed recovery to baseline, or altered responses to exercise or diet, could trigger targeted MS analysis to determine whether the signal reflects a true biochemical change or a sensing artifact. This feedback loop would allow biomarker panels, calibration models, and sensing strategies to be refined iteratively.

Such systems would also require robust data infrastructure and translational standards. To move from laboratory demonstrations to routine use, future wearable molecular platforms must address four major domains: standardization, clinical validation, data infrastructure, and equitable deployment. Standardized biofluid sampling, calibration, normalization, and reporting protocols will be required to compare results across devices, users, and studies. Longitudinal clinical studies will be needed to connect temporal biomarker patterns to actionable health outcomes, including metabolic instability, cardiometabolic risk, or response to intervention. Secure and interoperable data architectures will be necessary as continuous molecular profiles enter personal health records. Finally, equitable deployment will require evaluating performance across populations with differences in skin properties, sweat rate, sex, age, disease status, and access to healthcare.

For wearable lipid profiling, these considerations define the path from proof of concept toward clinical relevance. The most valuable future outputs may not be isolated measurements of cholesterol or triglycerides, but dynamic lipid profiles that describe how individuals respond to meals, physical activity, medications, metabolic stress, and lifestyle

interventions. By combining MS-guided molecular discovery with wearable sensing, future platforms could shift lipid assessment from episodic blood-based snapshots toward continuous, personalized, and context-aware cardiometabolic monitoring.

Bibliography of Chapter 4

1. Frank, R. & Hargreaves, R. Clinical biomarkers in drug discovery and development. *Nat. Rev. Drug Discov.* 2, 566–580 (2003).
2. Guo, T., Steen, J. A. & Mann, M. Mass-spectrometry-based proteomics: from single cells to clinical applications. *Nature* 638, 901–911 (2025).
3. Danzi, F. et al. To metabolomics and beyond: a technological portfolio to investigate cancer metabolism. *Signal Transduct. Target. Ther.* 8, 137 (2023).
4. Gao, W. et al. Fully integrated wearable sensor arrays for multiplexed in situ perspiration analysis. *Nature* 529, 509–514 (2016).
5. Brasier, N. et al. Applied body-fluid analysis by wearable devices. *Nature* 636, 57–68 (2024).
6. Heng, W. et al. A smart mask for exhaled breath condensate harvesting and analysis. *Science* 385, 954–961 (2024).
7. Chen, S. et al. Wearable flexible microfluidic sensing technologies. *Nat. Rev. Bioeng.* 2, 950–971 (2023).
8. Brunmair, J. et al. Finger sweat analysis enables short interval metabolic biomonitoring in humans. *Nat. Commun.* 12, 5993 (2021).
9. Delgado-Povedano, M. D. M., Calderón-Santiago, M., Priego-Capote, F., Jurado-Gámez, B. & Luque De Castro, M. D. Recent advances in human sweat metabolomics for lung cancer screening. *Metabolomics* 12, 166 (2016).
10. Kuwabara, H. et al. Salivary metabolomics with machine learning for colorectal cancer detection. *Cancer Sci.* 113, 3234–3243 (2022).
11. Ben, S. et al. Tear fluid-based metabolomics profiling in chronic dacryocystitis patients. *J. Proteome Res.* 24, 224–233 (2025).

12. Jiang, X., Wilkerson, E. C., Bailey, A. O., Russell, W. K. & Lillehoj, P. B. Microneedle-based sampling of dermal interstitial fluid using a vacuum-assisted skin patch. *Cell Rep. Phys. Sci.* 5, 101975 (2024).
13. Ibrahim, W. et al. Visualization of exhaled breath metabolites reveals distinct diagnostic signatures for acute cardiorespiratory breathlessness. *Sci. Transl. Med.* 14, eab15849 (2022).
14. Mueller, D. C., Piller, M., Niessner, R., Scherer, M. & Scherer, G. Untargeted metabolomic profiling in saliva of smokers and nonsmokers by a validated GC–TOF–MS method. *J. Proteome Res.* 13, 1602–1613 (2014).
15. Ossoliński, K. et al. Targeted and untargeted urinary metabolic profiling of bladder cancer. *J. Pharm. Biomed. Anal.* 233, 115473 (2023).
16. Wu, J. et al. Glaucoma characterization by machine learning of tear metabolic fingerprinting. *Small Methods* 6, e2200264 (2022).
17. Xu, W. et al. Diagnosis of Parkinson’s disease via the metabolic fingerprint in saliva by deep learning. *Small Methods* 7, e2300285 (2023).
18. Tu, J. et al. A wireless patch for the monitoring of C-reactive protein in sweat. *Nat. Biomed. Eng.* 7, 1293–1306 (2023).
19. Xu, C. et al. A physicochemical-sensing electronic skin for stress response monitoring. *Nat. Electron.* 7, 168–179 (2024).
20. Naik, A. R. et al. Printed microfluidic sweat sensing platform for cortisol and glucose detection. *Lab Chip* 22, 156–169 (2022).
21. Kunnel, B. P. & Demuru, S. An epidermal wearable microfluidic patch for simultaneous sampling, storage, and analysis of biofluids with counterion monitoring. *Lab Chip* 22, 1793–1804 (2022).
22. Lim, H.-R. et al. Smart bioelectronic pacifier for real-time continuous monitoring of salivary electrolytes. *Biosens. Bioelectron.* 210, 114329 (2022).
23. Gao, Y. et al. A flexible multiplexed immunosensor for point-of-care in situ wound monitoring. *Sci. Adv.* 7, eabg9614 (2021).
24. Koh, A. et al. A soft, wearable microfluidic device for the capture, storage, and colorimetric sensing of sweat. *Sci. Transl. Med.* 8, 366ra165 (2016).

25. Kim, J. et al. A skin-interfaced, miniaturized microfluidic analysis and delivery system for colorimetric measurements of nutrients in sweat and supply of vitamins through the skin. *Adv. Sci. (Weinh.)* 9, e2103331 (2022).
26. Jiang, X. & Lillehoj, P. B. Microneedle-based skin patch for blood-free rapid diagnostic testing. *Microsyst. Nanoeng.* 6, 96 (2020).
27. Yetisen, A. K. et al. Scleral lens sensor for ocular electrolyte analysis. *Adv. Mater.* 32, e1906762 (2020).
28. Li, X. et al. Lactate metabolism in human health and disease. *Signal Transduct. Target. Ther.* 7, 305 (2022).
29. Heikenfeld, J. et al. Accessing analytes in biofluids for peripheral biochemical monitoring. *Nat. Biotechnol.* 37, 407–419 (2019).
30. McElvaney, O. J., Curley, G. F., Rose-John, S. & McElvaney, N. G. Interleukin-6: obstacles to targeting a complex cytokine in critical illness. *Lancet Respir. Med.* 9, 643–654 (2021).
31. Alseekh, S. et al. Mass spectrometry-based metabolomics: a guide for annotation, quantification and best reporting practices. *Nat. Methods* 18, 747–756 (2021).
32. Mintun, M. A., Vlassenko, A. G., Rundle, M. M. & Raichle, M. E. Increased lactate/pyruvate ratio augments blood flow in physiologically activated human brain. *Proc. Natl Acad. Sci. USA* 101, 659–664 (2004).
33. Abujrais, S., Vallianatou, T. & Bergquist, J. Untargeted metabolomics and quantitative analysis of tryptophan metabolites in myalgic encephalomyelitis patients and healthy volunteers: a comparative study using high-resolution mass spectrometry. *ACS Chem. Neurosci.* 15, 3525–3534 (2024).
34. Wang, F. et al. Plasma metabolomic profiles associated with mortality and longevity in a prospective analysis of 13,512 individuals. *Nat. Commun.* 14, 5744 (2023).
35. Calderón-Santiago, M. et al. Human sweat metabolomics for lung cancer screening. *Anal. Bioanal. Chem.* 407, 5381–5392 (2015).
36. Nowak, C. et al. Glucose challenge metabolomics implicates medium-chain acylcarnitines in insulin resistance. *Sci. Rep.* 8, 8691 (2018).

37. Sonner, Z. et al. The microfluidics of the eccrine sweat gland, including biomarker partitioning, transport, and biosensing implications. *Biomicrofluidics* 9, 031301 (2015).
38. Bandodkar, A. J. et al. Battery-free, skin-interfaced microfluidic/electronic systems for simultaneous electrochemical, colorimetric, and volumetric analysis of sweat. *Sci. Adv.* 5, eaav3294 (2019).
39. Harshman, S. W. et al. Rate normalization for sweat metabolomics biomarker discovery. *Talanta* 223, 121797 (2021).
40. Nyein, H. Y. Y. et al. A wearable patch for continuous analysis of thermoregulatory sweat at rest. *Nat. Commun.* 12, 1823 (2021).
41. Shin, S. et al. A bioinspired microfluidic wearable sensor for multiday sweat sampling, transport, and metabolic analysis. *Sci. Adv.* 11, eadw9024 (2025).
42. Rakusanova, S., Fiehn, O. & Cajka, T. Toward building mass spectrometry-based metabolomics and lipidomics atlases for biological and clinical research. *Trends Analyt. Chem.* 158, 116825 (2023).
43. Kim, T. et al. A hierarchical approach to removal of unwanted variation for large-scale metabolomics data. *Nat. Commun.* 12, 4992 (2021).
44. Reeder, J. T. et al. Waterproof, electronics-enabled, epidermal microfluidic devices for sweat collection, biomarker analysis, and thermography in aquatic settings. *Sci. Adv.* 5, eaau6356 (2019).
45. Bi, H. et al. Electrostatic spray ionization-mass spectrometry for direct and fast wine characterization. *ACS Omega* 3, 17881–17887 (2018).
46. Udeshi, N. D. et al. Rapid and deep-scale ubiquitylation profiling for biology and translational research. *Nat. Commun.* 11, 359 (2020).
47. Roberg-Larsen, H., Wilson, S. R. & Lundanes, E. Recent advances in on-line upfront devices for sensitive bioanalytical nano LC methods. *Trends Analyt. Chem.* 136, 116190 (2021).
48. Bravo-Veyrat, S. & Hopfgartner, G. Mass spectrometry based high-throughput bioanalysis of low molecular weight compounds: are we ready to support personalized medicine? *Anal. Bioanal. Chem.* 414, 181–192 (2022).

49. Kim, M. et al. Laser-induced surface reconstruction of nanoporous Au-modified TiO₂ nanowires for in situ performance enhancement in desorption and ionization mass spectrometry. *Adv. Funct. Mater.* 31, 2102475 (2021).
50. Meister, I. et al. High-precision automated workflow for urinary untargeted metabolomic epidemiology. *Anal. Chem.* 93, 5248–5258 (2021).
51. Bian, Y. et al. Robust microflow LC–MS/MS for proteome analysis: 38,000 runs and counting. *Anal. Chem.* 93, 3686–3690 (2021).
52. Messner, C. B. et al. Mass spectrometry-based high-throughput proteomics and its role in biomedical studies and systems biology. *Proteomics* 23, e2200013 (2023).
53. Guzman, U. H. et al. Ultra-fast label-free quantification and comprehensive proteome coverage with narrow-window data-independent acquisition. *Nat. Biotechnol.* 42, 1855–1866 (2024).
54. Dayon, L. et al. Relative quantification of proteins in human cerebrospinal fluids by MS/MS using 6-plex isobaric tags. *Anal. Chem.* 80, 2921–2931 (2008).
55. Ravindra, K. C. et al. Tandem mass tag-based quantitative proteomic profiling identifies candidate serum biomarkers of drug-induced liver injury in humans. *Nat. Commun.* 14, 1215 (2023).
56. Park, J.-M. et al. MALDI-TOF mass spectrometry based on parylene-matrix chip for the analysis of lysophosphatidylcholine in sepsis patient sera. *Anal. Chem.* 91, 14719–14727 (2019).
57. Lancaster, N. M. et al. Fast and deep phosphoproteome analysis with the Orbitrap Astral mass spectrometer. *Nat. Commun.* 15, 7016 (2024).
58. Demichev, V. et al. dia-PASEF data analysis using FragPipe and DIA-NN for deep proteomics of low sample amounts. *Nat. Commun.* 13, 3944 (2022).
59. Meier, F. et al. Online parallel accumulation–serial fragmentation (PASEF) with a novel trapped ion mobility mass spectrometer. *Mol. Cell. Proteomics* 17, 2534–2545 (2018).
60. Ding, S. et al. A fingertip-wearable microgrid system for autonomous energy management and metabolic monitoring. *Nat. Electron.* 7, 788–799 (2024).
61. Friedel, M. et al. Opportunities and challenges in the diagnostic utility of dermal interstitial fluid. *Nat. Biomed. Eng.* 7, 1541–1555 (2023).

62. Arakawa, T. et al. A wearable cellulose acetate-coated mouthguard biosensor for in vivo salivary glucose measurement. *Anal. Chem.* 92, 12201–12207 (2020).
63. Xu, J., Tao, X., Liu, X. & Yang, L. Wearable eye patch biosensor for noninvasive and simultaneous detection of multiple biomarkers in human tears. *Anal. Chem.* 94, 8659–8667 (2022).
64. Shirzaei Sani, E. et al. A stretchable wireless wearable bioelectronic system for multiplexed monitoring and combination treatment of infected chronic wounds. *Sci. Adv.* 9, eadf7388 (2023).
65. Wang, C. et al. Wound management materials and technologies from bench to bedside and beyond. *Nat. Rev. Mater.* 9, 550–566 (2024).
66. Wang, C. et al. A microfluidic wearable device for wound exudate management and analysis in human chronic wounds. *Sci. Transl. Med.* 17, eadt0882 (2025).
67. Mimee, M. et al. An ingestible bacterial-electronic system to monitor gastrointestinal health. *Science* 360, 915–918 (2018).
68. Min, J. et al. Continuous biochemical profiling of the gastrointestinal tract using an integrated smart capsule. *Nat. Electron.* 8, 844–855 (2025).
69. Kim, S. et al. Soft, skin-interfaced microfluidic systems with integrated immunoassays, fluorometric sensors, and impedance measurement capabilities. *Proc. Natl Acad. Sci. USA* 117, 27906–27915 (2020).
70. ICH. Validation of analytical procedures Q2(R2). https://database.ich.org/sites/default/files/ICH_Q2-R2_Document_Step2_Guideline_2022_0324.pdf (2022).
71. U.S. Department of Health and Human Services, FDA, CDER & CVM. Bioanalytical method validation guidance for industry. <https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf> (2018).
72. Brasier, N. et al. The molecular signature of heat stress in sweat reveals non-invasive biomarker candidates for health monitoring. *Commun. Biol.* 8, 650 (2025).
73. Mahato, K. et al. Hybrid multimodal wearable sensors for comprehensive health monitoring. *Nat. Electron.* 7, 735–750 (2024).

Materials

Polyimide films (~12–75 μm thick) were purchased from DuPont. PET films (~12–250 μm thick) were purchased from McMaster-Carr. Medical adhesives (M-tapes, 3M 468MP) were purchased from 3M. Cholesterol esterase (ChEs) (123 U mg^{-1}), cholesterol oxidase (ChOx) (18.2 U mg^{-1}), lipase (LIP) (33.3 U mg^{-1}), glycerol kinase (GK) (102 U mg^{-1}) and glycerol-3-phosphate oxidase (G3PO) (23.8 U mg^{-1}) were purchased from Toyobo Co. Potassium chloride (KCl), nickel chloride (NiCl_2), potassium (III) ferricyanide ($\text{K}_3[\text{Fe}(\text{CN})_6]$), agarose, carbachol, glucose oxidase from *Aspergillus niger* (216 U mg^{-1}), bovine serum albumin (BSA), polyvinyl butyral resin BUTVAR B-98 (PVB), aniline, iron (III) chloride, sodium hydroxide, sodium chloride (NaCl), lithium perchlorate, creatine monohydrate (CR), and polypyrrole were purchased from Sigma-Aldrich. Glutaraldehyde (25% aqueous solution), uric acid (UA), and choline chloride (Chi) were purchased from Alfa Aesar. Amplex Red Cholesterol Assay kit, Tetrachloroauric acid (HAuCl_4), 10x phosphate buffer solution (PBS), hydrochloric acid (HCl), methanol (MeOH), Adenosine 5-triphosphate disodium salt hydrate (ATP), and dextrose (D-glucose) anhydrous were purchased from Thermo Fisher Scientific. Triglyceride quantification assay was purchased from Abcam. Triolein was purchased from Tokyo Chemical Industry.

Fabrication and assembly of wearable sensors**Printed electrodes**

Polyimide (PI) films served as the substrate for inkjet printing (DMP-2850, Fujifilm). Silver ink was utilized to print the interconnects, connection pads, interdigitated sweat rate sensor electrodes, and reference electrode. Carbon ink was used to print the iontophoresis electrodes, counter electrode, and working electrodes (on top of the connection pads). The reference and working electrodes were further modified for electrochemical sensing using an electrochemical station (CHI 840D).

Microfluidics

A 50 W CO₂ laser cutter (Universal Laser System) was used to pattern the microfluidic layers. M-tape (3M 468MP) and PET layers were used for layered patterning based on the specifications from the computer aided designs. The fabricated microfluidic layers were assembled onto the PI substrate for sweat collection and transport.

IP gels

The iontophoresis anode and cathode gels were prepared by heating deionized (DI) water containing 3% w/w agarose at 200 °C under continuous stirring to create homogeneous solutions. The solutions were then cooled to 165 °C. Once at 165 °C, carbachol (1% w/w) was added to the anode mixture, whilst KCl (1% w/w) was added to the cathode mixture. The prepared mixtures were then poured into the IP electrode molds of the assembled microfluidic sensor patches.

Biosensor development and characterization

Enzymatic sensors

Gold nanodendrites were grown on the surface of the carbon working electrodes by applying pulsed voltage from -0.9 to 0.9 V for 1500 cycles at a frequency of 50 Hz using a 50 mM tetrachloroauric acid and 50 mM HCl solution. A Prussian blue redox mediator was then electrochemically deposited on the surface of the Au layer by performing cyclic voltammetry from -0.2 to 0.6 V at a scan rate of 50 mV s⁻¹ in a solution containing 2.0 mM iron (III) chloride, 2.5 mM K₃[Fe(CN)₆], 0.1 M KCl and 0.1 M HCl for 20, 6, and 20 cycles corresponding to the glucose, cholesterol, and triglyceride sensors. Lastly, a NiHCF layer was deposited on the surface of the PB using cyclic voltammetry from 0 to 0.8 V at a scan rate of 100 mV s⁻¹ for 8 cycles in a solution containing 5.0 mM NiCl₂, 2.5 mM K₃[Fe(CN)₆], 0.1 M KCl and 0.1 M HCl.

The enzyme solution for the glucose sensor was prepared by mixing 99.0 µl of 1.0% bovine serum albumin, 1.0 µl of 2.5% glutaraldehyde and 2.0 µl of 10 mg ml⁻¹ glucose oxidase in 1x PBS. The cholesterol sensor enzyme solution was prepared by mixing 8.40 µl of 1.0% bovine serum albumin, 0.50 µl of 1.0% glutaraldehyde, 3.3 µl of 1.62 mg ml⁻¹ cholesterol esterase and 3.3 µl of 10.98 mg ml⁻¹ cholesterol oxidase in 1x PBS. The enzyme solution for

the triglyceride sensor was prepared by mixing 17.5 μl of 1.0% bovine serum albumin, 0.50 μl of 2.5% glutaraldehyde, 5.8 μl of 36 mg ml^{-1} LIP, 5.8 μl of 6 mg ml^{-1} GK and 5.8 μl of 9.6 mg ml^{-1} G3PO in 1x PBS. For all enzymatic sensors, 3 μl enzyme cocktail was then drop-cast on the surface of the modified electrodes and left to dry overnight at 4 $^{\circ}\text{C}$.

pH sensor

A gold layer was deposited on the surface of a carbon working electrode by applying a constant potential of 0 V for 30 s while submerged in a 50 mM tetrachloroauric acid and 50 mM HCl solution. After, a polyaniline layer was electropolymerized on the surface of the modified electrode via cyclic voltammetry sweeps from -0.2 to 1.0 V at a scan rate of 50 mV s^{-1} for 50 cycles.

ATP sensor

A 0.01% w/w carbon nanotube (CNT) solution in ethanol was prepared via probe sonication for 2 h to form a uniform dispersion of CNTs. Then, 2 μl of the CNT solution was drop-cast onto an inkjet-printed carbon working electrode. Lastly, 1 μl of a Nafion solution (0.1% v/v in ethanol) was drop-cast onto the sensor electrode surface and dried overnight.

Reference electrode

First, 0.3 μl of 0.1 M FeCl_3 solution was drop-cast on the surface of the inkjet-printed Ag reference electrode for 30 s to form a Ag/AgCl layer and then rinsed thoroughly with DI water. Then, 1.5 μl of a reference solution, made by sonicating 79.1 mg PVB and 50.0 mg NaCl in 1 ml methanol for 2 h, was drop-cast on the surface of the Ag/AgCl electrode. The modified reference electrode was left to dry overnight.

In vitro biosensor characterization

In vitro characterization of the biosensors was performed using an electrochemical workstation (CHI840E, CH Instruments for single electrode experiments and a CHI 1430, CH Instruments for multichannel studies) at room temperature. A commercial platinum wire was used as the counter electrode and a commercial Ag/AgCl electrode was used as the reference electrode. Chronoamperometric studies to characterize the enzymatic biosensors were performed at a constant potential of 0 V.

For multichannel experiments, amperometric sensors (Glu, CH, and TG) were operated continuously at 0 V versus Ag/AgCl, while potentiometric sensors were recorded under open-circuit conditions to prevent current injection into the electrolyte. ATP measurements were performed intermittently using differential pulse voltammetry with brief scans (approximately 20–30 s) every 10 min, enabling time-multiplexed operation. The DPV were used under the following conditions: potential range, 0.8–1.6 V; incremental potential, 0.04 V; pulse amplitude, 0.05 V; pulse width, 0.05 s; pulse period, 0.5 s. The counter electrode was designed to maintain uniform spacing relative to each working electrode, thereby minimizing unintended current pathways and reducing electrochemical coupling between sensing channels. In addition, because analyte concentrations are in the low micromolar range, the corresponding H_2O_2 production at each sensing site is inherently low, further limiting its potential to perturb neighboring electrodes or induce measurable pH shifts, with any generated H_2O_2 further reduced at the PB transducer interface.

The Glu, CH, and TG sensors were conditioned in PBS until a stable signal was observed and then the solutions were spiked to achieve concentrations of 0 to 200 μM of Glu, 0 to 3 μM of CH, and 0 to 150 μM of TG, respectively. For the TG sensor testing in vitro, the buffer solution contained 200 μM of ATP, to realize the enzymatic reaction. The pH dependency studies were performed by altering the solution's pH (from 7 to 9). Selectivity studies were performed by spiking the sensors with a known concentration of their corresponding target (100 μM , 3 μM and 50 μM for the Glu, CH and TG sensors, respectively) and spiking known concentrations of the potential interferents within the physiological ranges found in sweat. Shelf stability was assessed by storing an array of biosensors at 4 °C, and testing a new subset ($n=4$) at weekly intervals. The characterization of the pH sensor was achieved by performing open-circuit potential studies using McIlvaine buffers with pH values ranging from 3 to 8. The pH of each buffer solution was independently measured using a calibrated commercial pH meter prior to testing. The open-circuit potential of the integrated pH sensor was recorded after stabilization at each pH value, and calibration curves were constructed to evaluate sensitivity and linearity.

Sweating rate sensor and characterization

For in-vitro flow tests, a syringe pump (Braintree Scientific Inc.) was used to flow various solutions into the microfluidic channels at different flow rates (0.25 to 3 $\mu\text{l min}^{-1}$). NaCl solutions with various concentrations (12.5 to 200 mM) were prepared in DI water. For characterization of sweat rate sensor under various pH, McIlvaine buffers were used. A ceramic hot plate (Thermo Fisher Scientific) was used to characterize dependence on temperature in sweat rate sensor.

Poly-CORE module synthesis and characterization

The PPy/ATP structures within the CORE unit were synthesized using an electrochemical workstation (CHI840E, CHI Instruments) at room temperature. A commercial platinum wire was used as the counter electrode and a commercial Ag/AgCl electrode was used as the reference electrode. Firstly, an electrolyte solution containing 0.14 M pyrrole and 0.07 M lithium perchlorate was prepared in DI water. This solution was utilized to deposit a thin PPy film onto the surface of an ink-jet carbon electrode by applying a constant voltage of 1.0 V for 150 s. To achieve the PPy/ATP structures, different current densities were ramped up (from 0.075 to 1.0 mA cm^{-2}) in a solution containing 0.07 M lithium perchlorate, 0.20 M ATP and 0.15 M pyrrole, until a voltage of 0.6 V or higher was achieved to ensure polymerization. The total electrodeposition time for the characterization and on-body experiments was 30 min.

SEM images were taken to observe the morphological differences of the PPy/ATP structures synthesized in different electrodeposition conditions. A.C. Impedance studies were performed in a 0.1 NaCl solution using 0.01 V amplitude at a frequency range of 100 kHz to 1 Hz in a CHI 660 electrochemical station. A Braintree Scientific Inc. pump was utilized for all the in-vitro ATP releasing and TG sensing experiments. For the ATP release profile characterizations of the PPy/ATP structures, 1x PBS samples were collected from the microfluidic outlet of the sensing chamber to quantify the ATP concentrations of the samples at different time intervals. The aforementioned samples were measured via UV-vis (Thermo Scientific NanoDrop) at a wavelength of 260 nm. The PPy/ATP structures used for on-body experiments were electrodeposited the night prior to testing to ensure fresh ATP loading.

After fabrication, the devices were stored at 4 °C and protected from light until use. All ATP-containing precursor solutions were freshly prepared prior to each synthesis.

On-body evaluations of wearable platform and validation

Subject recruitment

The validation and evaluation of the sweat sensor were performed using human subjects in compliance with all the ethical regulations under a protocol (ID 19-0892) that was approved by the Institutional Review Board (IRB) at California Institute of Technology. Subjects were recruited from the campus and neighboring California Institute of Technology and the University of California at Los Angeles communities. Subjects from ages 21 to 65 were recruited. Normal weight individuals with a body mass index (BMI) of 18.5 to 24.9 kg m⁻² with fasting serum glucose <100 mg dl⁻¹ were enrolled. Overweight/obese individuals with a BMI of 25 to 35 kg m⁻² and fasting serum glucose ≥100 mg dl⁻¹ and <126 mg dl⁻¹ or HbA1c >5.7 % and <6.5 %; Overweight/obese individuals without prediabetes: BMI of 25 to 35 kg m⁻² and fasting serum glucose ≤100 mg dl⁻¹ or HbA1c <5.7 % were also eligible.

Subjects with a screening laboratory value outside of the laboratory normal range that was considered clinically significant for study participation by the investigator, taking blood thinning medications such as Warfarin or Coumadin, using tobacco products, unable or unwilling to comply with the study protocol, with a history of diabetes mellitus or other serious medical conditions as indicated by medical history, and subjects that were pregnant or breastfeeding were excluded from the study.

All subjects gave written informed consent before participating in the study.

On-body evaluations

On-body evaluations were performed using a multichannel electrochemical workstation (CHI 1430, CH Instruments). For the fasting study, the participant did not consume a meal for 12 hours prior to the beginning of the experiment. All the meals were consumed 30 minutes before placing the wearable device. The forearm of the subject was cleaned with an ethanol swab and DI water before attaching the sensing patch. IP was run for 5 minutes at a constant current of 1 mA to achieve localized sweat induction. Once the sweat filled the

microfluidic sensing chamber, the measurements started. For independent long-term on-body evaluations, a new sensing patch was used for each session and positioned at a neighboring site on the forearm to enable reproducible, extended duration sweat collection following iontophoresis.

Validation studies

The biomarker validation studies were performed by collecting sweat samples after induction via a Model 3700 Macroduct Sweat Collection System. Firstly, the forearm of the subject was cleaned with an ethanol swab before placing the commercial IP electrodes. After sweat induction, the subject's forearm was subsequently cleaned before placing the sweat collector. Sweat samples were collected for a maximum of 120 minutes. The collected samples were then analyzed using the commercial cholesterol and triglyceride quantification kits.

In parallel to the sweat collection, at the beginning of the study, a peripheral venous catheter was inserted into a suitable vein of each participant. Blood samples were collected through the catheter at 30-min intervals over the course of the study (180 min). These repeated blood draws provided discrete paired reference measurements for model training and validation, but do not constitute a continuous temporal gold standard at the same resolution as the sweat sensor. The blood samples were immediately stored for further processing. Participants consumed 75 grams of glucose during the study.

Causal inference framework for sweat-based biomarker prediction

Causal directed acyclic graph (DAG) construction

Task-specific causal DAGs were developed to represent hypothesized relationships among sweat biomarkers, physiological covariates, and blood lipid outcomes (**Fig. 3-1e-g**). Candidate variables were assembled from the demographic and physiological measurements collected in the preclinical cohort, including age, sex, BMI, glucose, blood pressure, HbA1c, body composition metrics (fat mass, muscle mass, fat-free mass), basal metabolic rate (BMR), total body water (TBW), together with sweat lipid measurements and sweat rate.

Pearson correlation analysis was performed between each candidate variable and the corresponding blood lipid endpoint (blood CH or blood TG) as an exploratory prioritization

step. Variables with $|r| \geq 0.2$ were considered for further evaluation, although this threshold was not used as a strict selection criterion.

Final inclusion and placement of variables in the DAGs were determined by combining these empirical trends with physiological domain knowledge, while accounting for multicollinearity and model parsimony. Sweat rate was treated separately as a dynamic secretion-related factor, whereas static body-composition effects were represented parsimoniously by BMI. Sex was additionally retained for the CH task based on both empirical trends and known physiological differences in lipid regulation.

The resulting adjustment sets satisfied the backdoor criterion, ensuring that major confounding pathways between treatment and outcome were controlled in downstream modeling.

Backdoor adjustment and model training

Backdoor adjustment followed Pearl's causal hierarchy to estimate unbiased causal effects. Two model architectures were used for each task: (i) dual-variable causal models that incorporated both sweat rate and the corresponding sweat lipid, and (ii) baseline regressors including simple linear, multivariate linear, Ridge, and Lasso models (**Fig. 3-1h-i**). Stratified analyses by BMI group and subject-wise response curves were performed to evaluate heterogeneity and individual-level robustness (**Fig. 3-1j-p**). Given the modest cohort size, model dimensionality was intentionally constrained through parsimonious covariate selection. Blood samples were collected at 30-minute intervals over 180 minutes, yielding repeated paired sweat–blood measurements within each participant, which increased the information available for model fitting without being treated as independent observations.

Dual-variable causal modeling

To capture joint effects between secretion dynamics and lipid concentration, two treatment variables were defined: sweat lipid (treatment 1) and sweat secretion rate (treatment 2). The joint causal model estimated their combined influence on blood lipid concentrations, accounting for correlations between the two predictors. This approach allowed simultaneous evaluation of dilution effects and metabolic turnover alongside concentration effects.

Heterogeneous treatment effect estimation

Subgroup-specific causal effects were calculated using sample-size-weighted correlations within strata defined by demographic or physiological variables. Bootstrap resampling ($n = 1000$) was used to assess effect stability and to construct confidence intervals. Effect magnitudes were classified as weak ($|r| < 0.1$), moderate ($0.1 \leq |r| < 0.3$), or strong ($|r| \geq 0.3$). Stratified causal estimates were compared with pooled models to assess effect heterogeneity across BMI.

Statistical analysis

Statistical testing was conducted using two-sided t-tests within each stratum, with a significance threshold of $\alpha = 0.05$. Interaction terms such as biomarker $\times$ BMI and biomarker $\times$ sex were included to the test for moderation effects. Model performance was evaluated using mean absolute error (MAE) and the coefficient of determination (R^2). All analyses were implemented in Python (v3.10) using the scikit-learn and statsmodels packages. Reproducible code pipelines were used, and results were validated through 5-fold cross-validation.