Towards an Accessible, Noninvasive Micronutrient Status Assessment Method: A Comprehensive Review of Existing Techniques

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Nutrients are critical to the functioning of the human body and their imbalance can result in detrimental health concerns. The majority of nutritional literature focuses on macronutrients, often ignoring the more critical nuances of micronutrient balance, which require more precise regulation. Currently, micronutrient status is routinely assessed via complex methods that are arduous for both the patient and the clinician. To address the global burden of micronutrient imbalance, innovations in assessment must be accessible and noninvasive. In support of this task, this article synthesizes useful background information on micronutrients themselves, reviews the state of biofluid and physiological analyses for their assessment, and presents actionable opportunities to push the field forward. By taking a unique, clinical perspective that is absent from technological research on the topic, we find that the state of the art suffers from limited clinical relevance, a lack of overlap between biofluid and physiological approaches, and highly invasive and inaccessible solutions. We present opportunities for future work to maximize the impact of a novel assessment method by incorporating clinical relevance, the holistic nature of micronutrition, and prioritizing accessible and noninvasive systems.

CCS Concepts: • **Applied computing** → **Health informatics**; **Health care information systems**; **Consumer health**; • **General and reference** → **Surveys and overviews**; • **Human-centered computing** → *Ubiquitous and mobile devices.*

Additional Key Words and Phrases: health sensing, mobile health, precision nutrition, micronutrients, nutrition assessment, malnutrition, point-of-care devices, accessibility

1 INTRODUCTION

Balanced nutrition is important for the development and functioning of the human body and can have many downstream health effects. The World Health Organization (WHO, Table 12) reports that individuals with proper nutrition have increased lifespans, are more likely to break cycles of poverty, and have lower risks of disease, which impacts productivity and mortality [139]. Malnutrition is the most critical issue related to nutritional intake, with undernutrition associated with about 45% of deaths in children younger than five years old. Anemia is a major resulting disease, affecting 37% of pregnant people and 40% of children under 5. The WHO defines malnutrition as nutrient imbalance, which consists of deficiencies or excesses in essential nutrients [206]. It is a condition that includes several interconnected factors: 1) being overweight, 2) obesity, 3) diet-related noncommunicable diseases like anemia, heart disease, and diabetes, and 4) undernutrition, which encompasses being underweight, wasting, and stunting, as well as a lack of essential micronutrients. Micronutrients are the vitamins (e.g. vitamins A, C, B12) and minerals (e.g. iron, calcium, iodine) which are critical to everyday human bodily function and can only be provided via dietary intake (i.e. they are not produced by the body).

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According to Hummell and Cummings [73], malnutrition can be caused by insufficient intake, malabsorption, acute and chronic diseases, increased nutrient need, and weight loss surgery. The impact of acute or chronic diseases and weight loss surgery is self-explanatory, whereas other factors are less visible. Insufficient intake in particular can be influenced by a wide range of variables, making it a challenging problem. These factors include income, access to nutritious foods, culture, and upbringing (malnutrition patterns can be passed down to generations). Insufficient intake is a particularly high risk for low-income families. Furthermore, there is often a lack of awareness about insufficient intake, making behavior change more difficult. Malabsorption can also be a cause, as the absorption of nutrients found in food and supplements is highly individual and can be affected by diseases, genetic issues, and one's stage of life. In addition, individual nutrient requirements change over time, and several factors can increase nutrient demand, such as growth from infancy to adulthood, pregnancy, lactation, and recovery from illness or other trauma.

Nutrients can be classified into two main categories: *macronutrients* and *micronutrients*. Both are essential in precise balance to prevent *malnutrition*, also referred to as *nutritional imbalance*. Macronutrients include carbohydrates, fats, and proteins, which are major energy sources and are required in larger amounts than micronutrients [67]. Although required in smaller amounts, optimal intake of micronutrients is crucial. Micronutrients include vitamins and minerals, and they play a critical role in chemical reactions that produce energy from macronutrients acquired from food, as well as other essential bodily functions [140]. Micronutrient intake through diet or supplements is crucial because our bodies cannot synthesize micronutrients, nor can they be substituted for one another [21]. A precise intake amount is required and slight deviations can result in either deficiency or excess, both with significant health impacts [67]. For these reasons, micronutrient imbalance is a problem that is unique and deserving of attention.

Micronutrient Deficiency. The estimated number of people with micronutrient deficiency is 2 billion worldwide [196]. It is estimated that micronutrient deficiencies are the cause of between 425,000 and 745,000 deaths in children under five years old [22]. An estimated 56% of preschool-aged children and 69% of non-pregnant females of reproductive age worldwide suffer from at least one micronutrient deficiency [179]. Globally, the most widespread micronutrient deficiencies occur in iron, iodine, folate, vitamin A, and zinc [11]. The population of the United States (US) has significant risk of micronutrient deficiency due to the prevalence of a high-energy, low-nutrient diet [41, 49]. The US' National Health and Nutrition Examination Survey (NHANES) estimates that 31% of the US population is at risk for micronutrient deficiency, with calcium, potassium, iron, and vitamins A, D, C, and E being of particular concern [41, 49]. Since NHANES is a survey of self-reported dietary intake with minimal biochemical testing, it is likely that this number is actually underestimating the true burden in the US [41].

Deficiencies can cause developmental issues, metabolic disorders, impaired immune system, altered endocrine and cognitive functioning, chronic disease, and more. For example, high magnesium intake and circulating status (via urine) is directly associated with a greater risk of cardiovascular disease (CVD) [156]. While developed countries are certainly at a risk for micronutrient deficiencies, those most at risk are people living in underdeveloped countries. Populations that also bear a significant burden include developing countries as well as children less than five years old, pregnant people, and victims of chronic disease [23, 41, 140, 179]. Risks of inadequate intake are exacerbated since different micronutrients are needed in different amounts at different stages in life [21]. Vitamin A, iron, and zinc are among the most significant micronutrient deficiencies observed in children [179]. In the first 1000 days of life, iron, iodine, folate, and vitamin D have high dietary requirements, and a failure to meet them could result in poor physical and cognitive development. During adolescence, iron, calcium, folate, and vitamin D intake is critical, especially for those who menstruate. Pregnancy sees an increase in iron, folate, vitamin B12, and vitamin D requirements. The elderly are more at risk for vitamin D, B12, and B6 deficiency, and medications

that influence micronutrient status or absorption must be heavily considered during this stage. Micronutrient deficiencies rarely manifest alone, and it is common to see multiple deficiencies arise simultaneously [11].

Micronutrient Excess. Like deficiency, micronutrient excess can also lead to health detriments, with the risk increasing as intake levels surpass an individual's upper limit [56]. Toxicity from micronutrient excess can occur in any person who exceeds this limit. However, there is an exceptional risk for vulnerable populations, namely infants, young children, and pregnant people [45, 56, 76, 147]. For example, an excess of iron increases the risk of diarrhea, sepsis, meningitis, and gut inflammation for infants and young children, with a lethal dose of 150 mg/kg [147]. Similarly, surplus iron can lead to an increased risk of gestational and type 2 diabetes for pregnant people [147]. Elevated health risks are also a concern with an excess of vitamin D in infants, calcium in pregnant people, vitamin A in infants, young children, and pregnant people, and iodine in all three of these groups in addition to breastfeeding parents [45, 147]. Individuals who are more likely to be taking supplements (especially multivitamins) to address an initial deficit could also be at a greater risk for micronutrient toxicity [24, 56]. Research suggests that ingesting an excess of micronutrients through diet alone is unlikely and may only occur for those who take supplements [24, 45, 147]. An excess of certain micronutrients can interfere with the absorption of others, potentially leading to secondary deficiencies. For instance, elevated calcium levels may reduce the absorption of magnesium. Additional information on micronutrient interactions are provided in Tables 13, 14, and 15. There is minimal research on micronutrient excess because it is usually limited to the above population groups. As a result, the work covered in this review largely focuses on micronutrient deficiency. For more information on excessive micronutrient intake and its specific effects for each micronutrient, we recommend referring to an up-to-date nutrition overview (e.g. Espinosa-Salas and Gonzalez-Arias [45]).

Micronutrition Assessment. When a micronutrient imbalance is identified, it is addressed through intervention. This involves modifying diet, providing micronutrient supplements, or on a population scale, fortifying food products such as iodized salt and fortified flour [11]. Interventions need to be carefully planned and monitored to avoid providing too little or too much of a particular micronutrient. There is a strong need for tools that can help guide intervention programs to effectively reach at-risk populations while minimizing the impact on those who have sufficient nutrient levels. This requires employing "different risk assessment methods to make the monitoring process more efficient, reliable, and cost-effective" [11]. *Nutritional status assessment* is a way to evaluate an individual's overall nutritional health and is necessary to identify, prevent, and address any imbalances. This review focuses on micronutrient status assessment methods in particular because we find that existing methods are insufficient and new techniques are under-researched.

We propose that an ideal assessment method is both *accessible* and *noninvasive*. An *accessible* method is one that is available to more individuals, while maintaining clinical effectiveness. This can be achieved through improvements in cost, efficiency, or mobility. Such a method should ultimately alleviate the need for laboratory tests, decrease reliance on high-effort and subjective surveys (such as dietary intake logging), and increase efficiency and accuracy for clinicians. A *noninvasive* method would either eliminate the need for a biofluid sample or obtain it in an unobtrusive manner. Noninvasiveness is therefore interconnected with accessibility, as a method that is invasive (e.g. requiring a blood draw) is less accessible (e.g. places burden on the patient and on the clinician). An ideal method that is both accessible and noninvasive could obtain micronutrient status regularly, without much effort for the patient or clinician. To maintain effective and clinically-relevant assessment methods, it is also essential to balance the accessibility and noninvasiveness of a method with its ability to be a *sensitive* (high true positive rate) and *specific* (high true negative rate) assessment of micronutrient status. Because every country in the world is impacted by malnutrition [206], it is clear that an accessible and noninvasive assessment method is needed to enable routine assessment globally. This is especially true in countries with high-fat, low-nutrient diets such as the US, in low- and middle-income nations, and in areas of the world where complex, blood-based analyses are generally infeasible. In our review, we aim to consider both clinical and

technical aspects of micronutrition assessment and explore the potential for novel, accessible, and noninvasive methods. We argue that such an interdisciplinary approach is key to finding optimal solutions.

In the subsequent sections, we discuss *biofluid* and *physiological* analysis methods for micronutrient status assessment. Within the context of this paper, biofluid analysis refers to techniques that measure the amount of a micronutrient biomarker present in a biofluid as a means to infer the underlying nutritional status of an individual, whereas physiological analysis focuses on the bodily signs and signals which are symptomatic a micronutritional imbalance. For the latter, we focus on providing a brief overview of clinical physical assessment in nutrition and describe relevant applications of optical sensing.

1.1 Paper Scope

Few existing papers that review novel micronutrient assessment methods are comprehensive and clinically relevant [26, 72, 83, 135, 171, 189]. Campuzano et al. [26] and Kalita et al. [83] focus particularly on electrochemical sensors, with the latter considering few vitamins and mostly out-of-body status. Huey et al. [72] focus on vitamin A assessment and emphasizes the limitations of relying on centralized laboratories and specialized equipment, underscoring the need for more portable and accessible diagnostic methods. Nimbkar et al. [135] focus on microfluidic assessment and Shi et al. [171] concentrate specifically on wearable sensors. Udhani et al. [189] provide a detailed review on biomarker-based micronutrient detection, focusing on the analytical chemistry aspect.

The novelty of our paper lies in its comprehensive review of accessible and noninvasive micronutrient assessment methods, uniquely emphasizing clinical relevance—an aspect often overlooked in prior work. It includes a background on micronutrients, tailored for a non-clinical perspective. We also explore a wide range of techniques for assessing biofluids and physiology, including assay-based technologies, electrochemistry-based methods, spectroscopy-based approaches, and analytic methods utilizing machine learning (ML). This review offers a high-level summary, with detailed methods available in dedicated sources. We contribute several relevant tables throughout the paper and provide a comprehensive reference about micronutrients and their assessment in the Appendix. Ultimately, this review calls for actionable potential opportunities to advance these methods.

This review consists mostly of methods that are both accessible and noninvasive, or at least one of these. We additionally include few methods that are neither accessible nor noninvasive, yet are interesting and valuable for future work within this scope. This review does not include methods for assessing micronutrient levels in entities outside of humans, such as food or pharmaceuticals. We note that while hair and nail samples can be valid subjects of micronutrient analysis, we focus on biofluids in our review of assessment techniques since nutritional analyses of hair and nails measure the metabolism of a nutrient in cells rather than the in-body status of the nutrient [14, 62]. Our focus is on vitamins and trace minerals (micronutrients), thus we do not discuss nutrients such as proteins, fats, and carbohydrates (macronutrients) or major minerals (e.g. potassium, calcium, etc.). These are sometimes considered separate from micronutrients because of their higher intake requirements and larger quantities within the body. Lastly, this review does not include alternative medicine techniques or methods, as this is out of the scope.

This review is structured as follows. We first present background information in Section 2. Next, we discuss micronutrient status assessment methods based on biofluid analysis in Section 3, and briefly touch on physiology-based methods in Section 4, focusing on optical sensing techniques. We summarize the current gaps in the literature and present suggestions for future work in Section 5. Finally, we end with a conclusion in Section 6, followed by reference tables in the Appendix (A).

2 BACKGROUND

In this section we describe micronutrient characteristics, their presence in biofluids, and their impacts on physiological functioning. Such a background is necessary to contextualize solutions to emerging micronutrient status assessment methods because of the variety of possible approaches. *In-body* assessment methods refer to the levels of a micronutrient present within the body, reflecting the current internal state of an individual's micronutrition. On the other hand, *out-of-body* assessment methods involve external indicators used to infer internal status, such as physiological symptoms measurable through wearable sensors or physical exams. We focus on both approaches, as each provides relevant insights into micronutrient status and offers the potential for non-invasive and accessible assessment methods.

2.1 Micronutrient Characteristics

Micronutrients are divided into three categories: water-soluble vitamins, fat-soluble vitamins, and minerals [25]. Water-soluble vitamins, such as vitamin C and the B vitamins, are absorbed directly into the bloodstream and are quickly excreted in urine. They are not stored for long periods in the body, so regular intake is necessary to prevent deficiencies, and there is less concern about toxicity from excess intake. On the other hand, fat-soluble vitamins like A, D, E, and K are absorbed into lymph vessels along with dietary fats. Fat-soluble vitamins are stored in larger quantities in fatty tissues and the liver, so deficiencies take longer to develop, and daily intake is less critical. However, due to their efficient storage and the lack of a rapid excretion mechanism, toxicity is more of a concern.

Minerals can be categorized as major or trace minerals based on the daily requirement [25]. Major minerals, such as sodium, potassium, chloride, phosphorus, and magnesium, are required in amounts greater than 100 mg per day, while trace minerals like iron, copper, zinc, selenium, iodine, chromium, fluoride, and manganese are needed in amounts of 100 mg or less per day. Minerals are water-soluble and are absorbed directly into the bloodstream, sometimes with the help of transport proteins. It is important to note that minerals have an electric charge, and their function and storage can be influenced by various factors. For example, certain minerals carry a positive charge, such as sodium (Na+) and potassium (K+), while others are negatively charged, like chloride (Cl-). Their electric charge plays a crucial role in mineral homeostasis, influencing absorption efficiency, competition for transport and storage, bioavailability, and electrolyte balance. For a more in-depth discussion of these interactions, we refer the reader to [174, 194]. A comprehensive summary of the characteristics of micronutrients is presented in the Appendix in Tables 13, 14, and 15.

Dietary guidelines for intake vary across organizations and countries, and are based on factors such as age and sex. These guidelines are not standardized due to the individualized nature of micronutrient metabolism, the diversity of micronutrients, their interactions, and the specific values at which they are needed. The US, for example, utilizes dietary reference intervals such as the Recommended Dietary Allowance (RDA) [134]. The RDA represents the average daily level of intake sufficient to meet the nutrient requirements of 97-98% of healthy individuals. The US National Institutes of Health (NIH) also defines terms to describe intake levels, such as an Upper Limit (UL), highlighting the potential for excess intake. In cases where there is insufficient evidence to establish these guidelines, there exists the more general term, Adequate Intake, which establishes a lower bound of nutritional intake necessary to meet a healthy nutritional state within a population [75]. For example, Adequate Intake for the nutrient biotin (vitamin B7) in human milk-fed infants is defined by the biotin content of human milk itself, because there is a lack of data available to scientifically determine an RDA for this population. Adequate Intake further emphasizes the uncertainty surrounding the appropriate levels of micronutrients in diet.

Micronutrient status assessment usually relies on the combination of the analysis of micronutrient biomarkers in biofluids as well as the physiological symptoms presented by an imbalance. Micronutrient biomarkers (predominantly measured by laboratory analyses of biofluids) are still debated, but can generally serve as a reliable *optimal*

reference standard for assessment methods [42]. An optimal reference standard is the clinically agreed-upon technique for determining a patient's in-body status of a micronutrient (e.g. vitamin B9), and describes the target biomarker (e.g. folate), biofluid matrix to be analyzed (e.g. blood serum), and analysis method (e.g. LC-MS/MS) to use for accurate assessment. These standards are continuously debated by the clinical community, and are typically defined by current knowledge of "the chemistry, absorption, distribution in the body, and metabolism" of a nutrient [42]. Because these dynamics can be highly individualized, validating a standard requires well-controlled studies that involve specific dietary interventions and the subsequent evaluation of the standard's "specificity, sensitivity, and suitability for various population subgroups" [42].

Certain micronutrient imbalances are associated with observable physical or physiological symptoms, such as those in the skin, eyes, autonomic functioning, etc., which may present opportunities for developing noninvasive assessment methods tailored to specific nutrient deficiencies [37, 44, 73, 119, 152]. These symptoms provide noninvasive insights into the bodily storage of micronutrients, internal processes, and can help identify appropriate biomarkers for further testing. Section 2.3 discusses micronutrient effects on physiological processes in more depth. It should be noted that some physical symptoms of micronutrient imbalances only arise when the imbalance becomes quite severe. This can happen slowly, in some cases over the course of several months (i.e. iron), and can result in irreversible symptoms (e.g. night blindness as a result of severe vitamin A deficiency; Tables 13 - 15). While physical symptoms still provide critical insights into the overall burden of micronutrient imbalance in a community, sole reliance on them for assessment can complicate preventative treatment. Quantitative, biofluid-based assessments for biomarkers are not immune to similar issues faced by physiological assessments (e.g. status of the biomarker plasma retinol decreases only after vitamin A stores in liver and eyes have nearly depleted), emphasizing the importance of considering both physiological and biofluid-based assessments in the practice of clinical nutrition.

2.2 Micronutrients in Biofluids

Biofluid analysis for micronutrient status remains challenging since it is often unclear how other biofluids reflect the optimal reference standard matrix for that micronutrient. Some biofluids include blood, saliva, sweat, tears, urine. Besides blood, each of these can be collected and analyzed noninvasively. Blood and urine are clinically relevant for representing in-body micronutrient status, as will be demonstrated in Section 3.1. However, evidence is less clear for saliva, sweat, and tears [74]. Although it will not be covered in depth, it is also worth mentioning human milk as a potential biofluid. Breast milk may have implications on the developmental outcomes of infants whose primary source of nutrition is human milk, but this is actively debated [109]. As mentioned earlier, iodine excess in breastfeeding parents can also express itself in human milk, placing infants at an increased risk [147].

In the clinical literature, the equivalence of saliva, sweat, and tears to blood and urine in micronutrient status assessment remains ambiguous. Some evidence for correlation of micronutrient levels in saliva with blood levels was found on a by-micronutrient basis [74]. For example, serum and saliva levels of vitamin D were found to have a correlation of 0.56, measured using a total vitamin D (25-hydroxy vitamin D) kit with the electrochemiluminescence technique [10]. Additionally, some correlation of iron levels in saliva and serum (a blood derivative) have been found. However, validity remains inconclusive as some sources have found a high positive correlation between salivary and serum levels [27, 61], while others report a high negative correlation [9, 48, 53]. The techniques used to study this correlation involve enzyme-linked immunosorbent assays (ELISAs), laboratory assays, spectrophotometry, and chemiluminescence methods.

Additional research aims to quantify micronutrient status with saliva as well as tears [159, 164]. The three types of human tears, basal, reflex, and emotional, differ in their chemical composition, which can influence which type is most appropriate to collect for specific analyses [106]. Sempionatto et al. [164] in particular argue that tears are a good biofluid for analysis since they are noninvasive, less complex than blood yet still contain a

"variety of biomarkers", and they "reflect concurrent blood levels" because of passive leakage of compounds from blood plasma. It is important to note, however, that neither of these works ([159, 164]) provide comparisons to the in-body status of their target micronutrients as measured by optimal reference standard, urine or blood-based assays. Additionally, it is important to consider the potential ethical implications of tear inducement and collection. One study used Schirmer strips, a common method for collecting tears in healthcare settings, and found that most participants considered the process acceptable [151]. Specifically, 70% did not mind the procedure, and 74% preferred tear collection over venous blood sampling or other forms of biofluid collection such as urine.

Sweat receives significant attention in emerging micronutrient detection methods, especially in wearables. The composition of collected sweat varies depending on whether it is produced actively (through exercise or heat exposure) or passively (through methods that encourage sweat production without physical activity, such as sweat patches or chemical stimulation) [95]. Active sweating in particular usually produces higher concentrations of sodium, chloride, and metabolites. *Eccrine sweat* is a clear, odorless fluid secreted by eccrine sweat glands, which are essential for thermoregulation [70]. It is the primary sweat type used for micronutrient analysis, as *apocrine sweat*—the other major type—is thicker and predominantly composed of lipids and proteins, making it less suitable for this purpose [66]. Therefore, when we discuss sweat, we refer to eccrine sweat, which is mostly sodium and chloride, with smaller amounts of micronutrients and metabolites (at the micro and nanomolar scale), similar to or smaller than their concentrations in blood plasma [13]. Micronutrients found in sweat include potassium, calcium, magnesium, iron, copper, zinc, vitamin C, and vitamin B1. Out of the compounds in sweat, mostly sodium and chlorine ions are well-studied. Some research has explored water-soluble vitamins in sweat, such as vitamin C and vitamin B1, but there is no such attention on fat-soluble vitamins. Many confounding factors can impact sweat composition, such as the contamination of sweat by skin-derived substances, notably iron [13]. This is combated by pre-rinsed skin, removal of initial sweat (concentrations stabilize after 20 to 30 minutes of sweating), and the analysis of cell-free sweat. The region and method of collection can also have an impact, as many micronutrient concentrations can vary two to four times depending on the region. Finally, sweat can be reabsorbed into the body. Skin temperature and the flow rate of sweat both impact the rate of this reabsorption.

The clinical literature notes a general "lack of association between dietary micronutrient intake and corresponding sweat micronutrient concentrations" [13]. This lack of an association exists in comparison to blood as well. A review by Baker and Wolfe [13] finds that there is no established correlation between sweat and blood composition, and there is "little support for using sweat as a surrogate for blood". Concentrations of minerals in sweat are much more varied than in plasma, likely because minerals bind to carrier proteins in blood. The review also reports little to no correlation between sweat and blood concentrations of vitamin C and iron status. This finding for iron is echoed in another paper that found both iron and calcium have no correlation between sweat and blood concentrations [12]. However, it was reported in the same paper that iron concentrations in sweat have been observed to be lower in anemic patients and higher in patients undergoing iron therapy.

Finally, it is important to consider the potential impact of time lag variability in biomarker expression across different body fluids. This variability arises from nutrient kinetics and bioaccessibility, particularly in relation to absorption, distribution, metabolism, and excretion (ADME) within the human body [59, 122]. However, studies described in this section do not explicitly account for these temporal effects, which are crucial for accurately correlating blood biomarkers with those present in other biofluids. At present, the temporal dynamics governing these relationships remain insufficiently understood, and require further investigation.

2.3 Micronutrient Effects on Physiological Processes

Most of the physiological effects of micronutrients are related to the autonomic functions of the body. Autonomic functioning refers to bodily functions controlled by the autonomic nervous system, which regulates involuntary

processes and rhythms such as breathing, heart rate, and digestion [202]. The specific importance of micronutrients to the proper functioning of the nervous system has been documented by several papers [16, 31, 54]. For example, a study of vitamin B12 deficiency compared responses to a 60-degree passive head up tilt test between a control group, a vitamin B12 deficient group, and a group with diabetes mellitus [16]. They found that the deficient group had comparable autonomic neuropathy to the diabetic group. Our exploration of observable impacts of deficiencies on autonomic functioning found three main affected areas relating to biofluid composition and physiological effects: general symptoms, cardiac function, and sleep.

2.3.1 General Symptoms. Deficiencies can be classified as either clinical or subclinical based on their severity [184]. Most deficiencies result in symptoms of general fatigue, lethargy, irritability, muscle pain, weakness, and headaches. Clinical deficiencies often have more distinguishable symptoms, while subclinical deficiencies are limited to the above non-specific ones. Deficiencies of vitamin C, B vitamins, iron, magnesium, and zinc have been linked to fatigue more so than others [8, 184]. While energy and fatigue are more subjective and can can rely on subject-reporting, there are some established and validated assessment methods such as the Multidimensional Fatigue Inventory [173] and the SF-36 Vitality Scale [200]. A comprehensive reference for physiological symptoms associated with deficiency is lacking in literature, so we provide one in Tables 16, 17, and 18 within the Appendix.

2.3.2 Cardiac Function. One main aspect of autonomic functioning affected by nutrient imbalance is cardiac functioning. Several studies explore the interaction between heart rate variability (HRV) and micronutrient deficiencies. Components of HRV are associated with parasympathetic (PNS) and sympathetic nervous system (SNS) activity [168]. High-frequency (HF) bands reflect PNS activity and correspond to the respiratory cycle, while low-frequency (LF) bands reflect PNS, SNS, and baroreceptor activity. Vitamin B12 deficiency is one of the most documented in terms of impact to HRV, with evidence that it lowers HRV overall, impacting sympathetic indices the most [7, 16, 111, 183]. Supplementation of B12 was also demonstrated to return HRV indices to a comparably normal state [7]. Deficiency of vitamin D was found to lower HRV as well [111]. Calcidiol (25(OH)D) levels, a form of vitamin D, was shown to be associated with the ratio of LF to HF HRV power [118]. This metric is sometimes called sympathovagal balance, and is intended to be a measure of 'balance' between SNS and PNS activity, but there has been debate over this interpretation [168]. Iron-deficiency anemia (IDA), an advanced form of iron deficiency, has more conflicting evidence of HRV impacts, with some studies finding no difference in HRV indices versus the control [188] while others were able to find a difference in the IDA group [80, 209].

Impacts of micronutrients on blood pressure have also been studied [16, 31, 185]. The supplementation of potassium, magnesium, zinc, vitamins C, D, B6, and a decreased intake of sodium and selenium can "positively modulate blood pressure levels" [31]. The aforementioned study involving responses to a head up tilt test in vitamin B12 deficient people found a drop in systolic blood pressure 60 beats after the test [16]. This finding aligns with previous work suggesting that a dip in blood pressure when standing up from sitting or lying down is a symptom of vitamin B12 deficiency [185].

As an aside, pulse-oximiters, smartwatches, and other health sensors or even smartphones can readily measure continuous cardiac function through photoplethysmography (PPG). Pulse rate (PR), HRV, and blood pressure can be derived from PPG [107, 126].

2.3.3 Sleep. Another area of research is the role of micronutrient status in sleep. Sleep duration is associated positively with iron, zinc, and magnesium and negatively with copper, potassium, vitamin A and vitamin B12 levels [19, 79]. Sleep quality increases with zinc, magnesium, and vitamin B9 status and is negatively associated with vitamin B12 status [19, 30, 77]. There are conflicting findings for iron. One study reports that iron status is not proven to be correlated with sleep quality [78], while another claims that supplementation had positive effects on sleep disorders [105]. Sleep deprivation is also connected to micronutrition through its influence on hormones that regulate stress and the immune system. Sleep deprivation can decrease levels of cortisol while increasing

ghrelin, a hormone linked to hunger [90]. This shift can lead to increased hunger, and a continuous lack of sleep was even found to have a positive correlation with obesity (which is interconnected with malnutrition [206]). Studies also found that after temporary sleep deprivation there were decreased magnesium levels measured via red blood cell testing and reduced zinc levels in plasma tests [112].

3 BIOFLUID ANALYSIS METHODS FOR MICRONUTRIENT STATUS ASSESSMENT

This section describes how biofluid analysis has been leveraged to assess micronutrient status. These assessment methods target particular biomarkers within a biofluid that are indicators of micronutrient status. Reliable biomarkers are a research challenge themselves (which AI techniques may address [34]), but clinical literature suggests that micronutrient biomarkers are more established and specific than macronutrient biomarkers [42]. Most micronutrients have one or two specific biomarkers associated with their circulating status that are considered to be the *optimal reference standard* for status assessment (Table 1). Although some optimal reference standards are still debated, their existence makes the evaluation of novel assessment methods more straightforward.

In this section we will discuss clinical biochemical analysis, followed by other innovative technologies. When reviewing the non-clinical methods (Sections 3.2 to 3.5), we particularly note what each approach claims to assess, the method by which they conduct this assessment, how their method was evaluated, and the clinical relevance of their implementation. Critical to accessibility is each method's platform. Point-of-care (PoC) devices are compact and portable enough to be deployed for use where needed, as opposed to traditional 'benchtop' technologies that are restricted to a laboratory setting. The term PoC is widely applicable, so we label methods more specifically to compare the advantages and disadvantages of each, while still acknowledging that they are considered PoC. Specifically, the terms 'portable', 'smartphone-based', and 'wearable' all imply PoC devices but also suggest varying levels of accessibility and ubiquity.

We further expand on clinical relevance by noting that this includes the target biomarker, the assessment method, and the concentrations of that biomarker that are evaluated. A clinically relevant method should closely align with the optimal reference standard on these factors (Table 1). To aid future work that may wish to integrate or innovate on a particular method, we explicitly mention when a study does not demonstrate this agreement (e.g. assessing RBP for vitamin A status) and/or what assessment methods were used during evaluation (e.g. ELISA). For micronutrients with rapid turnover (i.e. water-soluble vitamins) or without risks of excess, a lack of sensitivity in the upper spectrum of the clinically relevant concentrations (Table 1) are not a major limitation for methods which primarily aim to identify deficiency. Regardless, such a limitation is still noted for completeness. Last, we provide tables that group together similar works and summarize the pertinent details and quantitative results of their evaluation, if available. For conciseness, this information is omitted from the body of the text, and we encourage readers to instead refer to the relevant tables.

3.1 Clinical Biochemical Analysis

Clinical biochemical analysis involves laboratory testing of biomarkers found in urine, blood, or other biosamples [154]. Results can be influenced by several factors and need to be interpreted in the context of other aspects of the patient's health. Additionally, biochemical testing is often time- and resource-intensive. Despite this, biochemical analysis describes clinical optimal reference standard methods for quantifying the circulating micronutrient status in the body [74, 210]. These methods can be roughly separated into various types of assays, and liquid chromatography (LC)-coupled spectroscopy.

One type of assay which is popular for clinical micronutrient assessment is a microbiological inhibition assay. These assays work on the principle that specific micronutrients are needed for the growth of certain bacteria, and this growth can be measured to indicate the amount of a micronutrient present in a sample [210]. Microbiological inhibition assays were previously the widely-accepted optimal reference standard, but improvements in LC and

spectroscopy highlight their relatively poor precision and accuracy. These flaws have relegated microbiological inhibition assay methods to be used mostly during screening or in resource-constrained testing, except for some micronutrients, where they remain the standard. Another common type of assay applied in studies is the enzyme-linked immunosorbent assay (ELISA). An ELISA test is used for measuring antibodies in blood, and is a useful clinical screening tool for further testing [92]. While not the optimal reference standard for the assessment of micronutrient status, ELISA tests can yield valuable data for studies that are time or resource limited. Lastly, we find that antibody or immunoassays as well as colorimetric and fluorometric assays are popular in emerging accessible and non-invasive assessment technologies.

Modern optimal reference standards overwhelmingly apply LC-coupled spectroscopy [74, 210] (Table 1). LC is defined as "a separation process used to isolate the individual components in a mixture" [29]. High-performance LC (HPLC) uses pressure to facilitate the separation process, reducing the time required. It is commonly coupled with mass spectroscopy (MS) in optimal reference standard approaches [74, 210]. Spectroscopy is the "investigation and measurement of spectra produced by matter interacting with or emitting electromagnetic radiation" [1]. Every molecule reacts to the applied radiation in a unique way that allows us to "detect, determine, or quantify the molecular and/or structural composition of a sample" [1]. MS is the most important subfield of spectroscopy to understand for biochemical analysis. It measures the mass-to-charge ratio of the molecules in a sample as a way to determine and quantify the composition of molecules in the sample [1]. This is done by vaporizing the molecules in a sample into gas-phase ions, which are then sorted by their mass-to-charge ratios. We will discuss other forms of spectroscopy and their utility for micronutrient status assessment further in Section 3.4.

Matrices are the biosamples that are the subjects of the aforementioned methods of analysis. Most are blood based, but in a few cases urine is used in the optimal reference standard (mostly for water-soluble vitamins) [74]. Blood matrices are whole blood, washed red blood cells, plasma, and serum. Whole blood is blood as it is from the vein (venous blood). Washed red blood cells are red blood cells that have been separated from the other components of blood such as plasma, platelets, and white blood cells [85]. Plasma is obtained by adding an anticoagulant to whole blood and placing it in a centrifuge [182]. Serum is obtained similarly to plasma, except the blood is allowed to clot before centrifuging. However, blood can also be obtained from the capillaries (capillary blood) as opposed to the vein, often via a finger prick. Capillary blood is often easier and cheaper to obtain, especially via untrained personnel, but contains a mix of venous and arterial blood, together with interstitial fluid which surrounds cells in the body [158]. Because of this, test results on capillary vs venous blood can differ (e.g. hemoglobin concentrations are higher in capillary blood) and so the two should not be considered interchangeably.

Table 1. Optimal Reference Standard Methods of Assessing Micronutrient Imbalance. Information from [18, 74, 127, 133, 210].

Micronutrient	Method	Biomarker	Matrix	Intervals	Impact of Inflammation	Approx. Cost (US; Walk-In Lab)
Vitamin B1	Erythrocyte transketolase activity coefficient assay	Increase in erythrocyte transketolase activity	Washed red blood cells	Deficient: >25%; Insufficient: 15-25%; Sufficient: <15%	None on direct plasma levels	$65
Vitamin B2	Erythrocyte glutathione reductase activity coefficient assay	Increase in erythrocyte glutathione reductase activity	Washed red blood cells	Deficient: >40%; Insufficient: 20-40%; Sufficient: <20%	Decrease in plasma levels (erythrocyte assays are more stable)	$120

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Micronutrient	Method	Biomarker	Matrix	Intervals	Impact of Inflammation	Approx. Cost (US; Walk-In Lab)
Vitamin B3	LC-MS/MS	Niacin metabolites (NMN and 2-pyr, limited representation of stores and recent intake)	Urine	Deficient: <5.8; Insufficient: 17.5-5.8; Sufficient: >17.5 mcmol/day	Lack of evidence	$179
Vitamin B5	LC-MS/MS	Pantothenic acid (requires enzyme pretreatment)	Whole blood	Deficient: <0.22; Sufficient: 0.35-0.59 mg/L	Lack of evidence	$149
Vitamin B6	HPLC or LC-MS/MS	pyridoxal phosphate (PLP)	Plasma or serum	Sufficient: >4.94 or >7.41 μg/L plasma PLP (varies by source)	Decrease in plasma PLP, no effect on RBC concentration	$69
Vitamin B7 [43]	LC-MS/MS for biotin; gel densitometry for MCC/PCC [117]	Biotin (less sensitive); holo-MCC and holo-PCC (only reliable markers)	Urine for biotin; WBCs for MCC/PCC	Sufficient: 4.4-31 μg/day urinary biotin, 8.2 arbitrary units holo-MCC, 9.1 arbitrary units holo-PCC	None on biomarkers	$199
Vitamin B9	LC-MS/MS	Folate	Serum for altered exposure and recent intake; Red blood cell for long term/3 month status and storage levels	Sufficient: >3 ng/mL serum, >140 ng/mL RBC	Lack of evidence	$29
Vitamin B12 [3]	GC-MS	B12, confirmed with methylmalonic acid (MMA, also related to B2, B6, folate); no single 'optimal reference standard'	Plasma or serum	Deficient: <200-250 pg/mL B12, >0.03 mg/L MMA (some debate over this)	Association with increased B12 levels	$35
Vitamin C	HPLC	Ascorbate	Plasma (some claim serum should be avoided)	Deficient: 1.94; Insufficient: 2.11-4.05; Sufficient: 4.05 mg/L	Decrease in plasma ascorbic acid (rapid, decrease when CRP >10 mg/L, normal values not detected if CRP >40 mg/L)	$49
Vitamin A	LC-MS/MS	Retinol (only sensitive to deficiency or excess in storage, affected by infection and protein/zinc deficiency). Best method is to indirectly measure reserves in liver over several days of administration	Plasma or serum	Severely deficient: <0.1; Deficient: 0.1-0.2; Sufficient: 0.3-1; Toxic: >1 mg/L retinol	Decrease in serum retinol (adjustment equations exist but are not universally applicable, e.g. BRINDA [115])	$58
Vitamin D [157]	LC-MS/MS	25(OH)D (calcidiol)	Plasma or serum	Deficient: <12; Insufficient: 12-<20; Sufficient: 20-50; Toxic: >50 ng/mL (not definitively established/linked to clinical outcomes, varies based on assay and lab)	Decrease in plasma levels (all values below reference ranges with CBP >40 mg/L)	$59
Vitamin E	LC-UV	Ratio of Vit E to total blood lipids	Plasma or serum	Insufficient: <5.17 mg/L Vit E, <0.8 mg Vit E/g total lipid; Sufficient: 8.6-13 mg/L Vit E (adults have higher levels)	Some effects (blood concentrations less interpretable at CRP >80 mg/L)	$48
Vitamin K	Immuno-based assays	Plasma phylloquinone (usually for short term intake, no single 'optimal reference standard'); prothrombin time (time to blood clot, only clinically relevant measure); variety of other 'functional' biomarkers	Plasma	Deficient: <0.15; Sufficient (fasting): 0.15-1 μg/L	Status associated with lower inflammatory marker concentration	$96

Continued on next page

Micronutrient	Method	Biomarker	Matrix	Intervals	Impact of Inflammation	Approx. Cost (US; Walk-In Lab)
Iron [55, 58]	Electrochemiluminescence immunoassay (ECLIA)	Ferritin for deficiency (first phase, evaluates storage, inflated by infection); Iron increase after supplementation for malabsorption; Hemoglobin used to confirm IDA	Serum	Iron-deficiency anemia: <10, Deficiency: 10-30 μg/L ferritin	Ferritin may be inflated, falsely normal/misleading (adjustment equations exist but are not universally applicable, e.g. BRINDA [115])	$29
Copper	ICP-MS	Copper or ceruloplasmin (CP), neither reliable	Serum	Depletion: <50.8 (copper); Deficient: 50.8-76.2 (copper, high CRP); Sufficient: 63.5-158.9 μg/dL (copper), 180-400 mg/L (CP)	Increase in plasma concentrations	$33
Zinc [143]	Atomic Absorption Spectroscopy (AAS)	Zinc (levels are halved by Systemic Inflammatory Response Syndrome, can be normal with clinical symptoms present, levels vary with time of day so it is recommended that albumin and CRP changes are taken into account)	Plasma or serum	Deficient: 70 women, 74 men; Insufficient: 70/74-80; Sufficient: 80-120 μg/dL	Decrease in plasma levels (significant when CRP exceeds 20 mg/L, adjustment equations exist but are not universally applicable, e.g. BRINDA [115])	$38
Iodine	ICP-MS	Iodine	Urine (24h or random), serum less recommended	Depletion: <20, NA; Deficient: 20-100, <40; Sufficient: 100-300 μg/24hr urine, 40-100 μg/L serum (levels should be higher in those who are pregnant or lactating)	Lack of evidence	$89
Selenium	AAS, ICP-MS	Selenium (recent intake) or Selenoprotein P	Plasma or serum	Deficient: <60; Sufficient: >60; Toxicity: >474 to 948 μg/L Se (intervals vary by source and population: women and black people have naturally lower concentrations)	Decrease in plasma levels proportional to inflammation, can be adjusted for	$99
Magnesium	AAS	Magnesium	Serum (little correlation with overall status or tissue stores) and urine (after supplementation)	Deficient: <18.23; Sufficient: 18.23-23.1 mg/L serum Mg		$28

3.2 Assay-Based Technology

One of the largest areas of work applicable to micronutrient status explored by this review is quantitative assays. Although we cover many different types of quantitative assays, a comprehensive review of lateral flow quantitative assays by Urusov et al. [192] provides a robust background for how these devices work. Lateral flow (immunochromatographic) assays indicate that a target compound is either present in the sample or present in excess of a particular threshold, usually via staining on the test membrane. These types of assays are useful for tests that benefit from quick conclusions (e.g. pregnancy tests). The most prevalent approach for extracting quantitative information from lateral flow assays is via optical signal registration [192]. This method involves the analysis of absorbed and reflected light from the test surface and the staining upon it, similar to the practice of spectrophotometry. The test/control ratio is a common metric used to quantify the magnitude of this staining relative to a control or reference area. Some commercially-available devices are limited to automatically confirming the presence of the test line, while others can use line intensity to calculate analyte content. Urusov et al. [192] note that portability has become a recent focus in this market, and this is not just limited to specialized devices in a portable platform. Smartphones have been successfully used for optical signal registration, even with fluorescent labels that decrease detection limits. Some manufacturers provide their own smartphone apps for quantitative

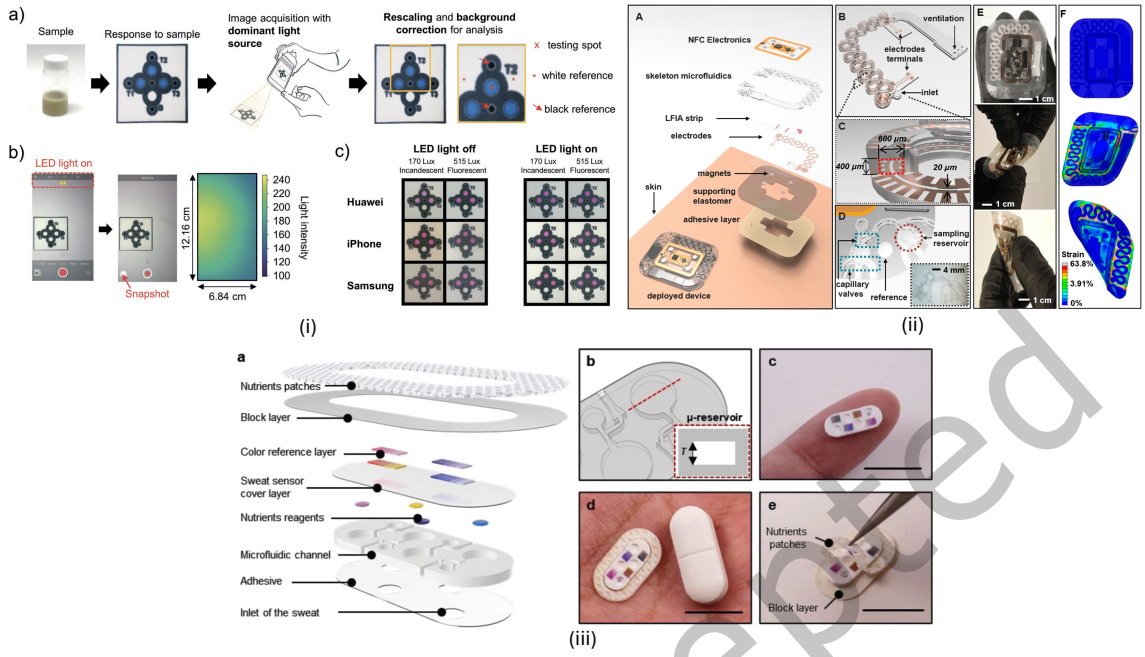

Fig. 1. (i) A smartphone camera and LED flash can be used to analyze results from a colorimetric assay. Used with permission from Kong et al. [98]. (ii) The design of a multiplexed sweat sensor using microfluidics. Used with permission from Kim et al. [89]. (iii) The design of a colorimetric sweat sensor using microfluidics. Used with permission from Kim et al. [88].

analysis, but controlling for lighting and positioning is a challenge. To address this issue, another approach is the use of a standardized scanning device to collect image data and the off-device analysis of the image by specialized software. More experimental approaches, such as magnetic and electrically conductive labels, have also surfaced but have yet to mature. Tables 2, 3, and 4 present a summary of assay-based methods developed for micronutrients.

3.2.1 Sweat-Based Colorimetric Sensors. Colorimetrics uses reagents to react with an analyte and change color to indicate concentrations of target substances. This color can be analyzed by a camera, such as in a smartphone as demonstrated in Figure 1(i) by Kong et al. [98]. Most sweat-based sensors use microfluidic devices for the collection of sweat [81]. These devices use very tiny valves and channels to "capture and store sweat on the surface of the subject's skin" via the natural pressure of sweat glands [81]. Sekine et al. [163] demonstrated a microfluidic skin patch with fluorometric probes that aimed to analyze chloride, sodium, and zinc in sweat. A smartphone attachment was designed to take light from the camera's flash and pass it through an excitation filter, allowing only a particular wavelength through. Similar to other quantitative assays, normalized intensity can be calculated against a reference and used to determine biomarker concentration and calibration curves for each nutrient were determined by known concentrations in spiked synthetic sweat. The authors claimed a strong correlation and accuracy, but do not report exact results. Field studies were conducted where sweat was induced in human volunteers (undisclosed sample size). Measured concentrations were compared to ion chromatography (chloride), atomic absorption spectrometry (AAS; sodium), and inductively coupled plasma mass spectrometry (ICP-MS; zinc). Again, no statistical analysis of results is presented and it appears zinc measurement was the most inaccurate and subject to the most variance.

A paper by Kim et al. [88] described on-body colorimetric measurement of vitamin C, calcium, zinc, and iron using sweat as a biofluid (Fig. 1(ii)). A bespoke colorimetric assay was used for each micronutrient, assessed with known concentrations in a buffer solution. Since temperature and pH can affect colorimetric results, the authors conducted tests within the normal range of body temperature and pH in sweat, finding only a slight shift in results. Uniquely, micronutrients could be supplemented transdermally through the patch itself. Multiple on-body tests were conducted with 7 volunteers (4 male). Sweat was induced by a sauna before and after supplementation (either orally or transdermally). Patch-based measurements were found to be correlated with ICP-MS results. Although the paper claimed that "sweat chemistry correlates, at least semiquantitatively, to plasma chemistry" for these micronutrients [88], this claim is based on the time dynamics of concentrations after supplementation rather than a comparison to an optimal reference standard status assessment (Table 1). Furthermore, the clinical literature points out that that vitamin C and iron concentrations in sweat have been shown to have little to no correlation with levels in blood [12, 13]. Therefore, we argue that this device provides insights into the rate of excretion of these micronutrients rather than their status. Lastly, there is no analysis of possible measurement bias induced by transdermal supplementation at the point of sweat collection.

3.2.2 Sweat-Based Multiplexed Sensors. Multiplexed analyses combine and analyze data from assays with multiple sensors such as electrocardiogram (ECG), temperature, electrodermal activity (EDA), HRV data and more. Thus, these multiplexed sensors can enable more complex physiologic monitoring and diagnosis. For example, EDA measures the change in skin conductance caused by sweat, an indicator of nervous system arousal [148], which can be associated with micronutrient status (Section 2.3).

Kim et al. [89] developed an on-body biosensing platform that could collect and analyze cortisol, glucose, and vitamin C in sweat using microfluidics (Fig. 1(iii)). The device included a lateral-flow assay for cortisol and fluorometric assays for glucose and vitamin C. Assay results were imaged with a smartphone (with special lenses in the case of fluorometry) and were analyzed to yield quantitative results. There were also electrodes for sweat rate and EDA. The focus in the paper was on stress indicators. Field-testing of the device for cortisol assessment involved subjecting 2 participants to "intensive work periods" (interrupted sleep schedule and caffeine intake) for 7 days then rest (regular schedule) for 14 days [89]. Additional tests with 2 different participants for all target biomarkers involved subjecting participants to intensive work followed by a regular schedule and vitamin C supplementation for 14 days. Spikes in vitamin C associated with intake could be observed under these conditions.

For a more in-depth review of the nuances of creating wearable multimodal sensors with sweat collection and analysis capabilities, we direct the reader to [208].

3.2.3 Smartphone-based Quantitative Assays. Smartphones are increasingly being used as analytical platforms for quantitative assays. Often, the phone is either used to photograph the assay results for quantitative analysis [40, 46, 103, 104, 149, 166, 167, 176], or it communicates with a more specialized and standardized sensing device [102, 113, 195]. Lee et al. [103] demonstrated the use of smartphones to image and quantify vitamin D (calcidiol) levels from an immunoassay. After the sample is deposited on the test and incubated for a few hours, the assay was imaged using a custom smartphone accessory. The device was evaluated using three levels of known concentrations that span from deficiency to sufficiency, but not excess, although this range is debated (Table 1). Results were compared to an ELISA test. The same researchers developed a smartphone-based assay method (dubbed "the Nutriphone") for B12 quantification [104]. Twelve human subjects provided capillary blood samples from a finger prick, and assay results were compared to an Immulite 2000 immunoassay system. On these samples, the Nutriphone failed to accurately determine B12 levels above 441 pg/mL. Unlike the Immulite, this solution appears to be insensitive to the upper spectrum of serum vitamin B12 concentrations (Table 1). In addition, while the authors do not specify the form of vitamin B12 their device aimed to investigate, the reported molecular weight is closest to the non-optimal reference standard cyanocobalamin. As reported in Table 1, vitamin B12 has no single optimal reference standard, but it is often confirmed with methylmalonic acid. The authors suggest that

Table 2. Assay-Based Methods Using Sweat

Method	Platform	Targets	Analytes	Evaluation	Control	Results	Notes	Source
Fluorometry	Wearable patch	Chloride, sodium, and zinc	Sweat	Human subjects	Ion chromatography (chloride), AAS (sodium), ICP-MS (zinc)	Zinc measurement was the most inaccurate and subject to the most variance	Statistical analysis of results is not presented	[163]
Colorimetry	Wearable	Vitamin C, calcium, zinc, and iron	Sweat	Human subjects	ICP-MS on diluted sweat samples and supplementation	Correlations with ICP-MS of 0.926 for Vit C, 0.743 for calcium, 0.895 for zinc, and 0.963 for iron, time dynamics of measurements after supplementation were in line with those of blood	Sensor can also supplement micronutrients transdermally	[88]
Fluorometric and lateral flow assays	Wearable with smartphone analysis	Cortisol, glucose, and vitamin C	Sweat	Human subjects	ELISA for cortisol, controlled stress and diet	Cortisol aligned with circadian rhythm changes and had R^2 of 0.7974 with ELISA, observed spikes in Vit C with intake, no trends in glucose	Measurement of sweat rate and EDA, with NFC and RF to power them and communicate results	[89]

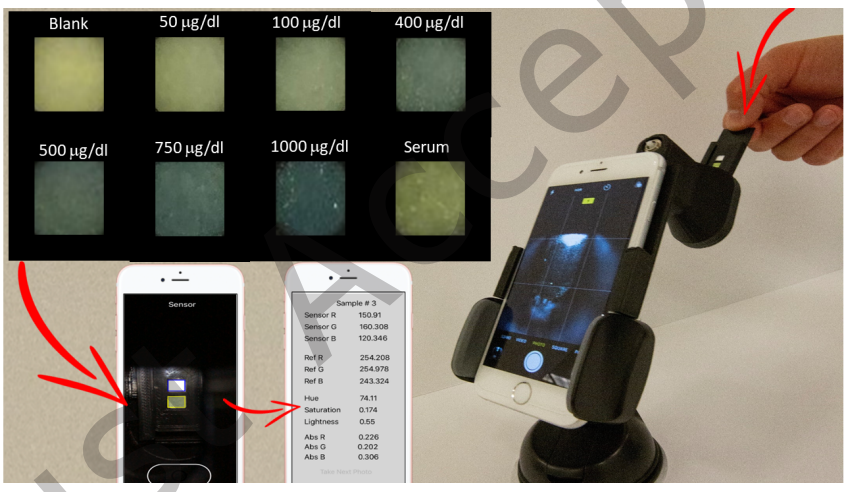

Fig. 2. The use of a smartphone-based assay for the quantitative assessment of iron in serum. Used with permission from Serhan et al. [167].

future work should aim to be more effective at lower limits of detection and better account for interferents in whole blood. An evolution of the Nutriphone assesses iron, as ferritin [176]. This assay was evaluated in-lab with known concentrations of ferritin in spiked buffer (n=27) and serum samples (n=12) to optimize performance. Human trials were also conducted (n=20) and results were compared to the Immulite 2000.

Serhan et al. [167] had a similar goal of using a smartphone-based assay to measure total iron in serum (Fig. 2). This paper focused on total iron instead of ferritin (the clinically-accepted biomarker for imbalance; Table 1) because it is "the most direct metabolite in the [iron] panel" [167]. Total iron can provide valuable insights into iron status, thus it is a worthwhile target even if it is not the optimal reference standard [132]. This is the

first work to consider the risks of excess iron, purposefully designing the assay to be sensitive to both deficiency and excess with a "dynamic range of 50-300 μg/dL" [167]. Twenty capillary blood samples were collected via a finger prick, and assay results were compared to optimized multi-well plate spectrophotometry. Additional analysis found that their approach had a coefficient of variance of 10.5%, compared to 2.2% for the lab tests. One unique form of validation here that did not appear in other work was specificity testing using interferent analytes. The preceding method was approved upon with a new system to measure total iron levels from whole blood, consisting of an iOS smartphone application, a 3D printed sensing chamber, and a vertical flow membrane-based sensor strip [166]. The smartphone application's accuracy and precision were tested against a reference imaging software (ImageJ) for the same colorimetric sensing strip. They compared their iron detection technique to a spectrophotometry-based laboratory test for iron detection on 14 venous blood samples from 9 volunteers (7 male) and found greater limit of detection (LoD) than the laboratory method (2.2 μg/dL) The authors discuss future steps of expanding this tool toward measuring total iron binding capacity and saturation levels.

Ferreira et al. [46] developed an assay for urine, a biofluid that is less invasive than blood and more representative of status than sweat. Their paper presented a paper-based colorimetric assay for urinary iron and quantification of results from images. The assay itself contained four columns of five sample units each. This design allows for replicate results and outlier exclusion. Blank (water) samples were used to obtain a baseline signal intensity for absorbance calculations and calibrate for urine color interference. Calibration curves were determined using iron standards in water and synthetic urine. It was found that the phosphate and citric acid in the synthetic urine significantly interfered with the slope of the calibration curve. The sensor was evaluated using volunteer urine samples (n=26) pre-treated with nitric acid. The results were compared to AAS and found to be similar. Dortez et al. [40] showcase another colorimetric assay for serum iron, quantified by smartphone images. The assay was developed using iron standard solutions and evaluated using diluted serum spiked with Fe3+. It was found that the assay could analyze multiple samples simultaneously, allowing for auto calibration of test samples against a certified reference control. Prakobdi et al. [149] claimed to present a noninvasive saliva-based screening test for IDA using a nitrocellulose lateral flow system to measure iron (Fe3+) levels in spiked saliva. A reaction's color change was analyzed, and the results indicate a linear response in the 100-2000 μg/dL range (falling far above the lower limit for 'normal' serum iron results [28]). The study used pooled commercial saliva, and it did not compare measured iron levels with a clinical optimal reference standard for iron status assessment. In addition, we note the general ambiguity of whether saliva levels of iron are an accurate indicator of circulating blood levels (Section 2.2).

The smartphone moved into a supporting role in Lee et al. [102]. The authors implemented a paper-based microfluidic immunoassay for vitamin A (as retinol binding protein or RBP), iron (as ferritin), and C-reactive protein (CRP) in a unique platform that resembles a flat, portable index-card design. Most notably, it was able to analyze whole blood with minimal pre-treatment because of built-in plasma separation (similar to [166]). The device included competitive assays for CRP and RBP and a sandwich assay for ferritin. Sandwich-based assays are more sensitive, while competitive assays are more effective for analytes of larger concentrations. CRP is a useful inclusion since both iron and vitamin A assessment are impacted by inflammation (Table 1). On-device light emitting diodes (LEDs) and photodetectors analyzed the assay, sending results and various test metrics to a smartphone app over NFC. The added ability to store this data in a remote server made the device a powerful tool for population-level screening. The device was evaluated on whole blood samples (n=95), each run 3 times, and compared to ELISA. A documented 84.4% of sample was male, and 6 samples were spiked with ferritin, CRP, and RBP to assess a wider range of concentrations. For evaluation, a physiologically-relevant cutoff of 15 μg/L was set for ferritin deficiency (although this is insensitive to the deficiency upper limit of 30 μg/L; Table 1). The decision to make this device single-use is perplexing, especially since a reusable version was used for testing. We would also like to note that RBP is not the clinically-accepted biomarker for vitamin A in serum, which should be measured directly instead (Table 1).

The approach in Lu et al. [113] achieved simultaneous quantification of vitamin A (as RBP), iron (as ferritin), and CRP on a single test strip using multiple fluorescent markers and immunoassays. The paper proposed a reusable, standalone reader (the TIDBIT). Interestingly, quantitative results were only presented to the user if they are within a "physiologically relevant dynamic range" of 2.2-20 μg/mL for RBP, 12-200 μg/L for ferritin, and 0.5-10 μg/mL for CRP [113]. We note that the ferritin detection range sufficiently covers iron deficient status, but does not extend into the threshold which indicates iron-deficiency anemia (IDA; <10 μg/L; Table 1). Although a complete blood count (CBC) test that measures hemoglobin is the standard method for diagnosing anemia more generally, an additional ferritin assessment is still useful to determine if the patent suffers from IDA (the most common type of anemia) [131]. Furthermore, ferritin status can inform the clinician whether iron supplementation is an appropriate treatment for the anemia, as over-supplementation and excess can lead to adverse effects [130]. To assess the TIDBIT device, forty-three human serum samples were purchased from a commercial vendor for testing and compared against ELISA [113]. While the R^2 for the RBP assay was lower than for the other biomarkers, the authors explained that the assay was optimized for high sensitivity and specificity near the diagnostic cutoff for vitamin A deficiency, rather than for precise quantification across the entire physiological range.

This TIDBIT device was applied further in Vemulapati et al. [195], which examined vitamin D (as 25(OH)D3) via the assessment of capillary blood from a finger prick with no pre-treatment. In testing, the test/control ratio was highly correlated to vitamin D concentrations in standard solutions. Commercial serum standards highly correlated with assay results (4-parameter logistic curve), with coefficient of variance (CoV) of 2.63% at 34 ng/mL and 11.2% at 0 ng/mL. Human trials with serum (n=21) and capillary blood (n=6) samples were conducted, and results were compared to LC-MS/MS measurements. The accuracy of deficiency detection was assessed for serum but not whole blood with an area under the curve (AUC) of 0.836 for deficiency cutoffs of 20 ng/mL and 1 for 12 ng/mL. Only the latter aligns with the general clinical threshold for deficiency (Table 1).

3.2.4 Commercial Products. Commercially-available devices have emerged in recent years to provide point of care (PoC) testing for some micronutrients [2, 5, 36, 47]. One study has explored the utility of a commercial iCheck FLUORO device to assess vitamin A concentrations in human milk (human milk vitamin A or HMVA) [2]. HMVA is critical since it is the primary source of vitamin A for breastfeeding children. If there is a vitamin A deficiency in human milk, it is likely to cause developmental issues for a child. The authors collected human milk samples and socio-demographic and anthropomorphic data from lactating mothers in the Mecha district, Ethiopia (n=104). This region was selected because prior studies applying this device for HMVA assessment recommended further investigation of populations at greater risk of vitamin A deficiency. Concentrations of vitamin A in human milk were measured by iCheck FLUORO and compared to HPLC. The commercial device was found to overestimate low HMVA concentrations and had a weak overall correlation with HPLC results. Therefore, the paper concluded that studies which assess vitamin A intake among breast-feeding children in developing countries should not assume average HMVA. It was argued that devices like the FLUORO are needed to monitor HMVA status, especially for intervention programs that typically assume average HMVA. Still, they must be "reliable across a range of HMVA concentrations" [2].

Albrecht et al. [5] likewise studied the efficacy of the Quidel Inc Sofia fluorescent immunoassay for serum vitamin D (as calciol). It should be noted that calciol is an inactive form of vitamin D, distinct from the optimal reference standard of calcidiol for vitamin D status. The assay was analyzed by the Sofia Analyzer, a PoC device for immunoassay analysis. A total of 324 samples were collected and 296 were used (229 female). Additionally, 433 tests were run using both frozen (208) and fresh samples (88). Notably, the researchers also assessed random error and inter-operator reliability for the device. Because only one sample had a concentration above 100 ng/mL, the authors recommend additional testing for concentrations above 80 ng/mL (well into the range of toxicity; Table 1).

Table 3. Assay-Based Methods Using Smartphones

Method	Platform	Targets	Analytes	Evaluation	Control	Results	Notes	Source
Quantitative immunoassay	Smartphone-based	Vitamin D (calcidiol)	Serum	Known sample solutions and human subjects	ELISA	Errors "at same order" as ELISA	No statistical analysis of results	[103]
Quantitative immunoassay	Smartphone-based	Vitamin B12 (cyanocobalamin)	Capillary blood sample	Human Subjects	Immulite 2000 Immunoassay	Correlation of 0.93 with control, 85% specificity and 60% sensitivity for deficiency detection	Used synthetic form of B12; poor accuracy outside of deficiency range	[104]
Quantitative immunoassay	Smartphone-based	Iron (total)	Capillary serum sample	Human Subjects	Multi-Well Plate Spectrophotometry	R^2 of 0.98 with control, CoV of 10.5%	Conducted specificity testing using interferent analytes; considered toxicity	[167]
Quantitative immunoassay	Smartphone-based	Iron (total)	Capillary blood sample	Human Subjects	Laboratory developed test: spectrophotometry-based technique	Correlation plot with slope of 1.09, R^2 of 0.96, and a mean bias of 5.3%	Improved on control LoD; manually diluted some samples to represent a low iron concentration	[166]
Quantitative immunoassay	Smartphone-based or portable device (TIDBIT)	Iron (ferritin)	Capillary blood sample	Human Subjects	Immulite 2000 Immunoassay	Correlation of 0.92 with control, sensitivity of 0.9 for deficiency detection		[176]
Quantitative immunoassay	Portable device (TIDBIT)	Vitamin D3 (calcidiol)	Serum and whole blood	Commercial standards; human subjects	Known solutions; LC-MS/MS	CoV with standards of 2.63% at 34 ng/mL and 11.2% at 0 ng/mL; R^2 of 0.91 for serum tests, 0.94 for capillary blood tests		[195]
Colorimetric assay	Paper-based	Iron (total)	Urine	Human subjects	AAS	RSD of 9.5%	Urine citric acid was found to interfere with results	[46]
Colorimetric assay	Paper-based	Iron (Fe3+)	Serum	Spiked, diluted from human subjects	Spiked known concentration	Error of 3.7% and RSD of 1%	LoD of 0.3 μg/mLl	[40]
Colorimetric assay	Paper-based	Iron (Fe3+)	Saliva	Spiked pooled commercial saliva	Spiked known concentration	R^2 of 0.99	It is debated whether salivary iron reflects circulating status	[149]
Opto-electronic immunoassay	Card	Vitamin A (RBP), Iron (ferritin), and CRP	Whole blood	Human subjects	ELISA	CVs of 2.5% for ferritin, 10.8% for RBP, and 3.9% for CRP	Cutoff for iron deficiency was insensitive to upper limit; RBP is not the clinically-accepted biomarker for vitamin A status; Device transmits results to a smartphone over NFC	[102]
Multiplexed quantitative assay	Portable device (TIDBIT)	Vitamin A (RBP), Iron (ferritin), and CRP	Serum	Human subjects	ELISA	R^2 of 0.56 for RBP, 0.92 for ferritin, 0.88 for CRP	Ferritin range does not cover anemia; RBP is not the clinically-accepted biomarker for vitamin A status; RMSE for RBP was 21 μg/mL	[113]

Bloom Diagnostics is a home use 'lab' device that analyzes single-use qualitative test strips to quantitatively assess the status of in-vitro (in-body) biomarkers, similar to the TIDBIT [36, 113, 195]. Tests available for Bloom include thyroid-stimulating hormone (TSH), ferritin, CRP, and estimated glomerular filtration rate with cystatin C. While Bloom approaches the goal of accessible nutrition assessment, its assays still require the user to collect a sample themselves. Depending on the test, this could involve a finger prick, coaxing the blood into a collection tube, and depositing it properly onto the assay. VitaScan is another commercial PoC device that tests for iron deficiency, and they validate results against the clinical optimal reference standard for in-body iron measurement [47]. The device is not yet released, but it is planned to assess vitamins B12, D, and A and CRP in the future. The method is still invasive as it utilizes capillary blood obtained from a finger prick and also requires the user to obtain the sample themselves.

Table 4. Commercial Assay-Based Methods

Method	Platform	Targets	Analytes	Evaluation	Control	Results	Notes	Source
Fluorometry	Portable device	Vitamin A (retinol and retinyl esters)	Breast milk	Human subjects	HPLC	Weak correlation (R^2=0.59, p<0.001), but mean difference was "not statistically different from zero"	Of major concern was the ability for the breast milk to satisfy the vitamin A requirements of children	[2]
Immuno-fluorescence	Portable device	Vitamin D (cholecalcif-erol)	Serum	Human subjects	Abbott Alinity i immunoassay	R^2 of 0.89; SE of 0.16 at 10 ng/mL, 0.19 at 12 ng/mL, and 0.35 at 30 ng/mL	Standard error lower than control; recom-mends additional testing for excess status; target is not the clinically-accepted biomarker for Vitamin D	[5]
Quantitative immunoassay	Home use device	Iron (ferritin), other tests	Capillary blood sample	Not published	Not published	Not published	Commercially available	[36]

3.2.5 Comparison. Although they are the most accessible and noninvasive, sweat-based assays are generally not a viable alternative to clinical methods of micronutrient status assessment given the current clinical knowledge about the correlation between sweat and blood concentrations of micronutrients. A critical tradeoff exists between accessibility and accuracy, yet until the clinical literature has reached an agreement on which alternative biosamples (i.e. not blood or urine) are most appropriate for the assessment of in-body status, it may be more productive to pursue accessibility through device design rather than through the biosample analyzed so as to not sacrifice clinical-relevance.

Microfluidics and smartphone-based quantification systems may deliver on accessibility and noninvasiveness. The former enables analysis on a small volume of a biosample, meaning that less of the sample needs to be collected (via potentially invasive means in the case of blood). The latter decreases reliance on commercial assay analyzers, which may only operate on proprietary assays, and enables accessibility through portability and cost-effectiveness.

As opposed to other types of assays (e.g. immunoassays), colorimetric assays have been overwhelmingly applied to the assessment of iron (with one expanding to sweat vitamin C, calcium, and zinc). This may indicate the poor versatility of colorimetric assays, whereas other assay techniques are able to assess a wider variety of biomarkers, largely through adjustments to the antibodies used in the assay. The trend of multiplexed assays in both sweat-based and non-sweat-based sensors is therefore a promising means to balance the pros and cons of different assay types. While multiplexed solutions may increase cost, we argue that the potential to assess

multiple biomarkers at once significantly decreases the burden on the patient and the clinician caused by the need to use multiple assays/devices.

3.3 Electrochemistry-Based Methods

Electrochemical analysis is a method that has been applied in literature to quantify levels of micronutrients in biofluids such as saliva, sweat, tears, urine, and blood. Huang et al. [71] summarizes the basis of electrochemical sensors with respect to vitamins, but we see work applying these ideas to minerals as well. The concentration of vitamins in an electrolyte (water or fat/organic solution that allows for the transfer of electrons) can be quantified by measuring electrical properties at a working electrode. The most common measurement techniques are *voltammetry* and *amperometry*. Voltammetry applies a varying voltage to the electrolyte and measures the resulting current, while amperometry applies a constant voltage and measures the resulting current over time [63]. Below, we dive into novel methods that were evaluated in biofluids, roughly divided into voltammetry and amperometry. For more detailed discussions, we direct the reader to recent reviews focusing on electrochemistry-based methods (e.g. [71, 93, 141, 160]). A summary of electrochemistry-based methods reviewed herein is found in Tables 5, 6, and 7.

3.3.1 Voltammetry. Voltammetry has been a popular method for the assessment of micronutrients in biofluids. Revin and John [155] proposed a novel electrode for the simultaneous measurement of vitamins B2 (riboflavin), B9 (folic acid), and C (ascorbic acid). Peak currents for each vitamin were well separated at mixtures of various concentrations. For vitamin C in particular, the tested linear range of the sensor was insensitive to the lower limit of sufficiency and below (Table 1). Additionally, the analyzed biomarkers for vitamins B2 and B9 differed from their clinical optimal reference standard. Selectivity analysis showed that linearity in each vitamin was maintained even in the presence of elevated concentrations of all other vitamins. No interference from other common physiological interferents was found. Two plasma samples from a clinical laboratory were diluted and tested before and after spiking with vitamin standards, with good recovery. Another electrode was developed by Jothimuthu et al. [82], examining zinc. Interestingly, a sample pH of 6 was optimal for zinc assessment, which is more acidic than most biofluids (e.g. blood has a pH of 7.35-7.45). The zinc content in both acetate buffer and spiked, HCl-diluted serum was evaluated. A square wave voltammetric sensor was developed for the simultaneous measurement of glutathione (GSH), nicotinamide adenine dinucleotide (NADH) and folic acid (vitamin B9) [153]. A single urine and serum sample was collected for evaluation and spiked with known amounts of the targets. The authors did not present serum results.

Kim et al. [87] designed a square wave anodic-stripping voltammetric sensor to monitor zinc in sweat during physical activity. The sensor itself was wearable, printed on tattoo transfer paper. The assessment was conducted with standard zinc solutions in a buffer medium. On-body experiments (7 participants, 5 male) demonstrated the sensor's ability to assess zinc in cycling-induced sweat, which was found to be close to the physiological range. Again, no clinical reference standard assessment methods for individual zinc status were used for comparison. However, this is less of a concern since this work explicitly focuses on providing insights into zinc excretion through sweat rather than determining in-body status.

Gao et al. examined zinc as well as copper using voltammetry Gao et al. [50]. A wearable electrochemical sensor was created to assess Zn, Cd, Pb, Cu, and Hg ions in sweat and urine. Uniquely, the sensor incorporated skin temperature measurement for calibration, and to compensate for the influence of temperature on electrochemical signals. This was important, as peak current was shown to increase with temperature. The device was developed with spiked synthetic sweat samples at concentrations an order of magnitude lower than is observed in blood (Table 1). Calibration curves demonstrate a linear relationship between peak current and concentration for the ions, but quantitative metrics were not reported. The authors conducted a human study with a single participant for on and off-body measurements with ICP-MS as a control. The measured and controlled concentrations for Zn

and Cu were similar, but statistical analysis of the results was not conducted. In Stanković et al. [178], a novel "boron-doped diamond electrode" for vitamin B12 (as cyanocobalamin) quantification was studied. Interference analysis showed a 10% signal change in the presence of a "10-fold excess of vitamin B6" [178]. The sensor was evaluated in four spiked urine samples, diluted, and pH adjusted to 2. In this case, the electrode analyzed cyanocobalamin, which is the synthetic form of vitamin B12.

Sempionatto et al. [164] developed an eyeglasses-based platform to conduct electrochemical analysis of tears, assessing the concentration of glucose, alcohol, vitamins B2, B6, and C. Tears were induced with menthol sticks before being collected and analyzed by the glasses. The sensor itself used square wave voltammetry (SWV) for vitamin measurement, demonstrated only as a proof of concept. After a baseline was acquired with the sensor, tears were induced from 3 participants and analysis was conducted every 30 minutes for 2 hours after taking a multivitamin. Peak potentials emerged for each vitamin and were verified with known concentrations of vitamins added to baseline tear samples. Because this was a proof of concept, no comparison to optimal reference standards with blood or efforts to quantify in-body vitamin levels were made.

A microfluidic, graphine-oxide-based sensor chip has been applied for the quantification of ferritin in serum [51]. Notably, the sample must be pumped through the sensor, where cyclic voltammetry was performed continuously with an external potentiostat. An evaluation was conducted with spiked serum samples (which were not sensitive to deficiency) and compared to ELISA. The sensor overestimated concentrations <100 μg/L by ~10% and underestimated the larger concentration by ~4%.

Sun et al. [180] focused on reusability in vitamin C assessment. Their device used cyclic voltammetry to determine whether the vitamin C content present in the sample is normal or deficient (<4.93 mg/L). This deficiency threshold was greater than what is reported by the clinical literature, exceeding even the limit for sufficiency (Table 1). Interestingly, their device was also self-powered, using vitamin C as a biofuel. In a trial for scurvy detection (n=22), the device correctly determined the 4 deficient individuals (ground truth by HPLC). The authors also demonstrated its potential to screen for patients exhibiting a medical condition, identifying 30 patients (total sample size unclear) who suffered from vitamin C deficiency during routine checkups. Of these, >85% had a medical condition associated with inflammation and oxidative stress.

On-body electrochemical sensing of vitamins B6 (pyridoxine), C (ascorbic acid), D3 (calciol), and E (alpha-Tocopherol), as well as 9 amino acids and several macros in sweat, was enabled by Wang et al. [199]. Of the biomarkers examined for each vitamin, only the vitamin C biomarker aligned with the clinical optimal reference standard biomarker. Sweat was passively sampled using iontophoresis in a watch-based platform. Voltammetry was used to detect vitamins indirectly. Quantitative results were not reported, but concentrations of vitamins appeared to linearly correlate with peak height current density. The authors noted the flexibility of this approach to measure numerous other biomarkers. Human trials were conducted with healthy volunteers and patients but only examined amino acids. This exemplifies the tendency of mainstream research to ignore micronutrients.

Lokesh Kumar et al. [110] developed a manganese dioxide nanoparticle–bimetallic metal-organic framework composite to detect vitamin D3 in spiked human plasma. Voltammetry measurements were compared to a optimal reference standard for vitamin D detection, HPLC with ultraviolet detection (HPLC-UV), and obtained similar values. Seker et al. [162] designed a touch-based sensor that simultaneously monitored zinc and ascorbic acid (vitamin C) levels after supplementation. The technique measured fingertip sweat and uses SWV for zinc detection and potentiometric measurement for ascorbic acid detection. Lastly, Shi et al. [170] assessed an NFC-powered sensor for riboflavin (B2) in sweat. Selectivity testing was conducted by the addition of common sweat molecules into the standard, which did not significantly influence results. Uniquely, a pH sensor was incorporated into the device to account or the influence of pH on measurements. Human trials involved subjecting participants to exercise (n=1) or heat stress (n=2) to induce sweat after supplementation. Sweat samples were analyzed by the device and compared to HPLC sweat measurements for the exercise trials and fluorescence spectroscopy for

the heat-stress trials. The time-dynamics of the device results followed the general trend of the control analysis methods, exhibiting more variance compared to urine results.

3.3.2 Amperometry. Vitamins B2, B9, C, and D, as well as the mineral iron, have been assessed in biofluids using amperometry [116, 136, 159, 165, 211, 212]. Maiyalagan et al. [116] confronted a major limitation of glassy carbon electrode-based sensors for vitamin B9 (folic acid): interference from vitamin C. Their nanofiber-modified electrode successfully avoided this interference. In evaluation, two serum samples were collected and evaluated before and after spiking with 4.41 μg/L of folic acid. The peak current increased accordingly, allowing for greater than 99% recovery. Vitamin C is examined by Sempionatto et al. [165], who deployed amperometry and immobilized ascorbate oxidase in a tattoo-based platform. Tests on human subjects (n=4) focused on the temporal characteristics of the current response, finding peak response in sweat 90 minutes after supplementation and a return to baseline 180 minutes after, in line with the plasma response of vitamin C. Tears and saliva were noted as other possible biofluids, with tears yielding a similar temporal profile on a single subject (albeit with different peak currents). The authors also experimented with supplementing vitamin C through orange juice, claiming that the response from sweat samples increased in line with increasing vitamin C content (n=2). Crucially, no statistical analysis was conducted on the results of the experiments, such as correlations between intake and measured current. There were also no comparisons made to clinical reference methods of vitamin C assessment, though this was stated as a subject for future work.

A wearable, electrochemical device to measure vitamin C levels in sweat, urine, and blood was proposed by Zhao et al. [211]. The device was used in a study where 6 male participants, aged 20-30, were given vitamin C as emergen-C brand supplements. The sensor was wearable, but no on-body measurements were made. Instead, urine and induced sweat were collected three hours post-intake and measured with the device. Blood samples were collected from a single participant in a separate study, analyzed with the device, and compared to results from urine and sweat. This research did not compare device results to any optimal reference standard for vitamin C assessment; instead, it relied on intake, which (as we will see in Section 4) is a poor equivalent for in-body status due to individual differences in micronutrient absorption. In addition, we note that the vitamin C supplement, emergen-C, contains several other nutrients that could influence the results of the analysis.

One paper proposed simultaneous measurement of vitamins C and D from a single saliva sample [159]. Their sensor combined an electrocatalytic vitamin C (ascorbic acid) amperometric assay and competitive vitamin D (25(OH)D3) immunoassay. Vitamin D was the clear focus of the paper, and the vitamin C sensor received little to no attention aside from some analysis of potential cross-talk between the two sensors. The sensor was applied in a study that supplemented vitamins to 3 participants and used the device to analyze saliva samples at increasing time intervals from intake. No optimal reference standard assessment method was used to evaluate the sensors, although the authors advocated for this evaluation and the development of truly quantitative sensors in future work. Another flexible, electrochemical sweat biosensor for vitamin C used polyaniline film modified with phytic acid [212]. The biosensor was validated with synthesized vitamin C samples of known concentrations. Four human subjects were given supplements and had their sweat collected 3 times over 90 minutes. The sensor detected a general increase in current from the sweat samples over time, with variation across subjects. Saliva tests were also conducted. Peak current occurred 60 minutes after supplementation, in line with results from [165]. Another team of researchers designed a finger-actuated wirelessly-charging wearable that measures vitamin C and levadopa (a central nervous system agent) levels from sweat [136]. The system had a microfluidic chip with a self-driven pump and anti-reflux valve, a flexible wireless circuit board, and a companion smartphone app. They ran a study with five healthy participants whose sweat was collected and measured after exercising and ingesting vitamin C tablets as well as fava beans [136]. The results were not compared to clinical results as a optimal reference standard baseline.

Table 5. Electrochemistry-Based Methods Using Voltammetry

Method	Platform	Targets	Analytes	Evaluation	Control	Results	Notes	Source
Voltammetry	Benchtop	Vitamins B2 (riboflavin), B9 (folic acid), and C (ascorbic acid)	Plasma	Human subjects	Spiked known concentrations	>99% recovery of spiked concentrations	Tested in 7.2 pH buffer, which is slightly lower than pH of blood (~7.4); direct riboflavin is not the gold-standard biomarker for vitamin B2; folic acid is the synthetic form of folate; Linear range for vitamin C did not reach below the lower limit for sufficiency	[155]
Anodic stripping voltammetry	Benchtop	Zinc	Serum	Human subjects	Spiked known concentrations	Peak current decreased with concentration, but were lower in magnitude than buffer	A sample pH of 6 was necessary for optimal performance	[82]
SWV	Benchtop	Vitamin B9 (folic acid), GSH, and NADH	Urine and serum	Human subjects (urine only)	Spiked known concentrations	Accurate recovery of spiked concentration	Simultaneous determination of targets; unclear whether pre-existing urine composition biased recovery; claims serum evaluation but this is not presented; folic acid is the synthetic form of folate	[153]
Square wave anodic stripping voltammetry	Wearable tattoo	Zinc	Sweat	Zinc stock solutions	Known zinc solutions	R^2 of 0.999 for measured current vs stock solutions, LoD of 0.05 μg/mL	Zinc content in actual sweat from single participant was close to physiological range	[87]
Square wave anodic stripping voltammetry	Wearable patch	Zn, Cd, Pb, Cu, and Hg ions	Sweat and urine	Human subjects	ICP-MS	Similar to control, but provided no statistical analysis	Included temperature sensor to account for the influence of skin temperature on peak current	[50]
SWV	Benchtop	Vitamin B12 (cyanocobalamin)	Urine	Diluted from human subjects	Spiked known concentrations	98-105% recovery	Cyanocobalamin is the synthetic form of B12	[178]
SWV and chronoamperometry	Eyeglasses	Vitamins B2, B6, and C, alcohol, and glucose	Tears	Human subjects	Breathalyzer BAC, commercial glucometer, vitamin supplementation	Correlations of 0.852 with BAC, 0.7 with glucometer, distinct voltage peaks for each vitamin	Glucose and alcohol was main focus, vitamin assessment included as a proof of concept; exact form of each vitamin is not known	[164]
Cyclic voltammetry	Benchtop	Iron (ferritin)	Serum	Spiked from human subjects	ELISA	R^2 of 0.966 and lower linearity than control; tended to overestimate concentrations <100 μg/L	Range of spiked concentrations was not sensitive to deficiency; studied the impact of pH and interferent compounds	[51]
Cyclic voltammetry	Portable	Vitamin C	Serum	Human subjects	HPLC	R^2 of 0.984 (p<0.001) and 100% accuracy in deficiency detection	Targets scurvy (extreme deficiency) but the threshold was set above sufficiency	[180]
Voltammetry	Wearable patch	Vitamins B6 (pyridoxine), C (ascorbic acid), D3 (calciol), E (alpha-Tocopherol), and other macronutrients and amino acids	Sweat	Not reported for vitamins	Not reported for vitamins	Observable linear relationship between vitamin concentration and peak height current density	Only vitamin C aligns with clinical standard, quantitative results for vitamins were not reported	[199]
Voltammetry	Benchtop	Vitamin D3	Plasma	Spiked from human subjects	Spiked known concentrations and HPLC-UV	LoD of 1.9 ng/mL; RSD of 0.3-2.6% and recovery of 96-102%	Exact form of D3 (gold-standard 25(OH)D3/calcidiol or calciol) not reported	[110]
SWV and potentiometric measurement	Portable	Zinc and vitamin C (ascorbic acid)	Fingertip sweat	Human subjects	Supplementation	Both micros could be analyzed over time simultaneously	No comparison to clinical assessment of status or statistical analysis of results; Vitamin C range far exceeded physiological concentrations in plasma	[162]
Differential pulse voltammetry	Wearable patch	Vitamin B2 (riboflavin)	Sweat	Human subjects	HPLC	R^2 of 0.9783 with sweat HPLC and 0.87 with urine fluorescence spectroscopy	Urine included as comparison to sweat status; incorporates pH sensor to control for influence of pH on measurements; direct riboflavin is not the gold-standard biomarker for Vitamin B2 status	[170]

Table 6. Electrochemistry-Based Methods Using Amperometry

Method	Platform	Targets	Analytes	Evaluation	Control	Results	Notes	Source
Amperometry	Benchtop	Vitamin B9 (folic acid)	Serum	Human subjects	Spiked known concentrations	>99% recovery of spiked concentration	Robust against interference from ascorbic acid; sensor performance peaked at pH 7.2, slightly lower than pH of serum; folic acid is the synthetic form of folate	[116]
Amperometry	Wearable tattoo	Vitamin C (ascorbic acid)	Sweat, tears, and saliva	Human subjects	Supplementation	Similar time dynamics to plasma for tears and sweat		[165]
Amperometry	Wearable sensor chip	Vitamin C (ascorbic acid)	Sweat, urine, and blood	Human subjects	Supplementation/ intake, blood measurements with the same sensor	Urine and sweat measurements increased after intake, with inter-trial variation. Correlations with blood sensor measurements were 0.81 for sweat and 0.72 for urine	Used emergen-C as a vitamin C supplement, which contains several other micronutrients	[211]
Amperometry	Wearable sensor chip	Vitamin C (ascorbic acid)	Sweat and saliva	Stock solutions and human subjects	Known solutions and supplementation	R^2 of 0.99 and LoD of 0.0299 μg/mL for stock solutions, was able to detect general increase in current after supplementation		[212]
Electrocatalytic vitamin C amperometric assay and competitive vitamin D immunoassay	Portable	Vitamins C (ascorbic acid) and D (calcidiol)	Saliva	Human subjects	Vitamin supplementation	Observable rise and drop in levels over time	No quantitative results; saliva sample must be pretreated; Vitamin C has little evaluation	[159]
Chronoamperometry	Wearable sensing system	Vitamin C (ascorbic acid) and Levodopa	Sweat	Human subjects	Vitamin supplementation	Detection correlation coefficients of both exceed 0.99; both sensors have a wide linear detection range of 0-17.6 mg/L and 0-1000 μM, respectively, and low detection limits of 0.05 mg/L and 17.9 μM, respectively.	The system is wireless, battery-free, flexible, finger-actuated, and self-pumping	[136]

3.3.3 Impedance Analysis. Heo et al. [69] focused on analyzing solely vitamin D (calcidiol) status using body impedance. They explored the correlation between vitamin D levels in blood, body composition, blood parameters from checkup, and arm impedance (from wrist to elbow) in 26 patients (14 male) to calibrate an impedance measurement frequency for vitamin D [69]. The motivation was that "body fat accumulates vitamin D," and body fat can be measured by impedance measurement [69].

3.3.4 Comparison. Across voltammetry and amperometry, vitamin C, B vitamins, and zinc are the most common targets of electrochemistry-based methods. However, a few do target vitamin D, which is the only target of the impedance analysis technique. Voltametric devices were more often applied to blood or urine biosamples, and the associated studies relied less on supplementation as a control compared to amperometry-based methods. Amperometric devices appeared to focus more on a wearable form-factor. The single study using impedance

Table 7. Other Electrochemistry-Based Methods

Method	Platform	Targets	Analytes	Evaluation	Control	Results	Notes	Source
Arm impedance measurement scan	Non-mobile, clinical device	Vitamin D (calcidiol)	Impedance measurement at 21.1 Hz	Human subjects	Vitamin D status by blood test (method unspecified)	R^2 of 0.75 (regression with Vitamin D level)	Also evaluated regression models with medical checkup and body composition analysis data	[69]

analysis reported results which were less accurate than those reported by voltametric or amperometric approaches to vitamin D assessment [69]. This may indicate that body impedance analysis is not a promising method for future research, but we add that the results of this study may be more trustworthy than others due to its larger sample size and evaluation in the context of optimal reference standard vitamin D status assessments using blood. For these reasons, we cannot conclude whether impedance analysis is less promising than amperometry or voltammetry.

3.4 Spectroscopy-Based Methods

Spectroscopy is the most common method of optimal reference standard micronutrient status assessment. However, there is still a lot of work to do to make spectroscopic approaches more accessible and less invasive. We begin with some definitions of common terms in the field. *Spectroscopy* is "the investigation and measurement of spectra produced by matter interacting with or emitting electromagnetic radiation" [1]. *Spectrometry* is the application of spectroscopy; the way in which quantitative measurements are obtained. When we speak of a *spectra*, we mean any measurement that is a function of wavelength or frequency. A sample will absorb or emit these spectra when electromagnetic radiation of a known wavelength is applied. Spectra are measured by a detector, the *spectrometer*. Because the level of radiation applied is known, analyzing the resultant spectra after it interacts with the sample provides information about the sample.

We have previously described MS and LC-coupled spectroscopy, the types of spectroscopy used by most optimal reference standard clinical biochemical analyses (Section 3.1. As mentioned, these methods are expensive, non-specific, complex, and often require an invasively-collected biosample. While there have been strides to make MS more accessible [33], we focus on alternative spectroscopic techniques that may yield micronutrient insights.

Categorized under emission spectroscopy, *fluorescence spectroscopy* has been demonstrated for the measurement of primarily B vitamins in non-biological samples such as multivitamins and energy drinks, although vitamin B1 was measured in urine [210]. The B vitamins continue to get attention in *near infrared (NIR) spectrophotometry* (750-2500 nm wavelength), where their measurement has been reported as well [210]. One review recognized the ability of *vibrational spectroscopy*, which includes *infrared (IR)* and *raman spectroscopy*, to act as a tool for biofluid analysis in precision nutrition [38] (Fig. 3). However, like other studies in nutrition, this review had few considerations for micronutrients and the approaches covered were largely concerned with general nutritional status or macronutrients. Tsiminis et al. [187] noted the potential of raman spectroscopy for the measurement vitamin B12, though this has yet to be realized at in-body concentrations due to the low sensitivity of raman spectroscopy. In biosamples, spectrophotometry was applied to measure vitamin C [74]. Spectroscopic skin tests and raman spectroscopy have also been noted as promising techniques in the assessment of provitamin A carotenoid status in the body [74]. Measurement of several water-soluble vitamins in synthetic mixtures and dosage forms was achieved with *derivative and multivariate spectrophotometry* [210]. Derivatives of UV

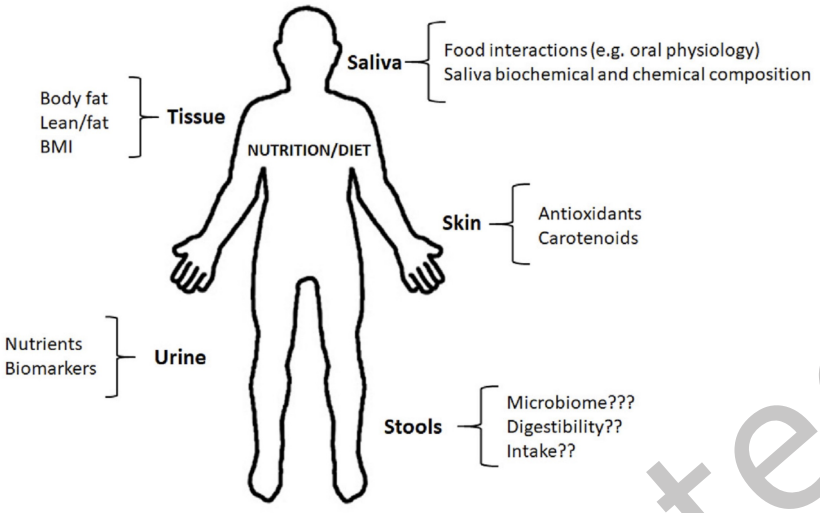

Fig. 3. Vibrational spectroscopy targeted at saliva, tissue, skin, and urine have current applications in precision nutrition, while stools hold potential nutritional insights (indicated by question marks). Used with permission from Dongdong and Cozzolino [38]

spectrophotometry (185-400 nm wavelength) have also been particularly useful in the analysis of caffeine and B vitamins in energy drinks. The open-source Lumos platform also enables on-body spectroscopy [201].

3.4.1 Accessible Spectroscopy. Significant progress has been made to make spectrometry as a study more accessible and compact [33]. Major subfields of spectrometry (visible, Raman, mid-IR, NIR, MS, and hyperspectral imaging) have seen the development of portable or handheld devices. In some cases, such as visible, near-IR, and hyperspectral imaging, these can even be smartphone-based. This paves the way for more accessible, noninvasive techniques. A summary of spectroscopy-based methods is found in Table 8.

There was one application of spectrometry for vitamin D (calcidiol) measurement in our interest area of accessible approaches [197]. With human serum samples in mind, their sensor used surface plasmon resonance (SPR) together with smartphone-based spectrophotometry to assess vitamin D content. The general design of the sensor involved an optical waveguide to direct light from the smartphone flash through one or more SPR sensors, a diffraction grating, and finally into the smartphone camera where a spectra of pixel intensities can be extracted. The device was evaluated on spiked serum samples. As the concentration decreased, the center of mass of the spectra shifted right, allowing for the detection of these concentrations. The paper claims a comparable LoD to optimal reference standard methods of LC-MS, but no quantitative estimates of vitamin D concentration, sample size, or statistical analysis of the results are provided.

Some benchtop approaches to spectroscopic analysis of biofluids for micronutrient assessment have also been demonstrated [20, 128, 145, 146]. Peterson et al. [146] presented a photonic crystal-based sensor for ferritin assessment. Photonic crystals were designed to accumulate a target biomolecule on their surface, which changes their reflected peak wavelength value (PWV) under a spectrophotometer. The authors subjected their sensor to robust evaluation, utilizing human liver ferritin, commercial serum controls (Liquichek), and three different ELISA tests. The developed sensor held up against the ELISA tests, but with a higher LoD (26 μg/L) that did not

cover the lower end of iron deficiency. Bias by Bland-Altman analysis was similar to that of the BioVendor ELISA, and recovery from known serum controls was greater than 94%.

Motivated by a specificity issue in the preceding sensor, Peterson et al. [145] employed iron-oxide nanoparticles to minimize non-specific signals. This time, the goal was soluble transferrin receptor (sTfR) quantification from serum, an indicator of iron supply to tissues [4]. It should be noted that sTfR is not influenced by inflammation to the same extent as serum ferritin, and is therefore a potentially more ideal biomarker. However, sTfR is more expensive to assay. For this experiment, biomolecule interaction on the assay was quantified using the Biomolecular Interaction Detection system from SRU Biosystems Inc [145]. The authors compared results from their assay on Liquichek control sera to ELISA and the previously developed photonic crystal assay. The authors claimed that the bias of the assay was not "statistically different from the reference ELISA tests" [145].

Moving from iron to vitamin C, Bi et al. [20] demonstrated the immobilization of ascorbate oxidase in a microfluidic channel, enabling the quantification of vitamin C with UV-visible spectroscopy. During analysis, the biosample was diluted in phosphate buffered saline (PBS) and "pumped through the microfluidic channel". A serum sample was obtained by a single healthy, female volunteer for evaluation. The sample was pretreated to remove proteins , and a few drops were added to the sensor. Even with extensive pretreatment, there was evidence of interference at 280 nm, close to the analysis peak of vitamin C at 266 nm. No optimal reference standard measurement was provided for comparison. Mughal et al. [128] mixed different electrolytic solutions with plasma and serum, and when paired with a novel, reduced graphene oxide, vitamins K1, K2, B6, and D3 could be individually identified using UV-visible spectrophotometry. This method did not compare the measured levels from 5 subjects with clinical values.

3.4.2 Comparison. A wide range of spectroscopic methods have been applied for the quantification of different micronutrients, making it difficult to compare each approach. Iron assessment appears to be feasible in serum, but further development would be useful to increase the accessibility of a spectroscopic approach that targets this nutrient. Furthermore, clinical research could consider alternative biosamples for iron, to increase the noninvasiveness of spectroscopic techniques. Studies using spectrophotometric methods have yet to be thoroughly validated so as to indicate the utility of spectrophotometry over any alternative method.

3.5 Biofluid Analytic Methods

AI and ML can be used to detect micronutrient levels in individuals. A common approach to estimating nutritional status is by making predictions from pre-existing biofluid analysis or demographic data using ML. Such methods have been applied to derive micronutrient-specific insights [86, 100, 114, 144, 150, 186]. A summary of biofluid analytic methods can be found in Table 9, where we observe a common trend of utilizing classical machine learning algorithms (logistic regression, gradient boost, naive bayes, random forest) for predictive modeling.

3.5.1 Single Micronutrient Malnutrition Detection. Some studies focus on detecting deficiency of a specific micronutrient. Two such papers investigate iron status [115, 150], while a third examines vitamin D [144]. Luo et al. [115] used hospital outpatient data collected over three months to predict whether a patient had normal or abnormal ferritin (iron) status using logistic regression. The collected data included age, sex, ferritin test results (used as markers), and other 'predictor' tests that were conducted in the main hospital lab only (n=5128, sex breakdown not reported). These authors considered the broader clinical usefulness of ML-powered insights, claiming that "predicted ferritin results may sometimes better reflect underlying iron status than measured ferritin" [115]. This conclusion was based on dual independent review by 2 pathologists on 26 selected cases where measured and predicted ferritin were 'highly discrepant'. They propose that predicted levels could be used to flag lab-measured ferritin for further review. Pullakhandam and McRoy [150] use gradient boost on NHANES complete blood count (CBC) data to classify and explain IDA (n=19995, sex breakdown not reported). They found

Table 8. Spectroscopic Methods

Method	Platform	Targets	Analytes	Evaluation	Control	Results	Notes	Source
SPR-coupled spectrophotometry	Smartphone-based	Vitamin D (calcidiol)	Serum	Spiked from human subjects	LC-MS	Claim comparable LoD to control, spectra shifted right with decreasing concentration	No quantitative vitamin D estimates or statistical analysis	[197]
Photonic crystal	Benchtop	Iron (ferritin)	Serum	Liquichek control sera	Known ferritin concentrations and multiple ELISA tests	Comparible recovery (>94%) and bias (by Bland-Altman analysis) to best-performing ELISA	LOD higher than cutoff for anemia, lower end of deficiency	[146]
Sandwich iron-oxide nanoparticle immunoassay	Portable	Iron (STfR)	Serum	Liquichek control sera	ELISA	SD of 0.45 mcg/mL vs ELISA		[145]
UV-vis spectrophotometry	Benchtop	Vitamin C (ascorbic acid)	Serum	Human subjects	None	Within phsyiologically-relevant concentrations	Strong focus on the effectiveness of the immobilization technique, not vitamin C measurement	[20]
UV-vis spectrophotometry	Benchtop	Vitamins K1 (phylloquinone), K2 (menaquinone), B6, D3 (cholecalciferol)	Serum/plasma	Human subjects	Various sensing techniques (SWV, HPLC-MS/MS (ESI), SWAdSV, Thermal wave transport analysis, DP AdSV, SWASV, DPV, Electrochemical, Colorimetric aptasensor)	Limits of detection of vitamins K1, K2, B6, and D3 are 0.075, 0.1, 0.12, and 0.15 ng/mL, respectively. Limits of quantification are 0.29, 0.3, 0.38, and 0.48 ng/mL for vitamins K1, K2, B6, and D3, respectively.	Clinical gold-standard biomarkers are not used for all vitamins (except for K, where LoD is too high for deficiency); used bismuth nanoparticle embedded polypyrrole nanocomposite (rGO/pPy/Bi NC) as an optical sensing material	[128]

that the most critical features for IDA are low levels of hemoglobin, higher age, and a higher red blood cell distribution width.

For the prediction of vitamin D deficiency, Patino-Alonso et al. [144] applied ML (logistic regression, naive bayes, and random forest) to anthropomorphic data of older Europeans (35-75 y/o; 50/50 males/females) given anthropomorphic features. A total of 501 participants contributed their "waist circumference (WC), body mass index (BMI), waist-to-height ratio (WHtR), body roundness index (BRI), visceral adiposity index (VAI), and the Clinical University of Navarra body adiposity estimator (CUN-BAE) for body fat percentage". Vitamin D as 25(OH)D was measured by immunoassay and the threshold of deficiency was set to be 20 ng/mL (34.7% prevalence). We note that this threshold more closely aligns with *insufficiency* (Table 1). Logistic regression analysis found that the most significant features differed by sex. All but CUN-BAE were associated with vitamin D deficiency in males, while only CUN-BAE was associated in females. ML models for deficiency prediction were trained on each feature individually. The authors discovered that Naive Bayes was the top performer by AUC for WC, BMI, WHtR, and BRI but was bested by logistic regression for VAI and CUN-BAE.

3.5.2 Multiple Micronutrient Detection. Since it is rare for micronutrient imbalance to occur in isolation, researchers have studied the ability to predict malnutrition of multiple micronutrients [100, 186]. Truijen et al. [186] focus on micronutrient malnutrition in older populations, citing how malnutrition in older adults is often diagnosed too late despite the existence of screening methods. The goal of the study was to use logistic regression

to classify each sample as having either no micronutrient deficiency or one or more deficiencies among vitamins C, B6, B12, selenium, and zinc, confirmed by blood tests. These particular micronutrients were selected because they interact less with each other (we add that B-vitamins do interact; Table 13), were among the most prevalent deficiencies, had clinically relevant cutoff points for deficiency. Logistic regression was applied to routine biochemical and diagnostic data from 9 years of United Kingdom NDNS for ages ≥50 (n=1518, 57.2% female). This dataset suffered from ethnic disparities, with the authors noting that ≥95% of NDNS participants were white.

Kurstjens et al. [100] aimed to develop a random forest algorithm to assess risk of low body iron storage (ferritin plasma levels) in anemic primary care patients using CBC and CRP test results from 3 medical laboratories (n=2,935, ~1,493 female. Two algorithms were developed, each based on laboratory ferritin results from different chemistry analyzers (from Siemens and Roche). Interestingly, the two most important features were both derived from CBC test results (Table 9). The authors took an important step to consider how such a model could assist a clinician by asking 4 professionals to indicate whether a patient had low ferritin based on CBC and CRP, with and without algorithm results. The found that the algorithm alone was more accurate than both scenarios. Detection of low vitamin B12 and B9 levels were also considered, but this yielded poor results with AUCs of 0.52 and 0.57 respectively.

3.5.3 Adjusting Biomarkers for Inflammation. When conducting biochemical analysis for micronutrient assessment, a common issue is the impact of inflammation on biomarker measurement. One R package aims to solve this problem and improve interpretability by adjusting biomarkers of micronutrients in the context of inflammation [114]. The package implements inflammation adjustment for retinol-binding protein, serum retinol, serum ferritin, sTfR, and serum zinc, using acid glycoprotein (AGP) and/or CRP as biomarkers for inflammation. The authors have also published a paper describing a procedure on when and how to apply their technique [114].

Table 9. Analytic Methods

Method	Targets	Data	Important Features	Ground Truth	Results	Notes	Source
Logistic regression	Normal or abnormal ferritin	Hospital outpatient data	"total iron-binding capacity, mean cell hemoglobin, and mean cell hemoglobin concentration" from Luo et al. [115]	Ferritin test results	AUC of 0.97	Predictions could be used to flag lab ferritin for review	[115]
Gradient boost	IDA	US NHANES (CBC and serum ferritin) dataset and Kenyan nutrition dataset (for evaluation)	Low blood level of hemoglobin, higher age, and higher red blood cell distribution width	Serum ferritin	Precision AUC of 0.87 (training); recall of 0.98 (training) and 0.89 (evaluation)	Heavy class imbalance (4.9% IDA vs. 95.1% non-IDA)	[150]
Logistic regression, naive bayes, and random forest	Vitamin D deficiency	Anthropormorphic measurements of older Europeans	CUN-BAE for females, all others for males	Blood 25(OH)D by immunoassay	Max AUC of ~0.53 for all features; LR best for VAI and CUN-BAE, NB for all others	Did not assess predictive ability of multiple features at once	[144]
Logistic regression	Presence of micronutrient deficiency	UK NDNS, ages ≥50	Low protein, energy intake, TC, hemoglobin, HbA1c, ferritin, vitamin D and high CRP	Blood test results for Vitamins C, B6, B12, selenium, and zinc	AUC of 0.79	≥95% of NDNS participants were white	[186]
Random forest	Classify low body iron storage (plasma ferritin)	CBC and CRP tests in anemic primary care patients	Mean corpuscular hemoglobin and mean corpuscular volume	Ferritin results from two laboratory chemistry analyzers (two separate models)	AUC of 0.9 and 0.92 for each model, models were more accurate than professionals with and without access to results	Attempted Vitamin B12 and B9 deficiency detection with poor results (AUCs of 0.52 and 0.57)	[100]

3.5.4 Comparison. Luo et al. [115] and Kurstjens et al. [100] both utilize data obtained from patients during the course of their health care, whereas other sources of data came from surveys (e.g. the US NHANES). Collecting data during health care can result in a large amount of data to mine for micronutritonal insights without needing to rely on national surveys (which may not contain a wide variety of features) or conducting an independent assessment of a population. The prediction of iron (as ferritin) appears to be more successful than the prediction of other biomarkers. This is likely due to iron's large influence on hemoglobin, which was an important feature for all ML models that were trained to predict ferritin status [100, 115, 150].

3.6 Biofluid Analysis Limitations

The largest general limitation of existing biofluid-based assessments is that methods effectively indicating in-body status of a micronutrient often utilize an invasively-collected biosample (i.e. blood) for analysis. While noninvasive biosamples such as sweat and saliva were applied, the clinical research on micronutrients suggests that these samples may not accurately reflect micronutrient status compared to blood. As such, validation studies are required. We also observe that biofluid-based assessments are more specialized in nature. Frequently, a bespoke assay, device, or sensor is implemented for the assessment of only a single micronutrient. This is understandable considering the inherent difficulties in detecting and quantifying micronutrients that have unique metabolic pathways, are present in such limited quantities, and play different roles in bodily function. Furthermore, there is also a common need for a specialized reagent, buffer, or electrolytic solution to enable the analysis of each micronutrient (especially for assays and electrochemical methods). It is for this reason that multiplexed or simultaneous detection of multiple micronutrients is limited, despite their potential to enable more holistic status monitoring. However, we acknowledge that combining several specialized assays and unique sensor designs adds complexity to the measurement process, creating barriers to commercialization as well as widespread adoption.

On the topic of commercialization, we posit that the lack of commercial, multiplexed, or PoC technologies for micronutrient assessment is a result of both the ingrained reliance on laboratory blood testing within health care institutions as well as the expensive and thorough human subjects evaluations necessary to bring an effective assessment technique to market. We find that current methods do not consistently test the clinical biomarker for a given micronutrient (e.g. measuring vitamin D via calciol instead of the optimal reference standard calcidiol a.k.a. 25(OH)D). Even when the proper biomarker is examined, the device itself may be evaluated on concentration intervals that are not pertinent to the clinical spectrum of deficiency to excess (e.g. the linear range of a sensor may be able to indicate sufficient status, but the LoD is too high to infer deficiency). Again, we recognize that some methods are specifically designed to accurately detect a deficient status in a resource-constrained environment, or assess micronutrients where excess does not pose a significant health risk. In these cases, a lack of sensitivity in the upper spectrum is less of an issue. Some research fails to include a clinically relevant biochemical test (e.g. ELISA or HPLC) for their target micronutrient as a scientific control. By contrast, a non-clinically relevant biochemical test either does not follow best practices in clinical nutrition, targets an alternative biomarker, or uses an alternative process from what is typically considered acceptable in modern clinical practice. Such a non-clinically relevant test can also simply rely upon supplementation rather than any substantive evaluation of micronutrient status (see below). Each of these oversights actively limits the clinical value, and therefore the real-world utility, of the proposed solution.

The lack of a clinically relevant biochemical test is confounded by the prevalence of supplementation-based experiments, where an assessment is conducted on a patient before, during, and/or after intake of the target micronutrient. The prescribed intake may be as specific as a supplement pill or as general as a food that is known to contain high amounts of a micronutrient. Because of individual variance in the absorption of different micronutrients, it is difficult to know the true impact of micronutrient intake, and therefore the practical accuracy of a status assessment, without also conducting a relevant biochemical test. Lastly, the study designs themselves

often feature low sample sizes (1-5 participants) that make it difficult to assess the analytical validity of each method. We recognize that design-oriented research faces a larger hurdle when attempting to conduct human-subjects experiments that are clinical in nature, and several studies echo this point. Biofluid analytic methods have sample sizes that are (necessarily) much larger, but share issues related to the equitable representation of demographic groups within their sample. Similar to the impact of omitting a relevant biochemical test, we argue that an emphasis on new technologies over more rigorous and larger-scale validation studies makes it difficult to judge the true effectiveness of a novel assessment method.

Moving to the specific approaches, we find that clinical biochemical analysis is invasive, expensive to analyze, and the methods of analysis and thresholds for imbalance are debated [154]. Additionally, biomarkers are generally sensitive but not specific, and their analysis requires considering an extensive list of factors that alter the ability of a marker to indicate nutrient status (such as inflammation, disease, or medications) [42].

Although there are several wearable, smartphone, and point of care assay devices, their accessibility in some cases is limited by specialized assay chips and most require invasive biofluids for analysis. Saliva and sweat have been proposed as alternatives to blood, but their ability to reflect in-body status of a given micronutrient is often unknown, debated, or disproved (Section 2.2). One survey offers additional insight into the issues faced by wearable sweat sensors: low sweat rates, sample evaporation, skin contamination impacting sweat content, and the difficulty to access fresh sweat [15]. Assays and electrochemical devices also suffer from being more specialized in nature (complicating manufacture and integration) and not utilizing more ubiquitous methods of health monitoring such as smartphones and smartwatches.

The field of spectroscopy shows great potential (especially IR and Raman), though we argue that there is not yet enough accessible, micronutrient-specific research that provides insights into in-body status. Most work in this area required benchtop analyzers instead of on-body approaches. With the latter however, one must take great care not to harm a user with the spectroscopic approach. UV spectroscopy in particular requires the application of UV light, which is broken into three types depending on its wavelength. Exposure to UVA (400 - 320 nm) and UVB (320 - 280 nm) radiation can cause damage to DNA and result in cancer, because of how this energy penetrates the skin [39]. UVC radiation from sunlight (280 - 100 nm) does not possess these same dangers, especially because it is absorbed by the skin, but direct exposure to artificial UVC radiation can still cause burns on the skin and eyes [191]. The exact amount of UV radiation which is considered harmful varies with the area of exposure, amount of melanin content in the skin, and more, so we direct the reader to Table 2 in D'Orazio et al. [39] for more information.

Prediction from clinical health data is able to combine and analyze a large breadth of features, but this data is often insufficient for micronutrition. The lack of micronutrition data availability poses a grand limitation for the ability to make strides in analytic techniques with AI/ML. As a result, Brown et al. [22] and others urge for more micronutrition data. Additionally, the insights provided by these solutions are limited to a small set of micronutrients and/or indicate only a binary deficiency status, rather than a continuous one.

4 PHYSIOLOGICAL SENSING FOR MICRONUTRIENT ASSESSMENT

Physiological assessment is as important as biofluid analysis, and some argue they should be considered together. Many physiological symptoms of micronutrient deficiency (Tables 16 to 18) only manifest in severe cases, but the assessment of physiological symptoms remains a critical step in nutritional practice for two central reasons. First, it is useful when estimating the burden of micronutrient malnutrition in a population that is difficult to assess via biofluid methods. Second, physiological assessments provide insights into patient health that are unique and complementary to quantitative, biofluid-based assessments. Though it is valuable, physiological analysis receives significantly less attention in emerging micronutrition research. A comprehensive overview of physical

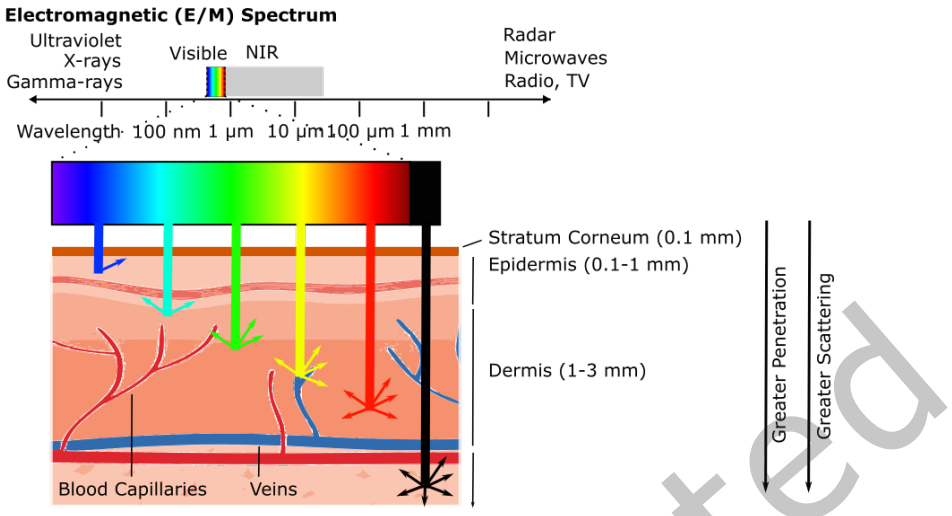

Fig. 4. As light is applied to the skin, it is absorbed and reflected in different ways depending on its wavelength. Importantly, skin melanin content can impact the absorbance of visible light. For more information, please refer to [6]. Used with permission from McDuff [120].

examination in clinical nutrition is out of scope for this review, so we direct the reader to Reber et al. [154] and Hummell and Cummings [73] for more information.

Physiological sensing enables automatic detection of symptoms linked to micronutrient deficiency (Section 2.3 and Tables 13 to 15), supporting NFPEs. Incorporating metrics like heart rate, activity, sleep, and temperature can enhance personalization and reveal health trends over time. King et al. [91] outline key criteria for effective wearable monitoring: noninvasive, user-friendly, reliable, and informative. Witt et al. [205] shows how raw data from sensors like PPG, ECG, accelerometers, EDA, and temperature can offer physiological insights about human physiology relevant to micronutrient imbalance. Yokus and Daniele [208] also provide valuable design considerations for future wearable micronutrient assessment tools. The remainder of this section highlights innovative methods of optical sensors in assessing physiological signals. While not explicitly wearables-focused, Yokus and Daniele [208] provide some useful considerations for wearable devices that should be applied to future micronutrient assessing devices.

4.1 Applications of Optical Sensors in Assessing Physiological Signals

McDuff [120] reviews how camera sensors can be used for noninvasive physiological measurement, which can allow for nutritional insights. The analysis of motion artifacts can reveal minute subtleties in body motion over time that are caused by various physiological mechanisms (e.g. breathing). Also, camera sensors can measure the intensity and wavelength of light absorbed and reflected by our bodies, especially skin (Fig. 4). Differences in measured light over time can be observed and associated with physiological signals (e.g. heartbeat) and status (e.g. low blood oxygen saturation). However, it is important to note that skin melanin content can impact how light is absorbed and reflected [6]. A failure to properly account for these differences can (and has) resulted in racial biases (for example, pulse oximetry [172] and wrist-worn heart rate sensors [96]).

The type of optical sensor has a large impact on the signals that can be derived from it [120]. RGB cameras are found in most smartphones and, therefore, are the most prevalent. These operate largely in the visible spectrum

of light (400 to 700 nm wavelengths), but they can often detect some light in the NIR range. NIR cameras are able to detect light in the 700 to 1000 nm range, and thermal cameras can go fully into the infrared spectrum of 2000 to 14000 nm. Thermal cameras, as their name suggest, can provide unique information over other sensors such as body temperature and sweat gland activation. However, this comes with a higher cost and lower resolution. Finally, multi and hyper-spectral cameras allow for the measurement of multiple wavelengths of light at once. This can also be achieved by combining signals from multiple sensors.

Cameras have been extensively applied to the measurement of physiological vital signs [60, 108, 120, 121, 129, 193]. One survey describes 5 physiological vital signs where optical sensor measurements been applied: pulmonary activity, EDA, blood oxygen saturation, glucose status, and cardiac activity [120]. PPG signal capture has been achieved with RGB cameras [60, 193]. NIR cameras are also useful for PPG in use cases like sleep monitoring that require environments with low visible light [120]. When measuring blood oxygen saturation, multi or hyper-spectral cameras are preferred. Measuring multiple wavelengths of light at once benefits the simultaneous measurement of oxy and deoxy-hemoglobin, and therefore blood oxygen saturation. Recently, Sharma et al. [169] proposed a smartphone-based hyperspectral imaging platform and used it to identify organic fruits. Optical sensor-based techniques could be use to detect and investigate other physiological symptoms in the fingertip [65, 190, 198], mouth [203, 207], and eyes [84, 99, 142, 181].

Optical sensors have been successfully applied to noninvasively assess a major protein, hemoglobin. Hemoglobin allows for oxygen transport in red blood cells and is produced by iron, vitamin B9, and vitamin B12 [177]. A deficiency in these micronutrients can result in lowered hemoglobin, which manifests as anemia. A review of the state of the art emphasizes the need for an affordable and accessible method of hemoglobin measurement, which can be realized with commodity smartphone cameras [64]. Various works have used smartphone cameras as a way to estimate hemoglobin status noninvasively [64, 65, 181, 198]. They report that PPG signals derived from the fingertip and conjunctiva (skin behind the lower eyelids) under NIR light in 1070 and 850 nm wavelengths contain the most critical features to hemoglobin estimation. Wang et al. [198] had users place their fingertip directly onto a smartphone camera while it recorded video to determine hemoglobin status. Analysis found that the blue spectra of plasma was the most important for protein composition, and therefore hemoglobin estimation. Classifiers were built to identify hemoglobin status as "normal" or "anemic" in the context of demographic averages. Optimal reference standard blood hemoglobin measurements (Masimo Pronto optical device) were used as ground-truth. Because camera-based assessment can exhibit bias with differences in skin color (see above), the authors carefully considered and reported the demographics of their study participants in addition to controlling for light absorption by skin tissue during experiments. A later paper devised a similar system, applying artificial neural networks (ANNs) and using only the smartphone's flash as a light source [65]. By combining frames within the videos, the researchers were able to identify regions with high variation in predicted levels.

The use of the conjunctiva as an ROI is gaining popularity due to the fact that it, like the fingernail bed and palmar creases, has no melanin and is devoid of "epidermis, dermis or subcutaneous fat which could impede the transmission of light" to deeper vascular layers [181]. This means the blood vessels are easier to analyze, and there may be less bias due to skin color. These properties have been leveraged by Suner et al. [181] to estimate hemoglobin concentration and screen for anemia using smartphone images of the conjunctiva. Spectral super-resolution (SSR) has been introduced to measure blood hemoglobin levels [142]. This method is based on a wealth of existing research reconstructing hyperspectral images from RGB signals. Statistical learning was applied to approximate a hyperspectral image of the conjunctiva from a simple smartphone camera image. Using the hyperspectral data, the hemoglobin content in blood can be computed more effectively than the RGB data alone. However, no analysis of skin color differences was conducted.

Techniques that use optical sensors benefit from the accessibility provided by the use of smartphones but their nutritional applications thus far are limited to macronutrients. They are also more susceptible to demographic biases in hardware, software, and datasets [120].

5 IMPLICATIONS FOR FUTURE MICRONUTRIENT STATUS ASSESSMENT METHODS

The state of the art reveals limitations that hinder the assessment of micronutrient status in individuals in the following ways: (1) the lack of *clinical relevance* in innovative approaches, (2) the absence of *comprehensive* assessment techniques, and (3) the deficiency of *accessible* and *noninvasive* methods. Future work could aim to address these issues while considering real-world integration into clinical and public health settings. Here, the requirements for medical diagnostic tests set by frameworks like REASSURED [101] are useful as they intersect with and expand beyond accessibility and non-invasiveness. REASSURED stands for Real-time connectivity, Ease of specimen collection, Affordable, Sensitive, Specific, User-friendly (including cultural acceptability), Rapid and robust, Equipment-free/Environmentally friendly, and Deliverable (i.e. accessible) to end-users [101]. First we briefly discuss how emerging methods align with these criteria, summarized in Table 10, then we discuss opportunities to address the aforementioned limitations, summarized in Table 11. We note that definitive conclusions remain challenging due to the early stage of many technologies.

5.1 Evaluating Emerging Methods

Assay-based methods like lateral flow immunoassays offer high sensitivity and specificity due to their targeted biomarker reactions but face issues with batch variability, limited range sensitivity, and environmental instability. Their performance can vary across sample types and demographics. While assay-based methods are familiar to clinicians, they may burden patients due to reliance on blood or urine samples.

Electrochemical methods are promising for PoC use due to speed and simplicity but can be affected by biofluid properties, electrode placement, and demographic differences. They often rely on less validated biosamples, limiting clinical adoption, and may require benchtop equipment, limiting practical deployment in limited resource settings.

Spectroscopy-based methods, such as LC-MS/MS, are the clinical optimal reference standard due to their high reproducibility, but they require complex lab equipment and protocols, limiting field use. Emerging on-body spectroscopic approaches could improve accessibility and cultural acceptability, especially when integrated with smartphones. However, research on noninvasive, accessible spectroscopy for in-body micronutrient assessment remains inadequate.

Biofluid analytic methods, including those applying AI/ML to physiological or multi-modal sensor data, may offer the highest patient acceptability by avoiding additional testing. While potentially fast and accurate, their effectiveness depends heavily on training data quality. Therefore, these approaches face challenges in reproducibility, bias, and scalability, especially across diverse and/or underserved populations. Collecting equitable, high-quality data for training is resource-intensive, and it is likely that effective models will have to be highly specific to the communities they serve, as opposed to being fully generalizable to micronutrient malnutrition globally.

Although emerging methods for accessible and noninvasive micronutrient status assessment have promising capabilities within this set of requirements, few of these approaches are currently standardized and ready for reproducible deployment across diverse geographic settings and clinical populations. The actionable future opportunities we propose in Table 11 aim to transition micronutrient status assessment from the laboratory to everyday use, enabling valuable micronutritional insights in a manner that is both easily accessible and noninvasive.

5.2 Future Opportunities to Address Limitations of Micronutrient Assessment

5.2.1 Clinically Relevant Innovations. We find that current innovative approaches to biofluid analysis for micronutrient status assessment lack clinical relevance (Sections 3.2 to 3.5). To overcome this limitation, future research could shift focus toward clinical relevance as a primary goal. This requires an interdisciplinary approach and

Table 10. Whether Methods Address Proposed and REASSURED [101] Criteria.

	Optimal Reference Standard	Assays				Electrochemical			Spectroscopic	Biofluid Analysis	
Criteria	-	Sweat Colorimetry	Multiplexed Sweat Sensors	Smartphone-based	Commercial	Voltammetry	Amperometry	Impedance	-	Single	Multiple
Clinical relevance	X	-	-	X	X	-	-	-	X	X	X
Comprehensive assessment	-	-	X	-	-	-	-	-	-	-	X
Noninvasive	-	X	X	-	-	X	X	X	-	X	X
Accessible	-	X	X	X	-	-	-	-	-	-	-
Reproducibility	X	-	-	-	X	-	-	-	X	-	-
Robustness	-	-	-	-	-	-	-	-	-	X	X
REASSURED											
Real-time connectivity	-	-	X	X	X	-	-	-	-	-	-
Ease of specimen collection	-	X	X	-	-	-	-	-	-	-	-
Affordable	-	X	X	X	-	X	X	X	-	-	-
Sensitive	X	-	-	X	X	-	-	-	X	-	-
Specific	X	-	-	X	X	-	-	-	X	-	-
User-friendly	-	X	X	-	-	-	-	-	-	X	X
Rapid	-	X	X	X	X	X	X	X	X	X	X
Equipment-free/ Environmentally friendly	-	X	X	-	-	-	-	-	-	-	-
Deliverable	-	X	X	X	-	-	-	-	-	-	-

recognition that technology is most effective when it complements clinical expertise, especially as micronutrition is inherently a clinical field where all solutions must be grounded in practical applications. Clinical, practical grounding can also be done by benchmarking new approaches against established optimal reference standards, detailed in Table 1. Comparative validation is essential to demonstrate a novel method's relevance and its potential as a viable substitute for the clinical optimal reference standard. We note that appropriate use of ELISA tests are sufficient in most cases.

We also find it is important for researchers to prioritize clinically relevant biofluids and ensure measurements reflect meaningful biomarker levels. For instance, reviewed papers claim to leverage sweat, yet sweat is an unreliable indicator of micronutrient status [12, 13]. Clinical relevance improves when methods align with the biomarkers used across the full spectrum of deficiency to excess. However, future research can also validate which biosamples and biomarkers may be suitable replacements for the optimal reference standard for specific

Table 11. Limitations of Existing Micronutrient Status Assessment Methods and Opportunities to Address Them.

Limitation	Opportunity
Limited clinical relevance	- Compare new approaches to the clinical optimal reference
	- Evaluate assessment performance in routinely-assessed patients
	- Adopt an interdisciplinary mindset when innovating
	- Understand and integrate clinically relevant biofluid samples
	- Measure clinically proven levels of circulating micronutrients
Lack of holistic and comprehensive approaches	- Employ precision nutrition by considering several types of data
	- Utilize multi-modal solutions
	- Gather many micronutrient statuses simultaneously
	- Collect data from diverse populations and make data available
Highly invasive and inaccessible	- Utilize commodity devices (smartphones, smartwatches) to collect data
	- Make designs open-source
	- Render insights actionable to non-experts
	- Bypass the need for biofluid samples by using wearables
	- Leverage less invasive biofluids such as urine

micronutrients, and in specific usage contexts. Since clinical validation can be challenging (Section 3.1), studies could apply new assessment methods in patient populations already monitored by clinicians, offering opportunities for collaboration and access to clinical reference data for validation. Targeting at-risk groups, like bariatric surgery candidates or individuals with diabetes, is especially useful as they are routinely assessed for nutritional status.

5.2.2 Comprehensive Approaches through Individualized and Multi-Modal Solutions. Micronutrition is complex and requires a holistic, individualized approach (Section 1). Advancing assessment technologies calls for precision nutrition and multi-modal sensing that account for differences in diet, metabolism, and lifestyle [94, 138, 175, 213]. While current methods often analyze biofluids (Section 3) or physiology (Section 4) separately, combining them with clinical data, like treatment history and anthropomorphic measurements, can uncover patterns and offer a more complete view of micronutritional status. Multi-modal sensing also enables simultaneous analysis of multiple micronutrients, essential due to their complex interactions [11]. (Tables 13, 14, 15). When single-device measurement is not feasible, future solutions could integrate multiple tools into a unified system.

We support calls for more micronutrition data [22], as access to individual nutrition profiles can accelerate progress toward precision assessment (Section 3.5). Innovative assessment devices and health sensors could be used in clinical studies to collect personalized micronutrient data over time. Valuable information includes demographics, clinical history, nutritional assessments, symptom images, biochemical results, and wearable data. Greater data availability would support deeper clinical investigations and aid early detection of imbalances. Disease etiology lies at the intersection of comprehensive clinical assessment, preventative care, and clinical

relevance. Etiology stresses the multifactorial (and deeply individual, social, and cultural) nature of disease progression [204]. Comprehensive assessments, and data thereof, can contribute to the etiological understanding of micronutrient malnutrition and ultimately drive precise prevention strategies.

AI and ML tools can help integrate these factors, uncovering latent patterns across a vast quantity and variety of micronutrition data [34]. We have discussed how micronutritional assessments are made more powerful when they are conducted and compared repeatedly over time. Accessible, multi-modal sensing would enable routine collection of health data relevant to micronutritional status, which can be combined and compared in real time, and *en masse*, by ML models. However, the computational and environmental costs of AI and ML models, especially large-scale generative AI models, must also be considered. It will also be essential to ensure demographic and cultural diversity to produce relevant and equitable models. Scalable and sustainable data collection through accessible PoC devices could be essential to achieving this.

5.2.3 Accessible and Noninvasive Point-of-Care Devices. This work highlights the critical need for accessible and noninvasive methods to assess micronutrient status. Such methods can generate valuable data though PoC assessments [94] and therefore improve the overall understanding of micronutrition. Current technologies fall short in accessibility, but potential solutions lie in leveraging commodity hardware, open-sourcing designs, prioritizing ease of use for non-experts, and utilizing less invasive biosamples. Smartphones and smartwatches, with built-in sensors, offer promising platforms for physiological insights. Their capabilities can be further expanded with features or accessories like electrochemical sensor chips or microfluidic pumps for biofluid analysis. As a result, multi-modal solutions with broad sensing and analysis capabilities will become more broadly available and low-cost.

Methods could be built considering some existing wearable devices. The Empatica E4 and Polar H10 offer high-fidelity HRV and autonomic data, which is associated vitamin B12 and iron status (Section 2.3) [57, 161]. Oura Ring, WHOOP, Fitbit, and Garmin devices track sleep, heart rate, and recovery metrics, which have been linked to nutrient-related fatigue and dysfunction [17, 68, 97, 123]. Additionally, Dexcom continuous glucose monitors, while focused on glucose, demonstrate the clinical viability of wearable biochemical sensing and may inform future designs for micronutrient monitoring [52]. While these tools vary in accuracy, especially across sensor types and populations, they highlight growing opportunities for accessible physiological monitoring relevant to micronutrition. Minimally invasive alternatives, such as urine testing (Section 2.2) instead of serum or plasma, can enhance accessibility. Even more promising are wearable, on-body sensing devices which use spectroscopy and ML to monitor micronutrients continuously without the need for biosamples [33, 124, 125, 201]. When care is taken to design innovative on-body, PoC assessments that are open-source and easy to use by laypeople (in addition to being accessible and noninvasive), comprehensive micronutrient status assessment can become viable for resource limited communities as well as individuals at home.

6 CONCLUSION

This article provides a comprehensive review of accessible and noninvasive methods for assessing in-body micronutrient status, focusing on biofluids (Section 3) and physiological (Section 4) approaches. We evlauate current techniques such as assays, electrochemistry, spectroscopy, optical sensors, and AI/ML for performance and clinical relevance. Key contributions include: (1) background on micronutrients for non-clinical audiences, (2) a synthesis of biofluid- and physiology-based methods, (3) future directions for noninvasive and accessible assessment, and (4) a unique focus on clinical applicability. Several summary tables provide an intuitive reference throughout.

This review synthesizes micronutrient status assessment methods based on biofluid and physiological analyses. Biofluid approaches benefit from established biomarkers but often rely on blood samples and lack clinical validation. Physiological assessments face challenges like weak symptom associations, self-report bias, and limited

micronutrient-specific research. From a clinical perspective, no current technology holistically integrates both types of analysis, despite this being standard in practice. Most research focuses on assessing a single micronutrient using either biofluid or physiological data alone. This review outlines three key opportunities to advance micronutrient status assessment: improving clinical relevance, adopting holistic multi-modal approaches, and reducing invasiveness to enhance accessibility (Table 11). Addressing these gaps through innovative, non-invasive, and individualized PoC solutions could empower individuals to better manage micronutrient malnutrition.

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REFERENCES

[1] 2019. Spectroscopy. https://www.pasco.com/products/guides/what-is-spectroscopy
[2] Zeweter Abebe, Gulelat Desse Haki, Florian J. Schweigert, Ina M. Henkel, and Kaleab Baye. 2019. Low breastmilk vitamin A concentration is prevalent in rural Ethiopia. *European Journal of Clinical Nutrition* 73, 8 (Aug. 2019), 1110–1116. doi:10.1038/s41430-018-0334-4
[3] Ada's Medical Knowledge Team. 2023. Vitamin B12 Deficiency: Signs, Causes and Treatments. https://ada.com/conditions/vitamin-b12-deficiency/
[4] Peter J. Aggett. 2012. Iron. In *Present knowledge in nutrition.* John Wiley & Sons.
[5] K. Albrecht, J. Lotz, L. Frommer, K. J. Lackner, and G. J. Kahaly. 2021. A rapid point-of-care assay accurately measures vitamin D. *Journal of Endocrinological Investigation* 44, 11 (Nov. 2021), 2485–2492. doi:10.1007/s40618-021-01575-8
[6] Evan Austin, Amaris N. Geisler, Julie Nguyen, Indermeet Kohli, Iltefat Hamzavi, Henry W. Lim, and Jared Jagdeo. 2021. Visible Light Part I. Properties and Cutaneous Effects of Visible Light. *Journal of the American Academy of Dermatology* 84, 5 (May 2021), 1219–1231. doi:10.1016/j.jaad.2021.02.048
[7] Kudret Aytemir, Serdar Aksoyek, Yahya Buyukasik, Ibrahim Haznedarogclu, Enver Alar, Necla Ozer, Kenan Ovunc, Ferhan Ozmen, and Ali Oto. 2000. Assessment of Autonomic Nervous System Functions in Patients with Vitamin B12 Deficiency by Power Spectral Analysis of Heart Rate Variability. *Pacing and Clinical Electrophysiology* 23, 6 (June 2000), 975–978. doi:10.1111/j.1540-8159.2000.tb00883.x
[8] Domenico Azzolino, Beatrice Arosio, Emanuele Marzetti, Riccardo Calvani, and Matteo Cesari. 2020. Nutritional Status as a Mediator of Fatigue and Its Underlying Mechanisms in Older People. *Nutrients* 12, 2 (Feb. 2020), 444. doi:10.3390/nu12020444
[9] Lokesh B, Vidhya Rathnavelu, Mythili Sabesan, Akila Ganesh, and Soumya Anandan. 2021. A Study to Assess the Levels of Salivary Ferritin in Iron Deficiency Anemia Subjects and Healthy Subjects. *Cureus* 13 (08 2021). doi:10.7759/cureus.17241
[10] Ayla Bahramian, Parisa Falsafi, Teymour Abbasi, Milad Ghanizadeh, Mehdi Abedini, Fatemeh Kavoosi, Maryam Kouhsoltani, Fatemeh Noorbakhsh, Fatemeh Dabbaghi Tabriz, Shahin Rajaeih, and Farzad Rezaei. 2018. Comparing Serum and Salivary Levels of Vitamin D in Patients with Recurrent Aphthous Stomatitis and Healthy Individuals. *Journal of Dentistry* 19, 4 (Dec. 2018), 295–300. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6338692/
[11] Regan L. Bailey, Keith P. West Jr., and Robert E. Black. 2015. The Epidemiology of Global Micronutrient Deficiencies. *Annals of Nutrition and Metabolism* 66, Suppl. 2 (2015), 22–33. doi:10.1159/000371618
[12] Lindsay B. Baker. 2019. Physiology of sweat gland function: The roles of sweating and sweat composition in human health. *Temperature* 6, 3 (July 2019), 211–259. doi:10.1080/23328940.2019.1632145
[13] Lindsay B. Baker and Anthony S. Wolfe. 2020. Physiological mechanisms determining eccrine sweat composition. *European Journal of Applied Physiology* 120, 4 (April 2020), 719–752. doi:10.1007/s00421-020-04323-7
[14] Varun Bali, Yugal Khajuria, Vidit Maniyar, Pradeep K. Rai, Upendra Kumar, Charles Ghany, M.A. Gondal, and Vivek K. Singh. 2023. Quantitative analysis of human hairs and nails. *Biophysical Reviews* 15, 3 (June 2023), 401–417. doi:10.1007/s12551-023-01069-2
[15] Mallika Bariya, Hnin Yin Yin Nyein, and Ali Javey. 2018. Wearable sweat sensors. *Nature Electronics* 1, 3 (March 2018), 160–171. doi:10.1038/s41928-018-0043-y
[16] Markus Beitzke, Peter Pfister, Jürgen Fortin, and Falko Skrabal. 2002. Autonomic dysfunction and hemodynamics in vitamin B12 deficiency. *Autonomic Neuroscience* 97, 1 (April 2002), 45–54. doi:10.1016/S1566-0702(01)00393-9
[17] Simone Benedetto, Christian Caldato, Elia Bazzan, Darren C Greenwood, Virginia Pensabene, and Paolo Actis. 2018. Assessment of the Fitbit Charge 2 for monitoring heart rate. *PLOS ONE* 13, 2 (2018), e0192691. doi:10.1371/journal.pone.0192691
[18] Mette M. Berger, Alan Shenkin, Anna Schweinlin, Karin Amrein, Marc Augsburger, Hans-Konrad Biesalski, Stephan C. Bischoff, Michael P. Casaer, Kursat Gundogan, Hanna-Liis Lepp, Angélique M.E. De Man, Giovanna Muscogiuri, Magdalena Pietka, Loris Pironi, Serge Rezzi, and Cristina Cuerda. 2022. ESPEN micronutrient guideline. *Clinical Nutrition* 41, 6 (June 2022), 1357–1424. doi:10.1016/j.clnu.2022.02.015

[19] May A. Beydoun, Alyssa A. Gamaldo, Jose A. Canas, Hind A. Beydoun, Mauli T. Shah, Jessica M. McNeely, and Alan B. Zonderman. 2014. Serum Nutritional Biomarkers and Their Associations with Sleep among US Adults in Recent National Surveys. *PLoS ONE* 9, 8 (Aug. 2014), e103490. doi:10.1371/journal.pone.0103490

[20] Hongyan Bi, Carla M. Duarte, Marina Brito, Vânia Vilas-Boas, Susana Cardoso, and Paulo Freitas. 2016. Performance enhanced UV/vis spectroscopic microfluidic sensor for ascorbic acid quantification in human blood. *Biosensors and Bioelectronics* 85 (Nov. 2016), 568–572. doi:10.1016/j.bios.2016.05.054

[21] K. Biesalski Hans and Tinz Jana. 2018. Micronutrients in the life cycle: Requirements and sufficient supply. *NFS Journal* 11 (June 2018), 1–11. doi:10.1016/j.nfs.2018.03.001

[22] Kenneth H Brown, Sophie E Moore, Sonja Y Hess, Christine M McDonald, Kerry S Jones, Sarah R Meadows, Mari S Manger, Jennifer Coates, Silvia Alayon, and Saskia Jm Osendarp. 2021. Increasing the availability and utilization of reliable data on population micronutrient (MN) status globally: the MN Data Generation Initiative. *The American Journal of Clinical Nutrition* 114, 3 (Sept. 2021), 862–870. doi:10.1093/ajcn/nqab173

[23] Maaike J Bruins, Roland Kupka, Michael B Zimmermann, Georg Lietz, Reina Engle-Stone, and Klaus Kraemer. 2016. Maximizing the benefits and minimizing the risks of intervention programs to address micronutrient malnutrition: symposium report. *Maternal & Child Nutrition* 12, 4 (Oct. 2016), 940–948. doi:10.1111/mcn.12334

[24] Maaike J. Bruins, Gladys Mugambi, Janneke Verkaik-Kloosterman, Jeljer Hoekstra, Klaus Kraemer, Saskia Osendarp, Alida Melse-Boonstra, Alison M. Gallagher, and Hans Verhagen. 2015. Addressing the risk of inadequate and excessive micronutrient intakes: traditional versus new approaches to setting adequate and safe micronutrient levels in foods. *Food & Nutrition Research* 59 (Jan. 2015), 10.3402/fnr.v59.26020. doi:10.3402/fnr.v59.26020

[25] Alice Callahan, Heather Leonard, and Tamberly Powell. 2020. Classification of Vitamins and Minerals. In *Nutrition: Science and Everyday Application, v. 1.0.* Open Oregon Educational Resources. https://openoregon.pressbooks.pub/nutritionscience/chapter/8a-classification-vitamins-minerals/ Book Title: Nutrition: Science and Everyday Application, v. 1.0.

[26] Susana Campuzano, Rodrigo Barderas, Maria Teresa Moreno-Casbas, Ángeles Almeida, and José M. Pingarrón. 2024. Pursuing precision in medicine and nutrition: the rise of electrochemical biosensing at the molecular level. *Analytical and Bioanalytical Chemistry* 416, 9 (April 2024), 2151–2172. doi:10.1007/s00216-023-04805-5

[27] Duran Canatan and Sevgi Kosaci Akdeniz. 2012. Iron and Ferritin Levels in Saliva of Patients with Thalassemia and Iron Deficiency Anemia. *Mediterranean Journal of Hematology and Infectious Diseases* 4, 1 (2012), e2012051. doi:10.4084/MJHID.2012.051

[28] Chad Haldeman-Englert, Raymond Turley Jr, and Tara Novick. [n. d.]. Iron and Total Iron-Binding Capacity. https://www.urmc.rochester.edu/encyclopedia/content.aspx?contenttypeid=167&contentid=iron_total_iron_binding_capacity

[29] Chemyx. 2017. Basic Principles of HPLC, MS & LC-MS. https://chemyx.com/support/knowledge-base/application-reference-by-topic/basic-principles-hplc-ms-lc-ms/

[30] Yoan Cherasse and Yoshihiro Urade. 2017. Dietary Zinc Acts as a Sleep Modulator. *International Journal of Molecular Sciences* 18, 11 (Nov. 2017), 2334. doi:10.3390/ijms18112334

[31] Hui-Fang Chiu, Kamesh Venkatakrishnan, Oksana Golovinskaia, and Chin-Kun Wang. 2021. Impact of Micronutrients on Hypertension: Evidence from Clinical Trials with a Special Focus on Meta-Analysis. *Nutrients* 13, 2 (Feb. 2021), 588. doi:10.3390/nu13020588

[32] James Collier. 2010. Storage of Micronutrients in the Body. https://www.dietetics.co.uk/micronutrient-storage.aspx

[33] Richard A. Crocombe. 2018. Portable Spectroscopy. *Applied Spectroscopy* 72, 12 (Dec. 2018), 1701–1751. doi:10.1177/0003702818809719

[34] Mélina Côté and Benoît Lamarche. 2022. Artificial intelligence in nutrition research: perspectives on current and future applications. *Applied Physiology, Nutrition, and Metabolism* 47, 1 (Jan. 2022), 1–8. doi:10.1139/apnm-2021-0448

[35] Dipankar De and Divya Seshadri. 2012. Nails in nutritional deficiencies. *Indian Journal of Dermatology, Venereology, and Leprology* 78, 3 (2012), 237. doi:10.4103/0378-6323.95437

[36] Bloom Diagnostics. 2021. Overview - Bloom Diagnostics. https://drive.google.com/file/d/19SXNAl_vvPfVa9XVU5Ety-EnDtVjnAlT/view?usp=sharing

[37] Michelle DiBaise and Sherry M. Tarleton. 2019. Hair, Nails, and Skin: Differentiating Cutaneous Manifestations of Micronutrient Deficiency. *Nutrition in Clinical Practice* 34, 4 (Aug. 2019), 490–503. doi:10.1002/ncp.10321

[38] Ni Dongdong and Daniel Cozzolino. 2023. Possibilities on the application of vibrational spectroscopy and data analytics in precision nutrition. *TrAC Trends in Analytical Chemistry* 163 (June 2023), 117067. doi:10.1016/j.trac.2023.117067

[39] John D'Orazio, Stuart Jarrett, Alexandra Amaro-Ortiz, and Timothy Scott. 2013. UV Radiation and the Skin. *International Journal of Molecular Sciences* 14, 6 (June 2013), 12222–12248. doi:10.3390/ijms140612222

[40] Silvia Dortez, Agustín G. Crevillen, and Alberto Escarpa. 2023. Integrated calibration and serum iron *in situ* analysis into an array microfluidic paper-based analytical device with smartphone readout. *Talanta* 253 (Feb. 2023), 123914. doi:10.1016/j.talanta.2022.123914

[41] Victoria Drake. 2018. Micronutrient Inadequacies in the US Population: an Overview. https://lpi.oregonstate.edu/mic/micronutrient-inadequacies/overview

[42] Ibrahim Elmadfa and Alexa L. Meyer. 2014. Developing suitable methods of nutritional status assessment: a continuous challenge. *Advances in Nutrition (Bethesda, Md.)* 5, 5 (Sept. 2014), 590S–598S. doi:10.3945/an.113.005330

[43] Wei Kay Eng, David Giraud, Vicki L. Schlegel, Dong Wang, Bo Hyun Lee, and Janos Zempleni. 2013. Identification and assessment of markers of biotin status in healthy adults. *The British Journal of Nutrition* 110, 2 (July 2013), 321–329. doi:10.1017/S0007114512005065

[44] Dema Halasa Esper. 2015. Utilization of Nutrition-Focused Physical Assessment in Identifying Micronutrient Deficiencies. *Nutrition in Clinical Practice* 30, 2 (April 2015), 194–202. doi:10.1177/0884533615573054

[45] Santiago Espinosa-Salas and Mauricio Gonzalez-Arias. 2024. Nutrition: Micronutrient Intake, Imbalances, and Interventions. In *StatPearls*. StatPearls Publishing, Treasure Island (FL). http://www.ncbi.nlm.nih.gov/books/NBK597352/

[46] Francisca T. S. M. Ferreira, Karina A. Catalão, Raquel B. R. Mesquita, and António O. S. S. Rangel. 2021. New microfluidic paper-based analytical device for iron determination in urine samples. *Analytical and Bioanalytical Chemistry* 413, 30 (Dec. 2021), 7463–7472. doi:10.1007/s00216-021-03706-9

[47] Joanna Fiddler, Michael McBurney, and Jere Haas. 2023. Using Capillary Whole Blood to Quantitatively Measure Ferritin: A Validation Trial of a Point-of-Care System. *Nutrients* 15 (03 2023), 1305. doi:10.3390/nu15061305

[48] Rostika Flora, Mohammad Zulkarnain, Nur Alam Fajar, Indah Yuliana, Risnawati Tanjung, Helfi Nolia, Sulaiman Sulaiman, and Aguscik Aguscik. 2021. Saliva Iron Levels to Assess Iron Status in Children. *Open Access Macedonian Journal of Medical Sciences* 9, E (2021), 843–846. doi:10.3889/oamjms.2021.6770

[49] The Institute for Functional Medicine. 2020. Micronutrient Malnutrition: Not Limited to Developing Nations. https://www.ifm.org/news-insights/micronutrient-malnutrition-not-limited-to-developing-nations/

[50] Wei Gao, Hnin Y. Y. Nyein, Ziba Shahpar, Hossain M. Fahad, Kevin Chen, Sam Emaminejad, Yuji Gao, Li-Chia Tai, Hiroki Ota, Eric Wu, James Bullock, Yuping Zeng, Der-Hsien Lien, and Ali Javey. 2016. Wearable Microsensor Array for Multiplexed Heavy Metal Monitoring of Body Fluids. *ACS Sensors* 1, 7 (July 2016), 866–874. doi:10.1021/acssensors.6b00287 Publisher: American Chemical Society.

[51] Mayank Garg, Martin Gedsted Christensen, Alexander Iles, Amit L. Sharma, Suman Singh, and Nicole Pamme. 2020. Microfluidic-Based Electrochemical Immunosensing of Ferritin. *Biosensors* 10, 8 (Aug. 2020), 91. doi:10.3390/bios10080091 Number: 8 Publisher: Multidisciplinary Digital Publishing Institute.

[52] Satish K. Garg, Mark Kipnes, Kristin Castorino, Timothy S. Bailey, Halis Kaan Akturk, John B. Welsh, Mark P. Christiansen, Andrew K. Balo, Sue A. Brown, Jennifer L. Reid, and Stayce E. Beck. 2022. Accuracy and Safety of Dexcom G7 Continuous Glucose Monitoring in Adults with Diabetes. *Diabetes Technology & Therapeutics* 24, 6 (2022), 373–380. doi:10.1089/dia.2022.0011

[53] Amr Gawaly, AbdA.Y El-Naby, and Ghada Alghazaly. 2020. Serum and salivary ferritin levels in iron-deficiency anemia - is there a difference? *The Egyptian Journal of Haematology* 45 (01 2020), 156. doi:10.4103/ejh.ejh_23_20

[54] Michael K. Georgieff. 2007. Nutrition and the developing brain: nutrient priorities and measurement. *The American Journal of Clinical Nutrition* 85, 2 (Feb. 2007), 614S–620S. doi:10.1093/ajcn/85.2.614S

[55] Gloria F. Gerber. 2024. Iron Deficiency Anemia. https://www.merckmanuals.com/home/blood-disorders/anemia/iron-deficiency-anemia

[56] Alison D. Gernand. 2019. The upper level: examining the risk of excess micronutrient intake in pregnancy from antenatal supplements. *Annals of the New York Academy of Sciences* 1444, 1 (2019), 22–34. doi:10.1111/nyas.14103 _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/nyas.14103.

[57] David Giles, Nick Draper, and William Neil. 2016. Validity of the Polar V800 heart rate monitor to measure RR intervals at rest. *European Journal of Applied Physiology* 116, 3 (2016), 563–571. doi:10.1007/s00421-015-3303-9

[58] Heather Gloria. no date. Iron Deficiency and Heart Rate. https://www.livestrong.com/article/322034-iron-deficiency-and-heart-rate/

[59] Michael J. Gonzalez, Jorge R. Miranda-Massari, Jorge Duconge, Jose A. Olalde, and Andrew W. Saul. 2023. New Concepts for Understanding Nutrient Pharmacokinetics: Nutri-kinetics and Nutri-dynamics. *Journal of Orthomolecular Medicine* 38, 1 (2023), 13–24. https://www.researchgate.net/publication/369033898_New_Concepts_for_Understanding_Nutrient_Pharmacokinetics_Nutri-kinetics_and_Nutri-dynamics

[60] Federico Guede-Fernández, Víctor Ferrer-Mileo, Marc Mateu-Mateus, Juan Ramos-Castro, Miguel Ángel García-González, and Mireya Fernández-Chimeno. 2020. A photoplethysmography smartphone-based method for heart rate variability assessment: device model and breathing influences. *Biomedical Signal Processing and Control* 57 (March 2020), 101717. doi:10.1016/j.bspc.2019.101717

[61] Fatemeh Haji-Sattari, Mahdieh Zarabadipour, Fatemeh Hajmanoochehri, and Monirsadat Mirzadeh. 2019. Salivary and Serum Ferritin Levels: Is There a Correlation? *Journal of Islamic Dental Association of IRAN* 31 (10 2019), 214–217. doi:10.30699/jidai.31.4.214

[62] Tae Hwan Han, Jin Lee, and Yong Joo Kim. 2016. Hair Zinc Level Analysis and Correlative Micronutrients in Children Presenting with Malnutrition and Poor Growth. *Pediatric Gastroenterology, Hepatology & Nutrition* 19, 4 (2016), 259. doi:10.5223/pghn.2016.19.4.259

[63] David Harvey. 2024. 11.4: Voltammetric and Amperometric Methods. https://chem.libretexts.org/Bookshelves/Analytical_Chemistry/Analytical_Chemistry_2.1_(Harvey)/11%3A_Electrochemical_Methods/11.04%3A_Voltammetric_and_Amperometric_Methods

[64] Md Kamrul Hasan, Md Hasanul Aziz, Md Ishrak Islam Zarif, Mahmudul Hasan, Mma Hashem, Shion Guha, Richard R Love, and Sheikh Ahamed. 2021. Noninvasive Hemoglobin Level Prediction in a Mobile Phone Environment: State of the Art Review and Recommendations. *JMIR mHealth and uHealth* 9, 4 (April 2021), e16806. doi:10.2196/16806

[65] Md Kamrul Hasan, Md Munirul Haque, Riddhiman Adib, Jannatul F. Tumpa, Azima Begum, Richard R. Love, Young L. Kim, and I. Ahamed Sheikh. 2018. SmartHeLP: Smartphone-based Hemoglobin Level Prediction Using an Artificial Neural Network. *AMIA ... Annual Symposium proceedings. AMIA Symposium* 2018 (2018), 535–544.

[66] Jason Heikenfeld, Wei Gao, Ali Javey, Wei Wei, Koji Sode, and Joseph Wang. 2023. Diving into Sweat: Advances, Challenges, and Future Directions in Epidermal Biosensing. *ACS Nano* 17, 9 (2023), 8353–8368. doi:10.1021/acsnano.4c10344

[67] Kristen Heitman. 2022. Micronutrients vs. macronutrients, and where to find them. https://health.osu.edu/wellness/exercise-and-nutrition/micronutrients-vs-macronutrients

[68] André Henriksen, Frode Svartdal, Sameline Grimsgaard, Gunnar Hartvigsen, and Laila Arnesdatter Hopstock. 2022. Polar Vantage and Oura Physical Activity and Sleep Trackers: Validation and Comparison Study. *JMIR Formative Research* 6, 5 (2022), e27248. doi:10.2196/27248

[69] Jin-Chul Heo, Doyoon Kim, Hyunsoo An, Chang-Sik Son, Sangwoo Cho, and Jong-Ha Lee. 2021. A Novel Biosensor and Algorithm to Predict Vitamin D Status by Measuring Skin Impedance. *Sensors* 21, 23 (Dec. 2021), 8118. doi:10.3390/s21238118

[70] Bonnie D. Hodge, Terrence Sanvictores, and Robert T. Brodell. 2022. *Anatomy, Skin Sweat Glands*. StatPearls Publishing, Treasure Island (FL). https://www.ncbi.nlm.nih.gov/books/NBK482278/

[71] Lei Huang, Shulin Tian, Wenhao Zhao, Ke Liu, and Jinhong Guo. 2021. Electrochemical vitamin sensors: A critical review. *Talanta* 222 (Jan. 2021), 121645. doi:10.1016/j.talanta.2020.121645

[72] Samantha L. Huey, Jesse T. Krisher, David Morgan, Penjani Mkambula, Bryan M. Gannon, Mduduzi N. N. Mbuya, and Saurabh Mehta. 2022. A review of portable quantitative and semi-quantitative devices for measurement of vitamin A in biological samples. *Current Research in Biotechnology* 4 (Jan. 2022), 253–274. doi:10.1016/j.crbiot.2022.04.003

[73] A. Christine Hummell and Michael Cummings. 2022. Role of the nutrition-focused physical examination in identifying malnutrition and its effectiveness. *Nutrition in Clinical Practice* 37, 1 (Feb. 2022), 41–49. doi:10.1002/ncp.10797

[74] Ulrich Höller, Stephan J.L. Bakker, Andre Düsterloh, Balz Frei, Josef Köhrle, Tobias Konz, Georg Lietz, Adrian McCann, Alexander J. Michels, Anne M. Molloy, Hitoshi Murakami, Dietrich Rein, Wim H.M. Saris, Karlheinz Schmidt, Kazutaka Shimbo, Soeren Schumacher, Cees Vermeer, Jim Kaput, Peter Weber, Manfred Eggersdorfer, and Serge Rezzi. 2018. Micronutrient status assessment in humans: Current methods of analysis and future trends. *TrAC Trends in Analytical Chemistry* 102 (May 2018), 110–122. doi:10.1016/j.trac.2018.02.001

[75] Institute of Medicine (US) Subcommittee on Interpretation and Uses of Dietary Reference Intakes and Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. 2000. Using the Adequate Intake for Nutrient Assessment of Groups. In *DRI Dietary Reference Intakes: Applications in Dietary Assessment*. National Academies Press (US). https://www.ncbi.nlm.nih.gov/books/NBK222886/

[76] Muhammad Umer Ishaq, Digbijay Kunwar, Abdul Qadeer, Aqsa Komel, Adnan Safi, Aqsa Malik, Linta Malik, and Anum Akbar. 2024. Effect of vitamin A on maternal, fetal, and neonatal outcomes: An overview of deficiency, excessive intake, and intake recommendations. *Nutrition in Clinical Practice* 39, 2 (April 2024), 373–384. doi:10.1002/ncp.11096

[77] Haitham Jahrami, Eman Alekri, Ahmed S. BaHammam, Ali Alsalman, Nicola Luigi Bragazzi, Omar Alhaj, and Zahra Saif. 2021. The association between micronutrient status and sleep quality in patients with depression: a case-control study. *Sleep and Breathing* 25, 3 (Sept. 2021), 1571–1579. doi:10.1007/s11325-020-02228-5

[78] Xiaopeng Ji. 2017. *Serum Micronutrient Status, Sleep And Neurobehavioral Function In Early Adolescents: A Cohort Study*. Ph. D. Dissertation. University of Pennsylvania. https://repository.upenn.edu/handle/20.500.14332/29281 Issue: 2370.

[79] Xiaopeng Ji, Michael A Grandner, and Jianghong Liu. 2017. The relationship between micronutrient status and sleep patterns: a systematic review. *Public Health Nutrition* 20, 4 (March 2017), 687–701. doi:10.1017/S1368980016002603

[80] Aniruddha Jibhkate and Richa Lath. 2019. Assessing severity of involvement of autonomic functions in iron-deficiency anemia patients. *National Journal of Physiology, Pharmacy and Pharmacology* 0 (2019), 1. doi:10.5455/njppp.2019.9.0307912032019

[81] Seongbin Jo, Daeun Sung, Sungbong Kim, and Jahyun Koo. 2021. A review of wearable biosensors for sweat analysis. *Biomedical Engineering Letters* 11, 2 (May 2021), 117–129. doi:10.1007/s13534-021-00191-y

[82] Preetha Jothimuthu, Robert A. Wilson, Josi Herren, Xing Pei, Wenjing Kang, Rodney Daniels, Hector Wong, Fred Beyette, William R. Heineman, and Ian Papautsky. 2013. Zinc Detection in Serum by Anodic Stripping Voltammetry on Microfabricated Bismuth Electrodes. *Electroanalysis* 25, 2 (2013), 401–407. doi:10.1002/elan.201200530 _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/elan.201200530.

[83] Hrishikesh Kalita, Mahima Kumari, Mayank Bhushan, Debananda Mohapatra, and Laishram Robindro Singh. 2022. Point-of-Care Nanobiosensors for Determining Vitamin Deficiency. In *Nanobiosensors for point-of-care medical diagnostics*, Manashjit Gogoi, Sanjukta Patra, and Debasree Kundu (Eds.). Springer Nature, Singapore, 173–193. doi:10.1007/978-981-19-5141-1_8

[84] Sivachandar Kasiviswanathan, Thulasi Bai Vijayan, Lorenzo Simone, and Giovanni Dimauro. 2020. Semantic Segmentation of Conjunctiva Region for Non-Invasive Anemia Detection Applications. *Electronics* 9, 8 (Aug. 2020), 1309. doi:10.3390/electronics9081309

[85] Amy K Keir, Dominic Wilkinson, Chad Andersen, and Michael J Stark. 2016. Washed versus unwashed red blood cells for transfusion for the prevention of morbidity and mortality in preterm infants. *Cochrane Database of Systematic Reviews* 2016, 1 (Jan. 2016). doi:10.1002/14651858.CD011484.pub2

[86] Jahidur Rahman Khan, Srizan Chowdhury, Humayera Islam, and Enayetur Raheem. 2021. Machine Learning Algorithms To Predict The Childhood Anemia In Bangladesh. *Journal of Data Science* 17, 1 (Feb. 2021), 195–218. doi:10.6339/JDS.201901_17(1).0009

[87] Jayoung Kim, William R. De Araujo, Izabela A. Samek, Amay J. Bandodkar, Wenzhao Jia, Barbara Brunetti, Thiago R.L.C. Paixão, and Joseph Wang. 2015. Wearable temporary tattoo sensor for real-time trace metal monitoring in human sweat. *Electrochemistry Communications* 51 (Feb. 2015), 41–45. doi:10.1016/j.elecom.2014.11.024

[88] Joohee Kim, Yixin Wu, Haiwen Luan, Da Som Yang, Donghwi Cho, Sung Soo Kwak, Shanliangzi Liu, Hanjun Ryu, Roozbeh Ghaffari, and John A. Rogers. 2022. A Skin-Interfaced, Miniaturized Microfluidic Analysis and Delivery System for Colorimetric Measurements of Nutrients in Sweat and Supply of Vitamins Through the Skin. *Advanced Science* 9, 2 (Jan. 2022), 2103331. doi:10.1002/advs.202103331

[89] Sungbong Kim, Boram Lee, Jonathan T. Reeder, Seon Hee Seo, Sung-Uk Lee, Aurélie Hourlier-Fargette, Joonchul Shin, Yurina Sekine, Hyoyoung Jeong, Yong Suk Oh, Alexander J. Aranyosi, Stephen P. Lee, Jeffrey B. Model, Geumbee Lee, Min-Ho Seo, Sung Soo Kwak, Seongbin Jo, Gyungmin Park, Sunghyun Han, Inkyu Park, Hyo-Il Jung, Roozbeh Ghaffari, Jahyun Koo, Paul V. Braun, and John A. Rogers. 2020. Soft, skin-interfaced microfluidic systems with integrated immunoassays, fluorometric sensors, and impedance measurement capabilities. *Proceedings of the National Academy of Sciences* 117, 45 (Nov. 2020), 27906–27915. doi:10.1073/pnas.2012700117

[90] Tae Won Kim, Jong-Hyun Jeong, and Seung-Chul Hong. 2015. The Impact of Sleep and Circadian Disturbance on Hormones and Metabolism. *International Journal of Endocrinology* 2015, 1 (2015), 591729. doi:10.1155/2015/591729 _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1155/2015/591729.

[91] Rachel C. King, Emma Villeneuve, Ruth J. White, R. Simon Sherratt, William Holderbaum, and William S. Harwin. 2017. Application of data fusion techniques and technologies for wearable health monitoring. *Medical Engineering & Physics* 42 (April 2017), 1–12. doi:10.1016/j.medengphy.2016.12.011

[92] Tricia Kinman. 2012. ELISA: Purpose, Procedure, and Results. https://www.healthline.com/health/elisa

[93] Mervenur Kirazoğlu and Birgul Benli. 2023. Recent Point of Care (PoC) Electrochemical Testing Trends of New Diagnostics Platforms for Vitamin D. *ChemistrySelect* (2023). https://api.semanticscholar.org/CorpusID:264066934

[94] Daniel Kirk, Cagatay Catal, and Bedir Tekinerdogan. 2021. Precision nutrition: A systematic literature review. *Computers in Biology and Medicine* 133 (June 2021), 104365. doi:10.1016/j.compbiomed.2021.104365

[95] L. Klous, C. de Ruiter, P. Alkemade, H. Daanen, and N. Gerrett. 2020. Sweat rate and sweat composition following active or passive heat re-acclimation: A pilot study. *Temperature (Austin)* 8, 1 (October 11 2020), 90–104. doi:10.1080/23328940.2020.1826287

[96] Daniel Koerber, Shawn Khan, Tahmina Shamsheri, Abirami Kirubarajan, and Sangeeta Mehta. 2023. Accuracy of Heart Rate Measurement with Wrist-Worn Wearable Devices in Various Skin Tones: a Systematic Review. *Journal of Racial and Ethnic Health Disparities* 10, 6 (Dec. 2023), 2676–2684. doi:10.1007/s40615-022-01446-9

[97] Bhanu Prakash Kolla, Subir Mansukhani, and Meghna P. Mansukhani. 2020. Consumer sleep tracking devices: A review of mechanisms, validity, and utility. *Expert Review of Medical Devices* 17, 7 (2020), 723–731. doi:10.1080/17434440.2020.1782214

[98] Tian Kong, Jae Bem You, Biao Zhang, Brian Nguyen, Farhang Tarlan, Keith Jarvi, and David Sinton. 2019. Accessory-free quantitative smartphone imaging of colorimetric paper-based assays. *Lab on a Chip* 19, 11 (2019), 1991–1999. doi:10.1039/C9LC00165D

[99] Devender Kumar, Steven Jeuris, Jakob E. Bardram, and Nicola Dragoni. 2021. Mobile and Wearable Sensing Frameworks for mHealth Studies and Applications: A Systematic Review. *ACM Transactions on Computing for Healthcare* 2, 1 (Jan. 2021), 1–28. doi:10.1145/3422158

[100] Steef Kurstjens, Thomas De Bel, Armando Van Der Horst, Ron Kusters, Johannes Krabbe, and Jasmijn Van Balveren. 2022. Automated prediction of low ferritin concentrations using a machine learning algorithm. *Clinical Chemistry and Laboratory Medicine (CCLM)* 60, 12 (Nov. 2022), 1921–1928. doi:10.1515/cclm-2021-1194

[101] Kevin J. Land, Debrah I. Boeras, Xiang-Sheng Chen, Andrew R. Ramsay, and Rosanna W. Peeling. 2019. REASSURED diagnostics to inform disease control strategies, strengthen health systems and improve patient outcomes. *Nature Microbiology* 4, 1 (Jan. 2019), 46–54. doi:10.1038/s41564-018-0295-3 Publisher: Nature Publishing Group.

[102] Stephen Lee, A. J. Aranyosi, Michelle D. Wong, Ji Hyung Hong, Jared Lowe, Carol Chan, David Garlock, Scott Shaw, Patrick D. Beattie, Zachary Kratochvil, Nick Kubasti, Kirsten Seagers, Roozbeh Ghaffari, and Christina D. Swanson. 2016. Flexible opto-electronics enabled microfluidics systems with cloud connectivity for point-of-care micronutrient analysis. *Biosensors and Bioelectronics* 78 (April 2016), 290–299. doi:10.1016/j.bios.2015.11.060

[103] Seoho Lee, Vlad Oncescu, Matt Mancuso, Saurabh Mehta, and David Erickson. 2014. A smartphone platform for the quantification of vitamin D levels. *Lab Chip* 14, 8 (2014), 1437–1442. doi:10.1039/C3LC51375K

[104] Seoho Lee, Dakota O'Dell, Jess Hohenstein, Susannah Colt, Saurabh Mehta, and David Erickson. 2016. NutriPhone: a mobile platform for low-cost point-of-care quantification of vitamin B12 concentrations. *Scientific Reports* 6, 1 (June 2016), 28237. doi:10.1038/srep28237

[105] Wayne Leung, Ishmeet Singh, Scout McWilliams, Sylvia Stockler, and Osman S. Ipsiroglu. 2020. Iron deficiency and sleep – A scoping review. *Sleep Medicine Reviews* 51 (June 2020), 101274. doi:10.1016/j.smrv.2020.101274

[106] Hao Liang, Songye Wu, Duo Yang, Jianhua Huang, Xiaolei Yao, Jingbo Gong, Zhixing Qing, Lijuan Tao, and Qinghua Peng. 2023. Non-targeted Metabolomics Analysis Reveals Distinct Metabolic Profiles Between Positive and Negative Emotional Tears of Humans: A Preliminary Study. *Cureus* 15, 8 (Aug 2023), e42985. doi:10.7759/cureus.42985

[107] Binbin Liu, Zhe Zhang, Xiaohui Di, Xiaoni Wang, Lin Xie, Wenjun Xie, and Jianbao Zhang. 2021. The Assessment of Autonomic Nervous System Activity Based on Photoplethysmography in Healthy Young Men. *Frontiers in Physiology* 12 (Sept. 2021), 733264. doi:10.3389/fphys.2021.733264

[108] Xin Liu, Girish Narayanswamy, Akshay Paruchuri, Xiaoyu Zhang, Jiankai Tang, Yuzhe Zhang, Yuntao Wang, Soumyadip Sengupta, Shwetak Patel, and Daniel McDuff. 2023. rPPG-Toolbox: Deep Remote PPG Toolbox. http://arxiv.org/abs/2210.00716 arXiv:2210.00716 [cs].

[109] Francesca Lockyer, Samantha McCann, and Sophie E. Moore. 2021. Breast Milk Micronutrients and Infant Neurodevelopmental Outcomes: A Systematic Review. *Nutrients* 13, 11 (Oct. 2021), 3848. doi:10.3390/nu13113848

[110] S. Lokesh Kumar, S. Kumar, and K.K.R. Tetala. 2023. A manganese dioxide nanoparticle–bimetallic metal organic framework composite for selective and sensitive detection of vitamin D3 in human plasma. *Microchimica Acta* 190, 345 (2023). doi:10.1007/s00604-023-05904-x

[111] Adrian L Lopresti. 2020. Association between Micronutrients and Heart Rate Variability: A Review of Human Studies. *Advances in Nutrition* 11, 3 (May 2020), 559–575. doi:10.1093/advances/nmz136

[112] Adrian L Lopresti. 2020. The Effects of Psychological and Environmental Stress on Micronutrient Concentrations in the Body: A Review of the Evidence. *Advances in Nutrition* 11, 1 (Jan. 2020), 103–112. doi:10.1093/advances/nmz082

[113] Zhengda Lu, Dakota O'Dell, Balaji Srinivasan, Elizabeth Rey, Ruisheng Wang, Sasank Vemulapati, Saurabh Mehta, and David Erickson. 2017. Rapid diagnostic testing platform for iron and vitamin A deficiency. *Proceedings of the National Academy of Sciences* 114, 51 (Dec. 2017), 13513–13518. doi:10.1073/pnas.1711464114

[114] Hanqi Luo, Jiaxi Geng, Madeleine Zeiler, Emily Nieckula, Fanny Sandalinas, Anne Williams, Melissa F. Young, and Parminder S. Suchdev. 2023. A Practical Guide to Adjust Micronutrient Biomarkers for Inflammation Using the BRINDA Method. *The Journal of Nutrition* 153, 4 (April 2023), 1265–1272. doi:10.1016/j.tjnut.2023.02.016

[115] Yuan Luo, Peter Szolovits, Anand S Dighe, and Jason M Baron. 2016. Using Machine Learning to Predict Laboratory Test Results. *American Journal of Clinical Pathology* 145, 6 (June 2016), 778–788. doi:10.1093/ajcp/aqw064

[116] Thandavarayan Maiyalagan, J. Sundaramurthy, P. Suresh Kumar, Palanisamy Kannan, Marcin Opallo, and Seeram Ramakrishna. 2013. Nanostructured α-Fe2O3 platform for the electrochemical sensing of folic acid. *Analyst* 138, 6 (Feb. 2013), 1779–1786. doi:10.1039/C3AN00070B Publisher: The Royal Society of Chemistry.

[117] Gaganpreet Kaur Mall, Yap Ching Chew, and Janos Zempleni. 2010. Biotin Requirements Are Lower in Human Jurkat Lymphoid Cells but Homeostatic Mechanisms Are Similar to Those of HepG2 Liver Cells. *The Journal of Nutrition* 140, 6 (June 2010), 1086–1092. doi:10.3945/jn.110.121475

[118] Michelle Mann, Derek Exner, Brenda Hemmelgarn, Darlene Sola, Tanvir Turin, Linda Ellis, and Sofia Ahmed. 2013. Vitamin D Levels Are Associated with Cardiac Autonomic Activity in Healthy Humans. *Nutrients* 5, 6 (June 2013), 2114–2127. doi:10.3390/nu5062114

[119] Kasuen Mauldin, John Gieng, Dania Saarony, and Catherine Hu. 2021. Performing nutrition assessment remotely via telehealth. *Nutrition in Clinical Practice* 36, 4 (Aug. 2021), 751–768. doi:10.1002/ncp.10682

[120] Daniel McDuff. 2021. Camera Measurement of Physiological Vital Signs. http://arxiv.org/abs/2111.11547 arXiv:2111.11547 [cs, eess].

[121] Daniel McDuff and Ethan Blackford. 2019. iPhys: An Open Non-Contact Imaging-Based Physiological Measurement Toolbox. http://arxiv.org/abs/1901.04366 arXiv:1901.04366 [cs].

[122] Carlo Mengucci, Alessandra Bordoni, and Francesco Capozzi. 2020. Understanding the kinetics of nutrients bioaccessibility by modelling foodomics data. *Current Opinion in Food Science* 31 (2020), 114–120. doi:10.1016/j.cofs.2020.04.002 Available online 15 April 2020, Version of Record 9 June 2020.

[123] Dean J Miller, Michele Lastella, Aaron T Scanlan, Clint Bellenger, Shona L Halson, Gregory D Roach, and Charli Sargent. 2020. A validation study of the WHOOP strap against polysomnography to assess sleep. *Journal of Sports Sciences* 38, 22 (2020), 2631–2636. doi:10.1080/02640414.2020.1797448

[124] Yulia B. Monakhova and Svetlana P. Mushtakova. 2017. Multicomponent quantitative spectroscopic analysis without reference substances based on ICA modelling. *Analytical and Bioanalytical Chemistry* 409, 13 (May 2017), 3319–3327. doi:10.1007/s00216-017-0275-0

[125] Yu B. Monakhova, S. P. Mushtakova, S. S. Kolesnikova, and S. A. Astakhov. 2010. Chemometrics-assisted spectrophotometric method for simultaneous determination of vitamins in complex mixtures. *Analytical and Bioanalytical Chemistry* 397, 3 (June 2010), 1297–1306. doi:10.1007/s00216-010-3623-x

[126] Seyedeh Somayyeh Mousavi, Mohammad Firouzmand, Mostafa Charmi, Mohammad Hemmati, Maryam Moghadam, and Yadollah Ghorbani. 2019. Blood pressure estimation from appropriate and inappropriate PPG signals using A whole-based method. *Biomedical Signal Processing and Control* 47 (Jan. 2019), 196–206. doi:10.1016/j.bspc.2018.08.022

[127] Charles Mueller (Ed.). 2017. *The ASPEN adult nutrition support core curriculum* (third edition ed.). American Society for Parenteral and Enteral Nutrition, Silver Spring, MD. OCLC: 1043948573.

[128] Zaib Un Mughal, Huma Shaikh, Shahabuddin Memon, and Siraj Uddin. 2024. Determination of Vitamins K1, K2, B6, and D3 using reduced Graphene Oxide fabricated with Bismuth nanoparticles embedded polypyrrole Nanocomposite based optical Sensor. *Materials Advances* 5 (02 2024). doi:10.1039/D3MA01149F

[129] Mohsen Nabian, Yu Yin, Jolie Wormwood, Karen S. Quigley, Lisa F. Barrett, and Sarah Ostadabbas. 2018. An Open-Source Feature Extraction Tool for the Analysis of Peripheral Physiological Data. *IEEE Journal of Translational Engineering in Health and Medicine* 6 (2018), 1–11. doi:10.1109/JTEHM.2018.2878000

[130] National Heart, Lung, and Blood Institute and National Institutes of Health. 2022. Anemia - Treatment and Management. https://www.nhlbi.nih.gov/health/anemia/treatment

[131] National Heart, Lung, and Blood Institute and National Institutes of Health. 2022. Iron-Deficiency Anemia. https://www.nhlbi.nih.gov/health/anemia/iron-deficiency-anemia

[132] National Institutes of Health. 2023. Iron Fact Sheet for Health Professionals. https://ods.od.nih.gov/factsheets/Iron-HealthProfessional/

[133] National Institutes of Health. no date. Dietary Supplement Fact Sheets. https://ods.od.nih.gov/factsheets/list-all/

[134] National Institutes of Health. no date. Nutrient Recommendations and Databases. https://ods.od.nih.gov/HealthInformation/nutrientrecommendations.aspx

[135] Shubham Nimbkar, M. Maria Leena, Jeyan Arthur Moses, and Chinnaswamy Anandharamakrishnan. 2024. Microfluidic assessment of nutritional biomarkers: Concepts, approaches and advances. *Critical Reviews in Food Science and Nutrition* 64, 15 (June 2024), 5113–5127. doi:10.1080/10408398.2022.2150597 Publisher: Taylor & Francis _eprint: https://doi.org/10.1080/10408398.2022.2150597.

[136] Qiang Ning, Shuo Feng, Qianqian Sun, et al. 2024. Finger-actuated wireless-charging wearable multifunctional sweat-sensing system for levodopa and vitamin C. *Nano Research* 17 (2024), 3096–3106. doi:10.1007/s12274-023-6197-6

[137] Academy of Nutrition and Dietetics. 2017. Micronutrient Exam: Clinical Interpretation of Nutrition Focused Physical Exam Findings. https://www.todaysgeriatricmedicine.com/archive/images/SO17p.22_Tablelg.pdf

[138] Harvard School of Public Health. 2020. Precision Nutrition. https://www.hsph.harvard.edu/nutritionsource/precision-nutrition/

[139] World Health Organization. 2024. Nutrition. https://www.who.int/health-topics/nutrition

[140] World Health Organization. 2025. Micronutrients. https://www.who.int/health-topics/micronutrients

[141] Pakeeza, Muhammad Umar Draz, Asim Yaqub, Ali Turab Jafry, Majid Khan, and Huma Ajab. 2024. Electrochemical sensing of B-complex vitamins: current challenges and future prospects with microfluidic integration. *RSC Advances* 14 (2024), 10331–10347. doi:10.1039/D4RA00555D

[142] Sang Mok Park, Michelle A. Visbal-Onufrak, Md Munirul Haque, Martin C. Were, Violet Naanyu, Md Kamrul Hasan, and Young L. Kim. 2020. mHealth spectroscopy of blood hemoglobin with spectral super-resolution. *Optica* 7, 6 (June 2020), 563. doi:10.1364/OPTICA.390409

[143] Pat Bass. 2023. Symptoms of Zinc Deficiency and How It's Treated. https://www.verywellhealth.com/zinc-deficiency-201070 Section: Verywell.

[144] Carmen Patino-Alonso, Marta Gómez-Sánchez, Leticia Gómez-Sánchez, Benigna Sánchez Salgado, Emiliano Rodríguez-Sánchez, Luis García-Ortiz, and Manuel A. Gómez-Marcos. 2022. Predictive Ability of Machine-Learning Methods for Vitamin D Deficiency Prediction by Anthropometric Parameters. *Mathematics* 10, 4 (Jan. 2022), 616. doi:10.3390/math10040616 Number: 4 Publisher: Multidisciplinary Digital Publishing Institute.

[145] Ross D. Peterson, Weili Chen, Brian T. Cunningham, and Juan E. Andrade. 2015. Enhanced sandwich immunoassay using antibody-functionalized magnetic iron-oxide nanoparticles for extraction and detection of soluble transferrin receptor on a photonic crystal biosensor. *Biosensors & Bioelectronics* 74 (Dec. 2015), 815–822. doi:10.1016/j.bios.2015.07.050

[146] Ross D. Peterson, Brian T. Cunningham, and Juan E. Andrade. 2014. A photonic crystal biosensor assay for ferritin utilizing iron-oxide nanoparticles. *Biosensors & Bioelectronics* 56 (June 2014), 320–327. doi:10.1016/j.bios.2014.01.022

[147] Vanessa Pike and Stanley Zlotkin. 2019. Excess micronutrient intake: defining toxic effects and upper limits in vulnerable populations. *Annals of the New York Academy of Sciences* 1446, 1 (2019), 21–43. doi:10.1111/nyas.13993 _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/nyas.13993.

[148] Hugo F. Posada-Quintero and Ki H. Chon. 2020. Innovations in Electrodermal Activity Data Collection and Signal Processing: A Systematic Review. *Sensors* 20, 2 (Jan. 2020), 479. doi:10.3390/s20020479

[149] Chirapha Prakobdi, Thaisa A. Baldo, Prakash Aryal, Jeremy Link, Phoonthawee Saetear, and Charles S. Henry. 2024. Non-invasive iron deficiency diagnosis: a saliva-based approach using capillary flow microfluidics. *Analytical Methods* 16, 16 (2024), 2489–2495. doi:10.1039/D3AY01933K

[150] Siddartha Pullakhandam and Susan McRoy. 2024. Classification and Explanation of Iron Deficiency Anemia from Complete Blood Count Data Using Machine Learning. *BioMedInformatics* 4 (03 2024), 661–672. doi:10.3390/biomedinformatics4010036

[151] Joanne Hui Min Quah, Louis Tong, and Sylvaine Barbier. 2014. Patient Acceptability of Tear Collection in the Primary Healthcare Setting. *Optometry and Vision Science* 91, 4 (2014), 452–458. doi:10.1097/OPX.0000000000000188

[152] Diane Rigassio Radler and Tracy Lister. 2013. Nutrient Deficiencies Associated With Nutrition-Focused Physical Findings of the Oral Cavity. *Nutrition in Clinical Practice* 28, 6 (Dec. 2013), 710–721. doi:10.1177/0884533613507284

[153] Jahan Bakhsh Raoof, Nader Teymoori, Mohammad A. Khalilzadeh, and Reza Ojani. 2015. A high sensitive electrochemical nanosensor for simultaneous determination of glutathione, NADH and folic acid. *Materials Science & Engineering. C, Materials for Biological Applications* 47 (Feb. 2015), 77–84. doi:10.1016/j.msec.2014.11.012

[154] Emilie Reber, Filomena Gomes, Maria F. Vasiloglou, Philipp Schuetz, and Zeno Stanga. 2019. Nutritional Risk Screening and Assessment. *Journal of Clinical Medicine* 8, 7 (July 2019), 1065. doi:10.3390/jcm8071065

[155] S. Brillians Revin and S. Abraham John. 2012. Simultaneous determination of vitamins B2, B9 and C using a heterocyclic conducting polymer modified electrode. *Electrochimica Acta* 75 (July 2012), 35–41. doi:10.1016/j.electacta.2012.04.056

[156] Nuria Rosique-Esteban, Marta Guasch-Ferré, Pablo Hernández-Alonso, and Jordi Salas-Salvadó. 2018. Dietary Magnesium and Cardiovascular Disease: A Review with Emphasis in Epidemiological Studies. *Nutrients* 10, 2 (Feb. 2018), 168. doi:10.3390/nu10020168

[157] A. Catharine Ross and Institute of Medicine (U. S.) (Eds.). 2011. *Dietary reference intakes: calcium, vitamin D.* National Academies Press, Washington, DC.

[158] Joshua T. Royal, Jason T. Fisher, Tinkara Mlinar, Igor B. Mekjavic, and Adam C. McDonnell. 2022. Validity and reliability of capillary vs. Venous blood for the assessment of haemoglobin mass and intravascular volumes. *Frontiers in Physiology* 13 (Nov. 2022), 1021588. doi:10.3389/fphys.2022.1021588

[159] Víctor Ruiz-Valdepeñas Montiel, Juliane R. Sempionatto, Eva Vargas, Eileen Bailey, Jennifer May, Andrea Bulbarello, André Düsterloh, Nathan Matusheski, and Joseph Wang. 2021. Decentralized vitamin C & D dual biosensor chip: Toward personalized immune system support. *Biosensors and Bioelectronics* 194 (Dec. 2021), 113590. doi:10.1016/j.bios.2021.113590

[160] Nargis Sardar, Umer Bin Tariq, Sikandar Ali Khan, Muhammad Haris, and Arsalan Rasheed. 2023. *Vitamin D Detection Using Electrochemical Biosensors: A Comprehensive Overview.* IntechOpen. doi:10.5772/intechopen.112212

[161] Angela A. T. Schuurmans, Peter de Looff, Karin S. Nijhof, Catarina Rosada, Ron H. J. Scholte, Arne Popma, and Roy Otten. 2020. Validity of the Empatica E4 Wristband to Measure Heart Rate Variability (HRV) Parameters: a Comparison to Electrocardiography (ECG). *J. Med. Syst.* 44, 11 (Sept. 2020), 11 pages. doi:10.1007/s10916-020-01648-w

[162] Sumeyye Seker, Dionysios Soulis, Jong-Min Moon, Anastasios Economou, and Joseph Wang. 2024. Decentralized touch-based micronutrition sensor towards personalized nutrition: Parallel detection of sweat ascorbic acid and zinc ions. *Sensors and Actuators B: Chemical* 408 (2024), 135525. doi:10.1016/j.snb.2024.135525

[163] Yurina Sekine, Sung Bong Kim, Yi Zhang, Amay J. Bandodkar, Shuai Xu, Jungil Choi, Masahiro Irie, Tyler R. Ray, Punit Kohli, Naofumi Kozai, Tsuyoshi Sugita, Yixin Wu, KunHyuck Lee, Kyu-Tae Lee, Roozbeh Ghaffari, and John A. Rogers. 2018. A fluorometric skin-interfaced microfluidic device and smartphone imaging module for in situ quantitative analysis of sweat chemistry. *Lab on a Chip* 18, 15 (July 2018), 2178–2186. doi:10.1039/C8LC00530C Publisher: The Royal Society of Chemistry.

[164] Juliane R. Sempionatto, Laís Canniatti Brazaca, Laura García-Carmona, Gulcin Bolat, Alan S. Campbell, Aida Martin, Guangda Tang, Rushabh Shah, Rupesh K. Mishra, Jayoung Kim, Valtencir Zucolotto, Alberto Escarpa, and Joseph Wang. 2019. Eyeglasses-based tear biosensing system: Non-invasive detection of alcohol, vitamins and glucose. *Biosensors and Bioelectronics* 137 (July 2019), 161–170. doi:10.1016/j.bios.2019.04.058

[165] Juliane R. Sempionatto, Ahmed A. Khorshed, Aftab Ahmed, Andre N. De Loyola E Silva, Abbas Barfidokht, Lu Yin, K. Yugender Goud, Mona A. Mohamed, Eileen Bailey, Jennifer May, Claude Aebischer, Claire Chatelle, and Joseph Wang. 2020. Epidermal Enzymatic Biosensors for Sweat Vitamin C: Toward Personalized Nutrition. *ACS sensors* 5, 6 (June 2020), 1804–1813. doi:10.1021/acssensors.0c00604

[166] Michael Serhan, David Jackemeyer, Karam Abi Karam, Kasyap Chakravadhanula, Mark Sprowls, Pinar Cay-Durgun, and Erica Forzani. 2021. A novel vertical flow assay for point of care measurement of iron from whole blood. *Analyst* 146, 5 (7 March 2021), 1633–1641. doi:10.1039/d0an02351e Publisher Copyright: © The Royal Society of Chemistry..

[167] Michael Serhan, David Jackemeyer, Mindy Long, Mark Sprowls, Ismael Diez Perez, Wolfgang Maret, Fang Chen, Nongjian Tao, and Erica Forzani. 2020. Total Iron Measurement in Human Serum With a Novel Smartphone-Based Assay. *IEEE Journal of Translational Engineering in Health and Medicine* 8 (2020), 1–9. doi:10.1109/JTEHM.2020.3005308

[168] Fred Shaffer and J. P. Ginsberg. 2017. An Overview of Heart Rate Variability Metrics and Norms. *Frontiers in Public Health* 5 (Sept. 2017), 258. doi:10.3389/fpubh.2017.00258

[169] Neha Sharma, Muhammad Shahzaib Waseem, Shahrzad Mirzaei, and Mohamed Hefeeda. 2023. MobiSpectral: Hyperspectral Imaging on Mobile Devices. In *Proceedings of the 29th Annual International Conference on Mobile Computing and Networking*. ACM, Madrid Spain, 1–15. doi:10.1145/3570361.3613296

[170] Zhenghan Shi, Peixue Deng, Li-ang Zhou, Meng Jin, Feiyue Fang, Tao Chen, Guang Liu, Hao Wen, Zijian An, Hao Liang, Yanli Lu, Jun Liu, and Qingjun Liu. 2024. Wireless and battery-free wearable biosensing of riboflavin in sweat for precision nutrition. *Biosensors and Bioelectronics* 251 (May 2024), 116136. doi:10.1016/j.bios.2024.116136

[171] Zhenghan Shi, Xin Li, Yifan Shuai, Yanli Lu, and Qingjun Liu. 2022. The development of wearable technologies and their potential for measuring nutrient intake: Towards precision nutrition. *Nutrition Bulletin* 47, 4 (2022), 388–406. doi:10.1111/nbu.12581 _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/nbu.12581.

[172] Michael W. Sjoding, Robert P. Dickson, Theodore J. Iwashyna, Steven E. Gay, and Thomas S. Valley. 2020. Racial Bias in Pulse Oximetry Measurement. *New England Journal of Medicine* 383, 25 (Dec. 2020), 2477–2478. doi:10.1056/NEJMc2029240 Publisher: Massachusetts Medical Society _eprint: https://www.nejm.org/doi/pdf/10.1056/NEJMc2029240.

[173] EMA Smets, Bert Garssen, B de Bonke, and JCJM De Haes. 1995. The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. *Journal of psychosomatic research* 39, 3 (1995), 315–325.

[174] N. W. Solomons. 1986. Competitive interaction of iron and zinc in the diet: consequences for human nutrition. *The Journal of Nutrition* 116, 6 (1986), 927–935. doi:10.1093/jn/116.6.927

[175] Balaji Srinivasan, Seoho Lee, David Erickson, and Saurabh Mehta. 2017. Precision nutrition — review of methods for point-of-care assessment of nutritional status. *Current Opinion in Biotechnology* 44 (April 2017), 103–108. doi:10.1016/j.copbio.2016.12.001

[176] Balaji Srinivasan, Dakota O'Dell, Julia L. Finkelstein, Seoho Lee, David Erickson, and Saurabh Mehta. 2018. ironPhone: Mobile device-coupled point-of-care diagnostics for assessment of iron status by quantification of serum ferritin. *Biosensors and Bioelectronics* 99 (Jan. 2018), 115–121. doi:10.1016/j.bios.2017.07.038

[177] Mayo Clinic Staff. 2023. Anemia - Symptoms and causes. https://www.mayoclinic.org/diseases-conditions/anemia/symptoms-causes/syc-20351360

[178] Dalibor M. Stanković, Darko Kuzmanović, Dragan Manojlović, Kurt Kalcher, and Goran Roglić. 2016. Electroanalytical Approach for Vitamin B12 Quantification Based on Its Oxidation at Boron Doped Diamond Electrode. *Journal of The Electrochemical Society* 163, 7 (April 2016), B348. doi:10.1149/2.1041607jes Publisher: IOP Publishing.

[179] Gretchen A. Stevens, Ty Beal, Mduduzi N. N. Mbuya, Hanqi Luo, and Lynnette M. Neufeld. 2022. Micronutrient deficiencies among preschool-aged children and women of reproductive age worldwide: a pooled analysis of individual-level data from population-representative surveys. *The Lancet Global Health* 10, 11 (2022), e1590–e1599. doi:10.1016/S2214-109X(22)00367-9

[180] Mimi Sun, Tong Xin, Zhiyong Ran, Xinyi Pei, Chongbo Ma, Jian Liu, Mengzhu Cao, Jing Bai, and Ming Zhou. 2021. A Bendable Biofuel Cell-Based Fully Integrated Biomedical Nanodevice for Point-of-Care Diagnosis of Scurvy. *ACS Sensors* 6, 1 (Jan. 2021), 275–284. doi:10.1021/acssensors.0c02335 Publisher: American Chemical Society.

[181] Selim Suner, James Rayner, Ibrahim U. Ozturan, Geoffrey Hogan, Caroline P. Meehan, Alison B. Chambers, Janette Baird, and Gregory D. Jay. 2021. Prediction of anemia and estimation of hemoglobin concentration using a smartphone camera. *PLOS ONE* 16, 7 (July 2021), e0253495. doi:10.1371/journal.pone.0253495

[182] Cell Guidance Systems. 2021. Serum vs Plasma: Do you know the difference? https://www.cellgs.com/blog/serum-vs-plasma-do-you-know-the-difference.html

[183] Ahmet Bilge Sözen, Şeref Demirel, Vakur Akkaya, Hasan Kudat, Tufan Tükek, Mustafa Yeneral, Mustafa Özcan, Özen Güven, and Ferruh Korkut. 1998. Autonomic dysfunction in vitamin B12 deficiency: A heart rate variability study. *Journal of the Autonomic Nervous System* 71, 1 (June 1998), 25–27. doi:10.1016/S0165-1838(98)00058-7

[184] Anne-Laure Tardy, Etienne Pouteau, Daniel Marquez, Cansu Yilmaz, and Andrew Scholey. 2020. Vitamins and Minerals for Energy, Fatigue and Cognition: A Narrative Review of the Biochemical and Clinical Evidence. *Nutrients* 12, 1 (Jan. 2020), 228. doi:10.3390/nu12010228

[185] S. Toru, T. Yokota, A. Inaba, M. Yamawaki, M. Yamada, H. Mizusawa, and M. Hayashi. 1999. Autonomic dysfunction and orthostatic hypotention caused by vitamin B12 deficiency. *Journal of Neurology, Neurosurgery & Psychiatry* 66, 6 (June 1999), 804a–805a. doi:10.1136/jnnp.66.6.804a

[186] Saskia P. M. Truijen, Richard P. G. Hayhoe, Lee Hooper, Inez Schoenmakers, Alastair Forbes, and Ailsa A. Welch. 2021. Predicting Malnutrition Risk with Data from Routinely Measured Clinical Biochemical Diagnostic Tests in Free-Living Older Populations. *Nutrients* 13, 6 (May 2021), 1883. doi:10.3390/nu13061883

[187] Georgios Tsiminis, Erik P. Schartner, Joanna L. Brooks, and Mark R. Hutchinson. 2017. Measuring and tracking vitamin B12: A review of current methods with a focus on optical spectroscopy. *Applied Spectroscopy Reviews* 52, 5 (May 2017), 439–455. doi:10.1080/05704928.2016.1229325

[188] Mustafa Tuncer, Yilmaz Gunes, Unal Guntekin, Hasan Ali Gumrukcuoglu, Beyhan Eryonucu, Niyazi Guler, Imdat Dilek, and Cengiz Demir. 2009. Variabilidade da frequência cardíaca em pacientes com anemia ferropriva. *Arquivos Brasileiros de Cardiologia* 92, 5 (May 2009). doi:10.1590/S0066-782X2009000500011

[189] Raveena Udhani, Charmy Kothari, and Jayrajsinh Sarvaiya. 2023. A Comprehensive Study: Traditional and Cutting-Edge Analytical Techniques for the Biomarker Based Detection of the Micronutrients & POC Sensing Directions for Next-Generation Diagnostic. *Critical Reviews in Analytical Chemistry* 0, 0 (Jan. 2023), 1–20. doi:10.1080/10408347.2023.2169823 Publisher: Taylor & Francis _eprint: https://doi.org/10.1080/10408347.2023.2169823.

[190] Usman Umar, Syafruddin Syarif, Ingrid Nurtanio, and Indrabayu. 2020. A real time non-invasive cholesterol monitoring system. *MATEC Web of Conferences* 331 (2020), 06005. doi:10.1051/matecconf/202033106005

[191] United States Food & Drug Administration. 2020. Ultraviolet (UV) Radiation. https://www.fda.gov/radiation-emitting-products/tanning/ultraviolet-uv-radiation

[192] Urusov, Zherdev, and Dzantiev. 2019. Towards Lateral Flow Quantitative Assays: Detection Approaches. *Biosensors* 9, 3 (July 2019), 89. doi:10.3390/bios9030089

[193] Koen M. Van Der Kooij and Marnix Naber. 2019. An open-source remote heart rate imaging method with practical apparatus and algorithms. *Behavior Research Methods* 51, 5 (Oct. 2019), 2106–2119. doi:10.3758/s13428-019-01256-8

[194] R. A. Varvara and D. C. Vodnar. 2023. Probiotic-driven advancement: Exploring the intricacies of mineral absorption in the human body. *Food Chemistry: X* 21 (2023), 101067. doi:10.1016/j.fochx.2023.101067

[195] S. Vemulapati, E. Rey, D. O'Dell, S. Mehta, and D. Erickson. 2017. A Quantitative Point-of-Need Assay for the Assessment of Vitamin D3 Deficiency. *Scientific Reports* 7, 1 (Oct. 2017), 14142. doi:10.1038/s41598-017-13044-5 Publisher: Nature Publishing Group.

[196] Klaus Von Grebmer, Amy Saltzman, Ekin Birol, Doris Wiesman, Nilam Prasai, Sandra Yin, Yisehac Yohannes, Purnima Menon, Jennifer Thompson, and Andrea Sonntag. 2014. *Synopsis: 2014 global hunger index: The challenge of hidden hunger*. Vol. 83. Intl Food Policy Res Inst.

[197] Johanna-Gabriela Walter, Lourdes S. M. Alwis, Bernhard Roth, and Kort Bremer. 2020. All-Optical Planar Polymer Waveguide-Based Biosensor Chip Designed for Smartphone-Assisted Detection of Vitamin D. *Sensors* 20, 23 (Nov. 2020), 6771. doi:10.3390/s20236771

[198] Edward Jay Wang, William Li, Doug Hawkins, Terry Gernsheimer, Colette Norby-Slycord, and Shwetak N. Patel. 2016. HemaApp: noninvasive blood screening of hemoglobin using smartphone cameras. In *Proceedings of the 2016 ACM International Joint Conference on Pervasive and Ubiquitous Computing*. ACM, Heidelberg Germany, 593–604. doi:10.1145/2971648.2971653

[199] Minqiang Wang, Yiran Yang, Jihong Min, Yu Song, Jiaobing Tu, Daniel Mukasa, Cui Ye, Changhao Xu, Nicole Heflin, Jeannine S. McCune, Tzung K. Hsiai, Zhaoping Li, and Wei Gao. 2022. A wearable electrochemical biosensor for the monitoring of metabolites and nutrients. *Nature Biomedical Engineering* 6, 11 (Nov. 2022), 1225–1235. doi:10.1038/s41551-022-00916-z Publisher: Nature Publishing Group.

[200] John E Ware and Mark Kosinski. 2001. *SF-36 physical & mental health summary scales: a manual for users of version 1*. Quality Metric.

[201] Amanda Watson, Claire Kendell, Anush Lingamoorthy, Insup Lee, and James Weimer. 2022. Lumos: An Open-Source Device for Wearable Spectroscopy Research. *Proceedings of the ACM on Interactive, Mobile, Wearable and Ubiquitous Technologies* 6, 4 (Dec. 2022), 1–24. doi:10.1145/3569502

[202] Joshua A. Waxenbaum, Vamsi Reddy, and Matthew A. Varacallo. 2023. Anatomy, Autonomic Nervous System. In *StatPearls [Internet]*. StatPearls Publishing, Treasure Island (FL). http://www.ncbi.nlm.nih.gov/books/NBK539845/

[203] Roshan Alex Welikala, Paolo Remagnino, Jian Han Lim, Chee Seng Chan, Senthilmani Rajendran, Thomas George Kallarakkal, Rosnah Binti Zain, Ruwan Duminda Jayasinghe, Jyotsna Rimal, Alexander Ross Kerr, Rahmi Amtha, Karthikeya Patil, Wanninayake Mudiyanselage Tilakaratne, John Gibson, Sok Ching Cheong, and Sarah Ann Barman. 2020. Automated Detection and Classification of Oral Lesions Using Deep Learning for Early Detection of Oral Cancer. *IEEE Access* 8 (2020), 132677–132693. doi:10.1109/ACCESS.2020.3010180

[204] Franklin White. 2020. Application of Disease Etiology and Natural History to Prevention in Primary Health Care: A Discourse. *Medical Principles and Practice* 29, 6 (Dec. 2020), 501–513. doi:10.1159/000508718

[205] Daniel R. Witt, Ryan A. Kellogg, Michael P. Snyder, and Jessilyn Dunn. 2019. Windows into human health through wearables data analytics. *Current Opinion in Biomedical Engineering* 9 (March 2019), 28–46. doi:10.1016/j.cobme.2019.01.001

[206] World Health Organization. 2024. Malnutrition. https://www.who.int/health-topics/malnutrition.

[207] Zhiyun Xue, Paul C. Pearlman, Kelly Yu, Anabik Pal, Tseng-Cheng Chen, Chun-Hung Hua, Chung Jan Kang, Chih-Yen Chien, Ming-Hsui Tsai, Cheng-Ping Wang, Anil K. Chaturvedi, and Sameer Antani. 2022. Oral Cavity Anatomical Site Image Classification and Analysis. *Proceedings of SPIE–the International Society for Optical Engineering* 12037 (2022), 120370E. doi:10.1117/12.2611541

[208] Murat A. Yokus and Michael A. Daniele. 2021. Integrated non-invasive biochemical and biophysical sensing systems for health and performance monitoring: A systems perspective. *Biosensors and Bioelectronics* 184 (July 2021), 113249. doi:10.1016/j.bios.2021.113249

[209] Mehmet Yokusoglu, Oral Nevruz, Oben Baysan, Mehmet Uzun, Sait Demirkol, Ferit Avcu, Cem Koz, Turker Çetin, Adnan Hasimi, Ali Ugur Ural, and Ersoy Isik. 2007. The Altered Autonomic Nervous System Activity in Iron Deficiency Anemia. *The Tohoku Journal of Experimental Medicine* 212, 4 (2007), 397–402. doi:10.1620/tjem.212.397

[210] Yuan Zhang, Wei-e Zhou, Jia-qing Yan, Min Liu, Yu Zhou, Xin Shen, Ying-lin Ma, Xue-song Feng, Jun Yang, and Guo-hui Li. 2018. A Review of the Extraction and Determination Methods of Thirteen Essential Vitamins to the Human Body: An Update from 2010. *Molecules* 23, 6 (June 2018), 1484. doi:10.3390/molecules23061484

[211] Jiangqi Zhao, Hnin Yin Yin Nyein, Lei Hou, Yuanjing Lin, Mallika Bariya, Christine Heera Ahn, Wenbo Ji, Zhiyong Fan, and Ali Javey. 2021. A Wearable Nutrition Tracker. *Advanced Materials* 33, 1 (Jan. 2021), 2006444. doi:10.1002/adma.202006444

[212] Chonghui Zhu, Yu Bing, Qidai Chen, Bo Pang, Juan Li, and Tong Zhang. 2023. Nonenzymatic Flexible Wearable Biosensors for Vitamin C Monitoring in Sweat. *ACS applied materials & interfaces* 15, 15 (April 2023), 19384–19392. doi:10.1021/acsami.2c22345

[213] Niv Zmora and Eran Elinav. 2021. Harnessing SmartPhones to Personalize Nutrition in a Time of Global Pandemic. *Nutrients* 13, 2 (Jan. 2021), 422. doi:10.3390/nu13020422

A TABLES

Table 12. Alphabetized List of Abbreviations in Main Text

Abbreviation	Definition	Abbreviation	Definition
AAS	atomic absorption spectrometry	LoD	limit of detection
AGP	acid glycoprotein	MILCA	mutual information least dependent component analysis
AI	artificial intelligence	ML	machine learning
ANN	artificial neural network	MS	mass spectroscopy
ANS	autonomic nervous system	NADH	nicotinamide adenine dinucleotide
AUC	area under the curve	NDNS	National Diet and Nutrition Surveys
BAC	blood alcohol content	NFC	near-field communication
BIA	bio-electrical impedance analysis	NFPE	Nutrition-Focused Physical Exam
BMI	body mass index	NHANES	National Health and Nutrition Examination Survey
BP	blood pressure	NIR	near infrared
Bphen	bathophenanthroline	PBS	phosphate buffered saline
BRI	body roundness index	PNS	parasympathetic nervous system
CBC	complete blood count	PoC	point of care
CoV	coefficient of variance	PPG	photoplethysmogram
CRP	C-reactive protein	PR	pulse rate
CUN-BAE	Clinical University of Navarra body adiposity estimator	PRV	pulse rate variability
CVD	cardiovascular disease	PWV	peak wavelength value
ECG	electrocardiogram	RBP	retinol binding protein
EDA	electrodermal activity	RDA	Recommended Dietary Allowance
EDR	estimated daily requirement	RF	radio frequency
ELISA	enzyme-linked immunosorbent assay	RMSE	root mean square error
GSH	glutathione	ROI	region of interest
HF	high-frequency	SNS	sympathetic nervous system
HMVA	human milk vitamin A	SPR	surface plasmon resonance
HPLC	high-performance liquid chromatography	SSR	spectral super-resolution
HPLC-IR	HPLC with IR detection	sTfR	soluble transferrin receptor
HPLC-UV	HPLC with UV detection	SWV	square wave voltammetry
HR	heart rate	TSH	thyroid-stimulating hormone
HRV	heart rate variability	UL	Upper Limit
ICA	independent component analysis	US	United States
ICP-MS	inductively coupled plasma mass spectrometry	UV	ultraviolet
IDA	iron-deficiency anemia	VAI	visceral adiposity index
IR	infrared	WC	waist circumference
LC	liquid chromatography	WHO	World Health Organization
LED	light emitting diode	WhtR	waist-to-height ratio
LF	low-frequency		

Table 13. Characteristics of Micronutrients: Water-Soluble Vitamins. Information from [11, 18, 32, 127, 133]

	Overview				Interactions Impacting Status		
Micronutrient	Purpose	Storage	Risk of Excess	High Risk Populations	Micronutrients	Diseases (decrease)	Medications (decrease)
Vitamin B1 (thiamin); 3 forms (TMP, TTP, TPP)	Critical to energy metabolism and cell development, functionality	Small amounts in liver	Lack of evidence	Older adults	Absorption decreased by magnesium, folate deficiency	Alcoholism, Inflammatory bowel diseases, Obesity post bariatric surgery, chronic renal failure, critical illness, HIV/AIDS, diabetes	Furosemide, Fluorouracil
Vitamin B2 (riboflavin); 2 coenzyme derivatives (FMN and FAD)	Critical to energy metabolism, cell development and functionality, and metabolism of fats, drugs, and steroids (maintains homocysteine levels)	Small amounts in liver, heart, kidneys	Lack of evidence	Vegetarian athletes, pregnant and lactating people and their infants, people who are vegan and/or consume little milk, people with riboflavin transporter deficiency	Absorption decreased by copper, zinc, iron, manganese intake; deficiency associated with those of folate, pyridoxine, niacin	Alcoholism, Chronic intestinal failure	None
Vitamin B3 (niacin); 2 forms (NAD and NADP)	Critical to energy metabolism, NAD is needed in over 400 enzyme reactions	Some excess in red blood cells	Yes (in supplementation)	Those with undernutrition	Status decreased by inadequate riboflavin, pyridoxine, and/or iron intakes	Hartnup disease, carcinoid syndrome	Antidiabetes, isoniazid and pyrazinamide
Vitamin B5 (pantothenic acid)	Critical to energy metabolism, breaking down and making fats	Red blood cells and tissues	Lack of evidence	Those with a pantothenate kinase-associated neurodegeneration 2 mutation			
Vitamin B6 (pyridoxine); 3 forms (pyridoxine, pyridoxal, pyridoxamine)	Involved in a wide variety of enzyme reactions, protein metabolism, and cognitive development (maintaining homocysteine levels)	Majority bounded to Albumin	Yes (in supplementation)	Those with autoimmune disorders	Poor status associated with low concentrations of other B-complex vitamins	Alcoholism, Inflammatory bowel diseases, chronic renal failure, malabsorption (celiac, Crohn's, etc), homocystinuria	HIV therapy/treatment, therapies inhibiting vitamin activity, cycloserine, antiepileptics, theophyline
Vitamin B7 (biotin)	Critical to the metabolism of proteins, fats, and carbohydrates into energy	Most stored in liver	None	Those with biotinidase deficiency, chronic alcohol exposure, and pregnant and breastfeeding people		Alcoholism, chronic intestinal failure	Anticonvulsants
Vitamin B9 (folate)	Used to create DNA and RNA, facilitate cell division, as well as to metabolize amino acids (conversion of homocysteine)	15-30 mg with 50% in liver, rest in blood and body tissues	Yes (masks B12 deficiency)	Women of childbearing age, pregnancy, MTHFR genetic polymorphism	Absorption decreased by zinc deficiency, bioavailability increased by Vitamin C, excess can mask B12 deficiency	Alcoholism, chronic intestinal failure, Chronic (atrophic) gastritis, obesity post bariatric surgery, chronic renal failure	Methotrexate, antiepileptics, sulfasalazine

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	Overview				Interactions Impacting Status		
Micronutrient	Purpose	Storage	Risk of Excess	High Risk Populations	Micronutrients	Diseases (decrease)	Medications (decrease)
Vitamin B12 (cobalamin) [3]	Critical to CNS development and functionality, RBC formulation, DNA synthesis, conversion of homocysteine	80% in liver; 1-5 mg (thousands times more than daily consumption); can last 2-5 years, 1-3 by some sources	None	Women, elderly, black people, those with low socioeconomic status, who have had gastrointestinal surgery, are vegetarian/vegan	Absorption decreased by excess vitamin C	Alcoholism, chronic intestinal failure, chronic (atrophic) gastritis, Liver diseases, obesity post bariatric surgery, critical illness	Gastric acid inhibitors, metformin
Vitamin C (ascorbic acid)	Required in synthesis of collagen and neurotransmitters, used in protein metabolism, and critical to immune function	High concentrations in cells and tissues, WBC, eyes, adrenal glands, pituitary gland, and brain; total content 300 mg (near acute deficiency) to 2g	Yes (mild nausea, diarrhea, cramps)	Smokers, those with low food variety, any disease causing oxidative stress	Shown to regenerate other antioxidants (ex vitamin E)	Alcoholism, chronic (atrophic) gastritis, obesity post bariatric surgery, critical illness	Chemotherapy/radiation, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors

Table 14. Characteristics of Micronutrients: Fat-Soluble Vitamins. Information from [11, 18, 32, 127, 133]

	Overview				Interactions Impacting Status		
Micronutrient	Purpose	Storage	Risk of Excess	High Risk Populations	Micronutrients	Diseases (decrease)	Medications (decrease)
Vitamin A	Critical for vision, cell growth, immune and reproductive functions	Most in liver (about 6 months), some in eyes	Yes	Infants, pregnant people in low/middle income/developing countries	Absorption decreased by zinc deficiency	Alcoholism, chronic intestinal failure, Inflammatory bowel diseases, liver diseases, obesity post bariatric surgery, cystic fibrosis	Orlistat, retinoids (results in toxicity)
Vitamin D; 2 forms: 25(OH)D (calcidiol) and 1,25(OH)D (calcitriol) [157]	Bone growth and strength, absorption and control of calcium, reducing inflammation	Fatty tissue and liver	Yes	Breastfed infants, adults 20-39, those with kidney/liver dysfunction, with dark skin, limited sun exposure, conditions limiting fat absorption	Magnesium is critical to activation and binding, function is heavily interwoven with Calcium	Alcoholism, chronic intestinal failure, chronic (atrophic) gastritis, Inflammatory bowel diseases, liver diseases, obesity post bariatric surgery, chronic renal failure, critical illness	Orlistat, statins, steroids, thiazide diuretics
Vitamin E (alpha-tocotrienol form)	Function as antioxidants, aid in immune, cell signaling, metabolic processes	Liver (alpha-tocotrienol form)	Lack of evidence (UL of 1000 mg in adults)	Infants, those with fat malabsorption, dieting		Alcoholism, chronic intestinal failure, inflammatory bowel diseases, liver diseases, obesity post bariatric surgery	Anticoagulant, antiplatlet, simvastatin, niacin, chemotherapy/radio treatment
Vitamin K	Involved in blood clotting and bone metabolism	Low blood and tissue stores, carried in lipoproteins	None (except for K3)	Newborns and those with fat malabsorption	Excretion stimulated by excess Vitamin D, absorption decreased by excess Vitamin E and A	Alcoholism, chronic intestinal failure, inflammatory bowel diseases, obesity post bariatric surgery, chronic renal failure, bleeding disorders	Antibiotics and anticoagulants, bile acid sequestrants, orlistat

Table 15. Characteristics of Micronutrients: Minerals. Information from [11, 18, 32, 127, 133]

	Overview				Interactions Impacting Status		
Micronutrient	Purpose	Storage	Risk of Excess	High Risk Populations	Micronutrients	Diseases (decrease)	Medications (decrease)
Iron [55, 58]	Essential to oxygen transport through hemoglobin, energy metabolism, physical growth, neurological development, cell functioning, and hormone synthesis	60% in blood hemoglobin, rest as ferritin in liver, spleen, bone marrow, muscles	Yes (especially those with hemochromatosis and elderly)	Infants, young children, teen females, pregnant people (especially if Mexican-American or Black), premenopausal, in food-insecure households, have increased menstrual bleeding	Absorption increased by Vitamin C intake, Absorption decreased by zinc, calcium, manganese intake and copper deficiency	Chronic intestinal failure, chronic (atrophic) gastritis, inflammatory bowel diseases, obesity post bariatric surgery, critical illness, cancer, heart failure	Levodopa, levothyroxine, proton pump inhibitors
Copper	Cofactor in energy production, iron absorption, neuropeptide activation, and synthesis of connective tissue and neurotransmitters	50-120mg total, 95% carried by ceruloplasmin; 2-3 months in skeleton and muscle; tightly regulated, only 1mg/d loss in bile	Yes	Pregnant people	Absorption decreased by high zinc	Chronic intestinal failure, obesity post bariatric surgery, chronic renal failure, critical illness, celiac disease, menkes disease	
Zinc [143]	Physical growth and development, cellular metabolism, and immune functions	85% in skeletal muscle and bone, 0.1% in plasma where 70% of that is bound to albumin; 1.5g females, 2.5g males total	Yes	Children, teens, exclusively breastfed infants, pregnant people, vegetarian/vegan, have eating disorders, malabsorption, gastrointestinal disorders	Absorption decreased by high calcium/iron	Alcoholism, chronic intestinal failure, inflammatory bowel diseases, liver diseases, obesity post bariatric surgery, chronic renal failure, critical illness, sickle cell disease, HIV	Antibiotics, penicillamine, piuretics
Iodine	Thyroid gland function, protein synthesis, metabolism and enzyme activity	70-80% in thyroid gland; 15-20 mg total	Yes	Infants, pregnant people, use uniodized salt, are in regions with iodine-deficient soils	Absorption decreased by iron intake and selenium deficiency		Anti-thyroids, angiotensin-converting enzyme inhibitors, potassium-paring diuretics
Selenium	Reproduction, thyroid hormone metabolism, and DNA synthesis through selenoproteins; also acts as antioxidant	28-46% in skeletal muscle, most in selenomethionine form	Yes	Kidney dialysis patients, those in selenium deficient regions		Inflammatory bowel diseases, liver diseases, chronic renal failure, critical illness, obesity	Cisplatin
Magnesium	Regulates several chemical reactions, including blood glucose and blood pressure regulation, DNA, RNA, and protein synthesis, proper muscle and nerve functioning, bone development, and calcium and potassium ion transport	Approx 25g; 50-60% in bone, <1% in serum (tightly controlled), rest in soft tissue	Yes	Elderly	Absorption increased by Vitamin D	Alcoholism, gastrointestinal disease, bariatric surgery, T2D	Bisphosphonates, antibiotics, diuretics, proton pump inhibitors

Table 16. Physiological Symptoms of Micronutrient Deficiencies: Water-Soluble Vitamins. Information from [18, 35, 37, 127, 133, 137, 152, 154]

Micronutrient	Eye	Nail	Oral	Disease	Autonomic	Misc	Timeframe
Vitamin B1	Disability in eye movement (ophthalmoplegia)			Cardiomyopathies/ heart failure, Sarcopenia		Correlated with fatigue	Stores depleted within 20 days of insufficient intake
Vitamin B2	Conjunctiva inflammation/grittiness (angular blepharitis), Redness/fissures in eyelid corners (Angular Palpebritis), conjunctiva redness/irritation, swollen/sticky eyelid, photophobia		Bilateral cracks/redness at corners of lips/mouth (angular cheilosis), dry/swollen/ulcerated lips (cheilosis), redness in lips and tongue, swollen/inflamed/smooth tongue (glossitis), Atrophied papillae			Correlated with fatigue	
Vitamin B3	Redness/fissures in eyelid corners (Angular Palpebritis), conjunctiva redness/irritation, swollen/sticky eyelid		Bilateral cracks/redness at corners of lips/mouth (angular cheilosis), dry/swollen/ulcerated lips (cheilosis), redness in lips and tongue, swollen/inflamed/smooth tongue (glossitis), Atrophied papillae, inflamed gums (gingivitis)	Pellagra		Correlated with fatigue	Biomarkers indicate insufficiency far before clinical symptoms appear
Vitamin B5					Sleep issues, fall in diastolic bp and lability of systolic bp	Correlated with fatigue, numbness/burning in extremities	
Vitamin B6	Conjunctiva inflammation/grittiness (angular blepharitis), conjuntiva pallor, Redness/fissures in eyelid corners (Angular Palpebritis), conjunctiva redness/irritation, swollen/sticky eyelid	Excessive thinness, hapalonychia	Bilateral cracks/redness at corners of lips/mouth (angular cheilosis), swollen/inflamed/smooth tongue (glossitis), dry/swollen/ulcerated lips (cheilosis), Atrophied papillae, redness in lips and tongue	Anemia, cardiomyopathies/heart failure	Supplementation improves blood pressure, reported to help regulate SNS	Correlated with fatigue	Borderline and mild status may not present symptoms for months or years; Radler and Lister [152] say "deficiency often occurs within 2 months of inadequacy"
Vitamin B7	Excessive dryness, excessive thinness, brittleness			Multiple sclerosis		Correlated with fatigue	
Vitamin B9	conjuntiva pallor	Central ridges	redness in lips and tongue, swollen/inflamed/smooth tongue (glossitis), inflamed gums (gingivitis), dry/swollen/ulcerated lips (cheilosis), Aphthous Stomatitis (canker sores), inflamed/burning mouth, Atrophied papillae	Anemia, diabetes mellitus		Correlated with fatigue	

Continued on next page

Micronutrient	Eye	Nail	Oral	Disease	Autonomic	Misc	Timeframe
Vitamin B12 [3]	conjuntiva pallor	Pallor, clubbing (Koilonychia), transverse white lines (Muehrcke's lines), excessive dryness, darkness in nails, curved nail ends, central ridges, longitudinal melanonychia	Bilateral cracks/redness at corners of lips/mouth (angular cheilosis), swollen/inflamed/smooth tongue (glossitis), dry/swollen/ulcerated lips (cheilosis), pallor, Aphthous Stomatitis (canker sores), inflamed/burning mouth, Atrophied papillae, redness in lips and tongue, bleeding gums, tooth loss, tooth cavities	Anemia, Osteoporosis, sarcopenia	Deficiency lowers HRV measurements, levels negatively correlated with sleep duration, levels positively correlated with sleep movement and self-assessed quality, night sweats, oxidative stress	Correlated with fatigue	Clinical symptoms can take years (typically 2-5) to appear because of storage levels, glossitis may present initially
Vitamin C		Splinter hemorrhage, excessive thinness, hapalonychia	Intraoral mucosa and tongue inflammation, inflamed gums (gingivitis), thrush, tooth loss, tooth cavities	Scurvy	Supplementation improves blood pressure, helps regulate SNS	Correlated with fatigue	Deficiency can occur after 3-6 months of poor intake, signs of scurvy appear within 1 month of <10mg/day intake

Table 17. Physiological Symptoms of Micronutrient Deficiencies: Fat-Soluble Vitamins. Information from [18, 35, 37, 127, 133, 137, 152, 154]

Micronutrient	Eye	Nail	Oral	Disease	Autonomic	Misc	Timeframe
Vitamin A	Bitot's spots, yellowish lumps around eyes (xanthelasma), cornea softening (kerotomalcia), night blindness	excessive dryness, excessive thinness, leukonychia, hapalonychia		Obesity (beta-carotene), measles	Depletion led to increased norepinephrine and epinephrine in heart and spleen of rats	Heavily associated with antioxidants and immune processes;	Plasma retinol lowers only after storage in liver and eyes are nearly depleted, then Xerophthalmia (progressive eye dryness leading to night blindness) develops after that
Vitamin D [157]		Beau's lines, longitudinal melanonychia, excessive thinness, hapalonychia	inflamed gums (gingivitis)	Cancer cachexia, cardiomyopathies/heart failure, Chronic obstructive pulmonary disease, osteoporosis, sarcopenia, critical to formation of hypocalcemia, depression	Deficiency lowers HRV measurements, calcidiol deficiency lowers resting sympathovagal balance, calcitriol deficiency to worse reactions to stress, supplementation improves blood pressure		
Vitamin E				Obesity			
Vitamin K				Osteoporosis		Impaired clotting and bleeding	

Table 18. Physiological Symptoms of Micronutrient Deficiencies: Minerals. Information from [18, 35, 37, 127, 133, 137, 152, 154]

Micronutrient	Eye	Nail	Oral	Disease	Autonomic	Misc	Timeframe
Iron [55, 58]	conjuntiva pallor, Redness/fissures in eyelid corners (Angular Palpebritis), conjunctiva redness/irritation, swollen/sticky eyelid, blue-tinted sclera	Pallor, clubbing (Koilonychia), transverse white lines (Muehrcke's lines), brittleness, excessive dryness, excessive thinness, darkness in nails, curved nail ends, central ridges, Onycholysis, onychorrhexis	Bilateral cracks/redness at corners of lips/mouth (angular cheilosis), pallor, swollen/inflamed/smooth tongue (glossitis), Atrophied papillae, dry/swollen/ulcerated lips (cheilosis), thrush, inflamed/burning mouth, redness in lips and tongue	Anemia, cardiomyopathies/heart failure, osteoporosis	Disrupts optimal function of endocrine and immune systems; positively correlated with sleep quality (disputed); IDA affects temp regulation and HRV (HRV disputed); low levels associated with higher HR	Status has relation to energy levels and fatigue according to some sources; critical to oxygen binding; weakness; impaired cognitive function	Multiple phases: depletion of stores (mild deficiency, can take several months), iron-deficiency erythropoiesis (erythrocyte production), then iron deficiency anemia (IDA)
Copper	Conjuntiva pallor			Anemia, chronic obstructive pulmonary disease, fatty liver disease, osteoporosis	negatively correlated with sleep quality, reported to help regulate sns	Abnormal lipid metabolism	Some weeks to develop and not readily recognized, Usually manifests in acute conditions
Zinc [143]	Conjunctiva inflammation/grittinesss (angular blepharitis)	Beau's lines, onychorrhexis, leukonychia, brittleness	Changes in taste (inconsistently observed), dryness (Xerostomia), inflamed gums (gingivitis)	Alcoholic hepatitis, cancer cachexia, chronic obstructive pulmonary disease, obesity, osteoporosis, sarcopenia, increased pneumonia risk	Deficiency linked to increased blood pressure, positively correlated with sleep quality, reported to help regulate SNS, critical to ANS functionality according to some sources	Light evidence of relationship between low dietary zinc and un-ideal metabolic response, correlated with fatigue	Symptoms after "several months of low levels"
Iodine		clubbing (Koilonychia)		Goiter, hypothyroidism		Critical to metabolic function	Hypothyroidism occurs when intake falls below 10-20 μg/d, goiter appears fairly quickly
Selenium		excessive dryness, excessive thinness, pallor		Cardiomyopathies/ heart failure, chronic obstructive pulmonary disease, obesity	Intake reduces hypertrophy and oxidative stress, negatively effects blood pressure	Effects on metabolism	
Calcium		Beau's lines, transverse leukonychia, brittleness, excessive dryness, excessive thinness, onychomadesis, onychorrhexis, hapalonychia		Osteoporosis, rickets, osteomalacia, congestive heart failure, seizures		Hypocalcemia can be asymptomatic or have a wide range of symptoms; most common are numbness, tingling, muscle spasms	

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Micronutrient	Eye	Nail	Oral	Disease	Autonomic	Misc	Timeframe
Magnesium		Excessive dryness, excessive thinness, brittleness	inflamed/burning mouth	Cardiovascular disease, hypertension, metabolic syndrome, type 2 diabetes, depression, hypocalcemia, hypokalemia, seizures	Abnormal heart rhythms observed	Overt signs of clinical deficiency are not routinely recognized; correlated with fatigue, nausea, numbness, tingling, muscle spasms	

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