

International Journal of Future Engineering Innovations

Photonics-Fueled Profiling: Label-Free Surface Plasmon Resonance and Localized Surface Plasmon Resonance Biosensors for Multiplexed Adipokine Detection in Obesity

Sunday Ameh ¹, Nkechi Asogwa ¹, Taiwo Awojulu ², Kelechi Asogwa ¹, Joseph Ezeani ³, Oscar Oturu ⁴

¹ Department of Chemistry, University of Benin, Benin City, Edo, Nigeria

² Department of Chemical Engineering, University of Benin, Benin City, Edo, Nigeria

³ Department of Chemical Engineering, University of Toledo, Toledo, Ohio, USA

⁴ Department of Chemistry, University of Jos, Jos, Plateau State, Nigeria

* Corresponding Author: Sunday Ameh

Article Info

ISSN (online): 3049-1215

Volume: 01

Issue: 03

May-June 2024

Received: 09-05-2024

Accepted: 12-06-2024

Page No: 30-41

Abstract

Obesity is a major global health concern linked to chronic inflammation, insulin resistance, and increased risk of metabolic diseases such as type 2 diabetes and cardiovascular disorders. Adipokines, a group of signaling proteins secreted by adipose tissue, play critical roles in regulating metabolic and inflammatory pathways. Accurate monitoring of adipokine levels can provide valuable insight into the onset and progression of obesity-related complications. However, traditional detection methods such as ELISA face limitations in sensitivity, throughput, and real-time analysis. Surface plasmon resonance (SPR) and localized surface plasmon resonance (LSPR) biosensors offer powerful label-free alternatives that enable dynamic and multiplexed detection of biomolecular interactions. This review presents the fundamental principles of SPR and LSPR, outlines key sensor components, and highlights the advantages of label-free detection. It also examines major adipokines relevant to obesity, current analytical challenges, and the urgent need for multiplexed biosensing in clinical applications. Engineering strategies for enhancing sensitivity, including nanostructured materials, surface functionalization, and signal amplification, are discussed alongside recent implementations of multiplexed SPR and LSPR platforms. Clinical studies demonstrating their utility in distinguishing obese from lean individuals and tracking metabolic status are reviewed. Finally, the paper addresses technical barriers, such as standardization and reproducibility, and explores future directions including portable devices, non-invasive sampling, and AI-driven data analysis. SPR and LSPR biosensors hold significant promise for advancing personalized diagnostics and improving the clinical management of obesity.

DOI: <https://doi.org/10.54660/IJFEI.2024.1.3.30-41>

Keywords: Surface Plasmon Resonance, Localized Surface Plasmon Resonance, Label-Free Biosensors, Adipokines, Obesity, Multiplexed Detection

1. Introduction

Obesity, a global epidemic, drives chronic diseases like diabetes and cardiovascular disorders via dysfunctional adipose tissue, now recognized as an endocrine organ secreting adipokines (Fuster *et al.*, 2016). In obesity, adipose tissue shifts adipokine secretion toward pro-inflammatory mediators (TNF- α , IL-6) and reduces protective adiponectin, exacerbating metabolic syndrome (Torres *et al.*, 2019). Current adipokine detection (ELISA, Luminex) is limited by single-analyte focus, cross-reactivity, and labor-intensive protocols (Fuster *et al.*, 2016).

Label-free photonic biosensors based on Surface Plasmon Resonance (SPR) and Localized Surface Plasmon Resonance (LSPR) offer a transformative approach to adipokine profiling (Butt *et al.*, 2023). By enabling the real-time detection of binding events on a functionalized surface without labels, these technologies allow for direct monitoring of molecular interactions, offering

valuable kinetic information in addition to concentration measurements (Takemura, 2021). The potential for high sensitivity, achievable through optimizing plasmonic nanostructures and employing signal amplification techniques, combined with the inherent capability for multiplexing through array fabrication, positions SPR and LSPR as powerful tools for simultaneously profiling multiple critical adipokines from a single sample. Recent studies demonstrating the successful detection of individual adipokines like leptin and adiponectin in complex matrices such as serum underscore the practical feasibility of this approach (Özcan and Sezginürk, 2021; Sankiewicz *et al.*, 2021).

This review aims to provide a comprehensive overview of the recent advancements in utilizing label-free SPR and LSPR biosensor technologies specifically for the detection and multiplexed profiling of adipokines in the context of obesity. The scope encompasses the fundamental principles of SPR and LSPR relevant to biosensing, a discussion of various SPR and LSPR platform designs applied to adipokine detection, examples of successful implementations, and an analysis of the current challenges and future perspectives in developing clinically viable photonic biosensors for obesity research and diagnosis. The review is structured to first introduce the biological context and diagnostic challenges, followed by a detailed exploration of the biosensing technologies, their application to adipokines, and concluding with a forward-looking perspective on the field.

2. Principles of SPR and LSPR Biosensing

Label-free photonic biosensors represent a powerful analytical paradigm shift, leveraging the unique optical properties of noble metals to detect biomolecular interactions in real-time without the need for modifying the target analyte or detection molecule with labels. Surface Plasmon Resonance (SPR) and Localized Surface Plasmon Resonance (LSPR) are two prominent techniques within this class, differing in the specific plasmonic phenomenon exploited but sharing the fundamental principle of detecting refractive

index changes near a metal surface induced by molecular binding events (Butt *et al.*, 2023).

2.1 Fundamentals of Surface Plasmon Resonance (SPR)

SPR is an optical phenomenon that occurs at the interface between a metal (typically a thin film of gold or silver) and a dielectric medium (e.g., buffer or sample). Under specific conditions, incident light can excite collective oscillations of free electrons at the metal surface, known as surface plasmon polaritons (SPPs). SPPs are electromagnetic waves that propagate along the interface, decaying evanescently into both media. Excitation of SPPs requires that the wavevector (momentum) of the incident light matches that of the SPPs (Anal, 2018).

In the commonly used Kretschmann configuration, P-polarized light is directed through a high-refractive-index prism onto the metal film. By varying the angle of incidence, the effective wavevector of the light parallel to the surface can be tuned. At a specific angle, known as the resonance angle, the wavevector matches the SPP wavevector, leading to energy transfer from the incident light into the SPPs. This results in a sharp decrease in the intensity of the reflected light. The resonance angle is highly sensitive to the refractive index of the dielectric medium in the immediate vicinity (typically within a few hundred nanometers) of the metal surface, as this influences the SPP wavevector (Yesudasu, Pradhan and Pandya, 2021).

In an SPR biosensor, the metal surface is functionalized with specific biorecognition elements (e.g., antibodies against a target adipokine). When a sample containing the target analyte flows over the surface, specific binding occurs. This binding event increases the mass concentration and thus changes the local refractive index near the sensor surface. The change in refractive index causes a shift in the resonance angle required for SPP excitation. By monitoring this shift in real-time, the binding interaction, including association and dissociation kinetics, can be tracked and quantified (Nguyen *et al.*, 2015).

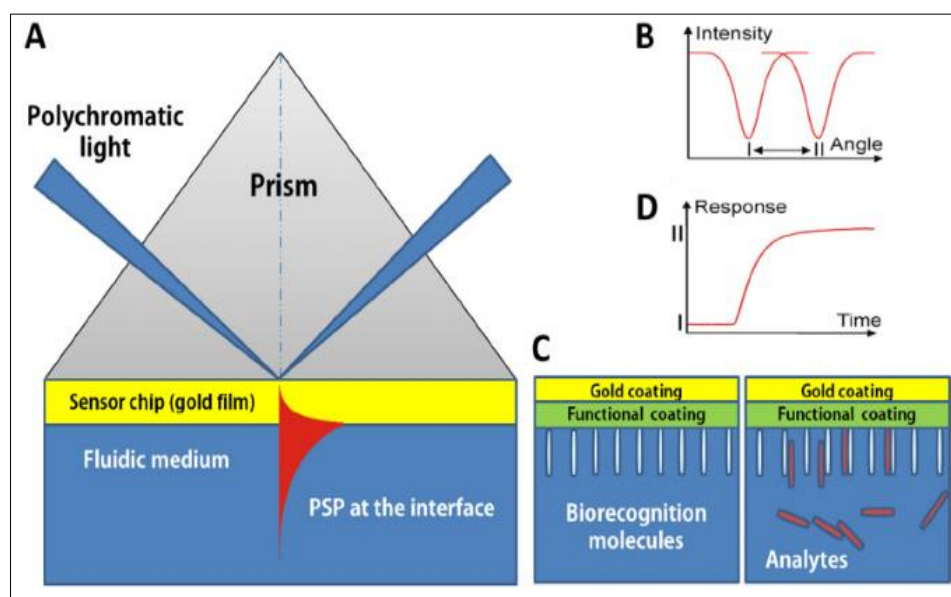


Fig 1: Concept of a surface plasmon resonance (SPR) biosensor: (A) Kretschmann geometry of the ATR method; (B) spectrum of reflected light before and after refractive index change; (C) analyte-biorecognition elements binding on SPR sensor surface and (D) refractive index changes caused by the molecular interactions in the reaction medium (Nguyen *et al.*, 2015).

2.2 Fundamentals of Localized Surface Plasmon Resonance (LSPR)

LSPR is related to SPR but involves the excitation of plasmons in metallic nanoparticles (typically gold or silver) rather than extended thin films. When light interacts with a noble metal nanoparticle smaller than the wavelength of the light, the conduction electrons within the nanoparticle oscillate collectively in response to the oscillating electric field of the light. This oscillation is localized to the nanoparticle surface (Hammond *et al.*, 2014).

Similar to SPR, this localized oscillation exhibits a strong resonance at a specific wavelength, known as the LSPR wavelength (Figure 2). This resonance is typically observed as a peak in the extinction (absorption and scattering) spectrum of the nanoparticle solution or array. The LSPR wavelength is extremely sensitive to the refractive index of the surrounding medium and the dielectric properties of molecules adsorbed onto the nanoparticle surface (Hammond *et al.*, 2014).

For LSPR biosensing, the surface of the plasmonic nanoparticle (either dispersed in solution or immobilized on a substrate) is functionalized with biorecognition elements. When the target analyte binds to these elements, it changes the local dielectric environment and refractive index immediately adjacent to the nanoparticle surface. This change causes a measurable shift in the LSPR peak wavelength. Monitoring this spectral shift allows for the detection and quantification of the binding event. LSPR typically probes an even smaller volume around the nanoparticle surface (tens of nanometers) compared to conventional SPR, which can offer advantages for detecting small molecules or specific binding events close to the particle surface (Unser *et al.*, 2015).

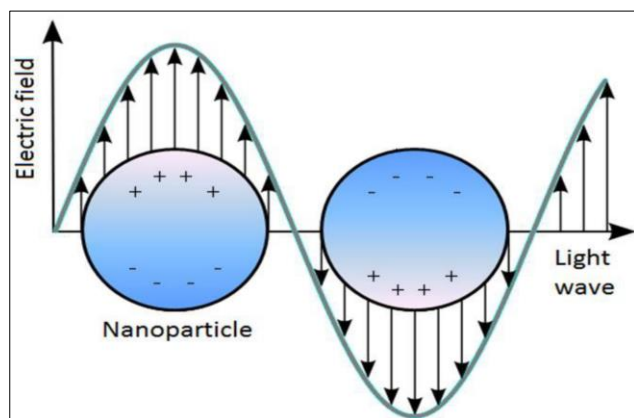


Fig 2: Schematic of LSPR where the free conduction electrons in the metal nanoparticle are driven into oscillation due to strong coupling with incident light (Hammond *et al.*, 2014).

2.3 Key Sensor Components

Noble Metal Substrates: The core transducer element relies on the unique optical properties of noble metals like gold (most common due to chemical inertness and ease of functionalization) or silver. In SPR, this is typically a thin film (e.g., 50 nm gold) deposited on a substrate like glass. In LSPR, it involves gold or silver nanoparticles of specific shapes and sizes (e.g., nanospheres, nanorods, nanotriangles) either in colloidal suspension or immobilized on a solid support (Nguyen *et al.*, 2015).

Bio-Recognition Elements: These are highly specific capture molecules immobilized on the metal surface or nanoparticle.

They are designed to selectively bind the target adipokine(s). Antibodies are widely used due to their high affinity and specificity, but other molecules like aptamers, peptides, or receptors can also be employed depending on the target and application (Hoyos-Nogués, Gil and Mas-Moruno, 2018; Ning, Hu and Lu, 2020). The specificity of the biorecognition element is paramount for accurate detection in complex biological samples (Agharazy Dormeny, Abedini Sohi and Kahrizi, 2020).

Surface Chemistry: A robust and reproducible surface chemistry is essential to effectively immobilize the biorecognition elements onto the metal surface while maintaining their biological activity and minimizing non-specific binding of other components in the sample matrix. Common strategies include direct thiol coupling to gold, formation of self-assembled monolayers (SAMs), covalent coupling via NHS/EDC chemistry to activated surfaces, or affinity-based immobilization (e.g., protein A/G, streptavidin-biotin) (Sun, 2021; Balzer and Whitehurst, 2023). Subsequent blocking steps using molecules like bovine serum albumin (BSA) or ethanolamine are typically employed to passivate remaining active sites and prevent unwanted binding.

2.4 Advantages of Label-Free Sensing

Real-Time Monitoring: SPR and LSPR allow for the continuous monitoring of binding events as they happen, providing dynamic information about the interaction between the analyte and the immobilized recognition element. This contrasts with endpoint assays that only provide a snapshot of the binding at a single time point. This capability is particularly advantageous for capturing transient or weak interactions that might be overlooked in endpoint assays, enabling a more comprehensive understanding of binding dynamics. Additionally, real-time data facilitates rapid optimization of experimental conditions, such as ligand density or buffer composition, during assay development (Zanchetta *et al.*, 2017).

Kinetic Profiling: The real-time data enables the determination of kinetic parameters, including association (K_{on}) and dissociation (K_{off}) rates, from which the equilibrium dissociation constant (K_D) can be derived. These parameters provide valuable insights into the strength and stability of the molecular interaction, which can be crucial for understanding biological mechanisms or evaluating potential drug candidates. Such detailed kinetic analysis is critical for comparing binding affinities across different biomolecules or engineered variants, supporting rational drug design and epitope mapping studies. Furthermore, these measurements can reveal subtle differences in binding mechanisms that correlate with biological outcomes or therapeutic efficacy (Samuel and Rao, 2022).

Native Interactions: By eliminating the need for fluorescent, enzymatic, or isotopic labels attached to the analyte or detection molecule, SPR/LSPR assays observe the binding event between molecules in a state closer to their native conformation and activity. This minimizes potential artefacts or alterations in binding behaviour that labels might introduce. This label-free approach also reduces sample manipulation steps, preserving the integrity of delicate analytes and enabling studies in physiologically relevant conditions. It allows direct measurement of binding events even in complex matrices like serum, where labels might interfere with detection or alter molecular behavior (Sang *et al.*

al., 2016).

Simplified Sample Preparation: The absence of a labeling step reduces assay complexity, cost, and time compared to many conventional methods. This minimizes opportunities for human error during labeling workflows and enables faster processing of large sample batches, enhancing scalability for clinical or research settings. While sample matrix effects (like those from serum) still need to be addressed (e.g., through dilution or pre-treatment), the core detection principle avoids tedious labeling procedures. The streamlined workflow also facilitates easier adaptation to diverse sample types, such as minimally processed biofluids, making the technology more versatile for point-of-care or resource-limited environments (Kabashin, Kravets and Grigorenko, 2023).

Potential for Regeneration and Multiplexing: Many SPR/LSPR sensor surfaces can be regenerated by using a mild buffer to dissociate the bound analyte, allowing the sensor chip to be reused for multiple measurements. Furthermore, the ability to array multiple distinct recognition elements on a single chip surface or integrate multiple sensing spots enables the simultaneous detection of multiple target analytes from a single sample (multiplexing), which is highly relevant for profiling panels of adipokines (Das, Devireddy and Gartia, 2023).

3. Adipokines in Obesity: Biosensing Targets

Understanding the complex interplay of adipokines is crucial for unraveling the pathophysiology of obesity and its associated metabolic disorders. As highlighted previously, adipose tissue actively secretes a multitude of these bioactive molecules, which exert both local and systemic effects. Profiling these molecules provides valuable insights into the inflammatory and metabolic state of an individual, making them prime targets for advanced biosensing applications.

3.1 Major Adipokines and their Pathophysiological Roles

Leptin: Leptin, an adipokine produced by adipose tissue, plays a key role in regulating appetite by signaling the brain to reduce food intake and increase energy expenditure. Leptin, along with other adipokines, influences various physiological processes, including hepatic steatosis and insulin sensitivity. Elevated leptin levels, often associated with increased fat mass, can contribute to conditions like atherogenesis when its signaling becomes impaired (Yadav *et al.*, 2013). Often considered the prototype adipokine, leptin is primarily produced by adipocytes and plays a critical role in regulating appetite, energy expenditure, and neuroendocrine function. Leptin levels generally correlate positively with body fat mass (Figure 3). However, in obesity, a state of "leptin resistance" often develops, where high levels of leptin fail to exert their anorexigenic effects, contributing to continued weight gain (Izquierdo *et al.*, 2019). This resistance is linked to impaired leptin receptor signaling

and reduced blood-brain barrier transport, limiting its central effects. Therapeutic strategies to overcome leptin resistance, such as leptin sensitizers, remain a focus of obesity research but face challenges in clinical translation.

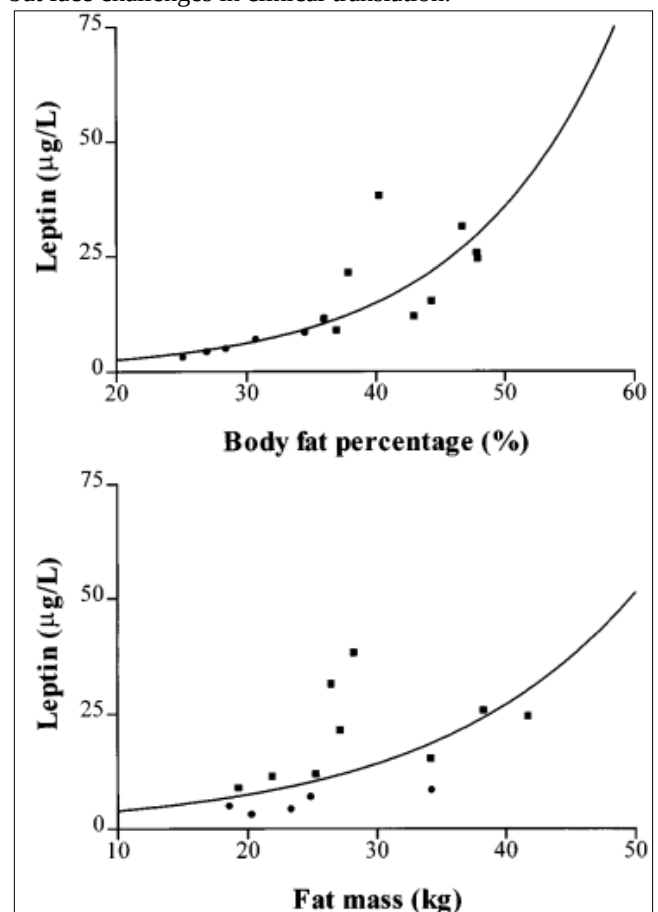


Fig 3: In the upper panel body fat percentage is plotted against serum leptin. The lower panel shows the exponential relation between fat mass and serum leptin (Janssen *et al.*, 1997).

Adiponectin: In contrast to leptin, adiponectin is an insulin-sensitizing and anti-inflammatory adipokine. Circulating levels of adiponectin are typically reduced in individuals with obesity, type 2 diabetes, and cardiovascular disease. Low adiponectin levels are strongly associated with insulin resistance and contribute to the pro-inflammatory state seen in dysfunctional adipose tissue. Adiponectin interacts with specific receptors to modulate energy homeostasis and protect against metabolic diseases (Figure 4). Adiponectin's protective effects include enhancing fatty acid oxidation in muscles and suppressing hepatic glucose production, which counteracts metabolic dysfunction. Its high-molecular-weight oligomers are particularly bioactive, and their selective decline in obesity may exacerbate metabolic risks.

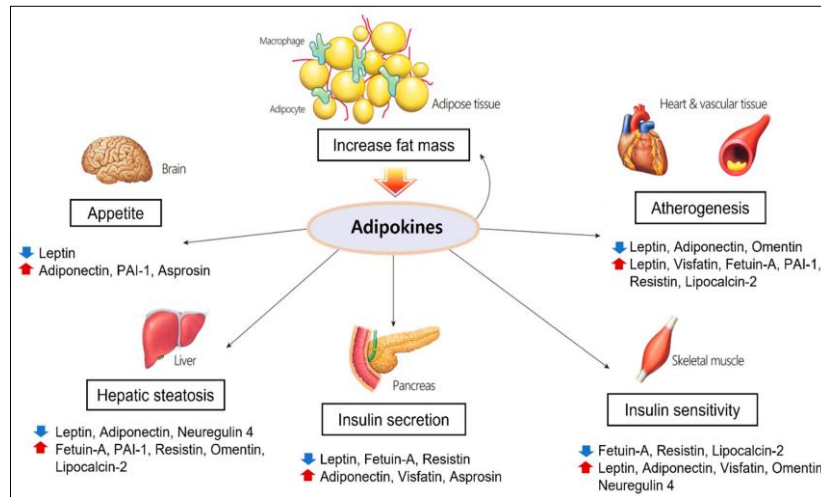


Fig 4: Physiological processes regulated by adipokines in each organ (Kim *et al.*, 2022).

Resistin: While its role is debated and varies across species, resistin has been linked to insulin resistance and inflammation in humans. Higher resistin levels are often found in obese and insulin-resistant individuals, although its specific contribution to the pathogenesis compared to other adipokines is still an area of active research (Figure 5).

Resistin is thought to promote hepatic gluconeogenesis and impair endothelial function, potentially linking obesity to diabetes and atherosclerosis. However, conflicting studies on its mechanistic pathways highlight the need for standardized assays to clarify its clinical relevance (Li *et al.*, 2021).

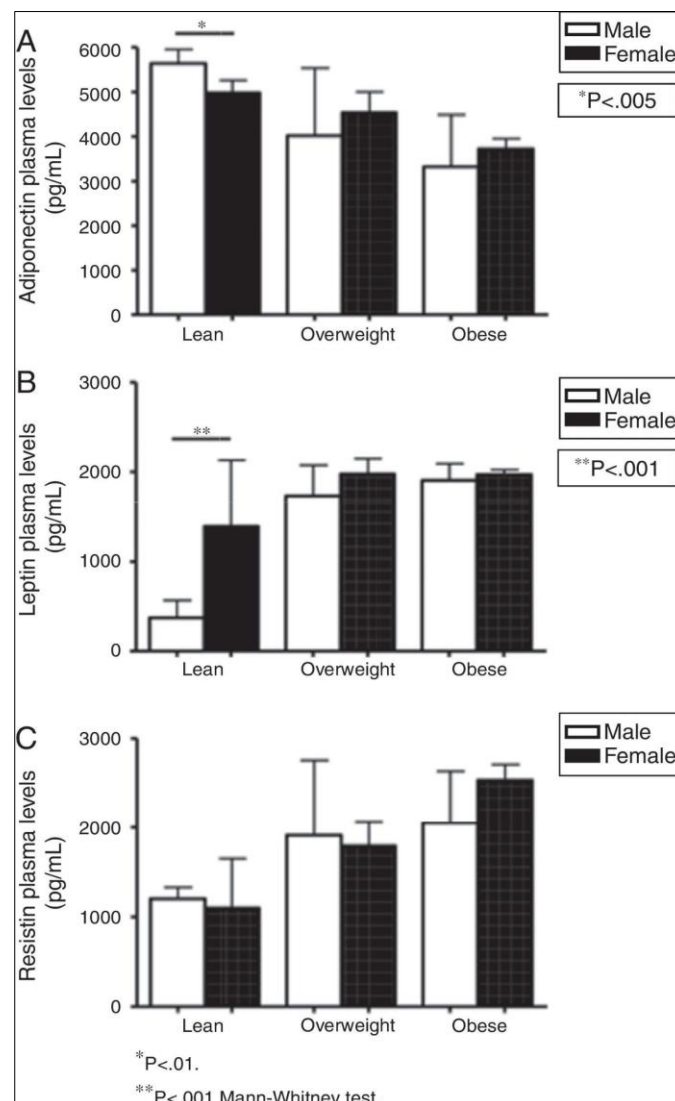


Fig 5: Plasma levels of adipokines (pg/mL) in lean (n=24), overweight (n=30), and obese subjects (n=50). A, adiponectin; B, leptin; C, resistin. * $p < 0.01$. ** $p < 0.001$ Mann-Whitney test (Mantovani *et al.*, 2016).

Tumor Necrosis Factor- α (TNF- α): Although not exclusively an adipokine (also produced by immune cells), TNF- α is significantly upregulated in the adipose tissue of obese individuals and contributes significantly to the chronic low-grade inflammation (Sethi and Hotamisligil, 2021). TNF- α impairs insulin signaling pathways and contributes to insulin resistance and metabolic dysfunction. It also promotes lipolysis, increasing free fatty acid release into circulation, which further exacerbates systemic insulin resistance. Despite its clear role in metabolic inflammation, therapeutic targeting of TNF- α in obesity has shown limited success, likely due to its pleiotropic effects (El Hini *et al.*, 2020).

The altered balance and absolute concentrations of these and other adipokines in obesity drive key pathological processes, making their accurate measurement essential for diagnosis, risk stratification, and monitoring therapeutic interventions.

3.2 Analytical Challenges

Low Analyte Concentrations: While some adipokines like leptin and adiponectin are present in the ng/mL to μ g/mL range, many crucial pro-inflammatory cytokines (e.g., TNF- α , IL-6) are typically found at much lower concentrations, often in the picogram per milliliter (pg/mL) range in healthy individuals, increasing to low ng/mL in diseased states (Aljafary and Al-Suhaimi, 2022). Detecting these low-abundance targets with sufficient sensitivity and accuracy is challenging.

Specificity: Biological fluids contain thousands of different proteins, lipids, salts, and other molecules. Ensuring that the detection method specifically recognizes and quantifies the target adipokine without cross-reactivity with other matrix components is critical. Non-specific binding to the sensor surface can lead to false positive signals or mask the actual target signal (Stenken and Poschenrieder, 2015).

Matrix Interference: The complex composition of serum or plasma can interfere with the assay principle itself. High protein concentrations can cause non-specific binding to the sensor surface, alter the refractive index background, or interfere with the binding kinetics of the target analyte. Lipids and other components can also foul the sensor surface over time, reducing sensitivity and reproducibility. Dilution of samples can mitigate some matrix effects but also reduces the concentration of the target analyte, exacerbating the challenge of detecting low-abundance targets (D'Agata *et al.*, 2021).

3.3 Need for Multiplexed, Sensitive, and Dynamic Adipokine Profiling in Clinical Samples

Multiplexed Detection: Obesity is characterized by dysregulation of a panel of adipokines, not just one. The collective profile and ratios of different adipokines (e.g., adiponectin/leptin ratio) often provide more clinically relevant information than single-analyte measurements. Therefore, simultaneously quantifying multiple adipokines from a single small-volume sample is highly desirable for comprehensive profiling and understanding their synergistic or antagonistic effects (Frühbeck *et al.*, 2019).

High Sensitivity: Detecting low-abundance adipokines like inflammatory cytokines (TNF- α , IL-6) or subtle changes in the levels of others, especially in early-stage disease or during therapeutic interventions, requires highly sensitive detection methods. Increased sensitivity allows for the analysis of smaller sample volumes and potentially less invasive sampling (Chait and den Hartigh, 2020).

Dynamic and Real-Time Information: Monitoring changes in adipokine levels over time in response to weight loss interventions, pharmacological treatments, or disease progression provides crucial dynamic information. While conventional methods offer snapshots, real-time biosensing allows for potentially tracking rapid changes or analyzing binding kinetics, which can provide deeper mechanistic insights and improve assay efficiency (Blüher, 2014).

Conventional methods like ELISA, while sensitive, are typically single-plex, requiring significant sample volume and time for profiling multiple targets. Multiplexed immunoassays like Luminex address the multiplexing need but still involve multiple steps, reagent labeling, and can be prone to matrix effects and cross-reactivity challenges. This underscores the potential of label-free SPR and LSPR biosensors, with their inherent multiplexing capabilities, high sensitivity potential (especially with signal amplification strategies), and real-time detection, to overcome the limitations of current methods and facilitate comprehensive adipokine profiling for better understanding and management of obesity (Chiu, 2022).

4. Engineering SPR and LSPR Biosensors for Adipokine Detection

Developing effective SPR and LSPR biosensors for adipokine detection involves careful selection and engineering of the sensor components and interface. Optimizing the plasmonic materials, surface chemistry, and detection schemes is critical to achieving the necessary sensitivity, specificity, and multiplexing capabilities required for profiling these biomarkers in complex biological samples.

4.1 Materials and Nanostructures

Gold (Au) and Silver (Ag) Films: For standard SPR, thin films (typically 45-55 nm) of gold are the most widely used due to their chemical stability, resistance to oxidation, and well-established surface modification chemistries, particularly thiol binding. Silver films can offer narrower resonance dips, potentially leading to higher bulk sensitivity, but are less stable in ambient conditions and biological media. These films are typically deposited via evaporation or sputtering onto glass substrates (Khurana and Jaggi, 2021).

Plasmonic Nanoparticles: LSPR biosensing utilizes localized surface plasmons excited in metallic nanoparticles. Gold nanoparticles (AuNPs) are the preferred choice for biomedical applications due to their biocompatibility and surface chemistry. Various nanoparticle shapes are used, including nanospheres, nanorods, nanodisks, nanocubes, and nanotriangles, each exhibiting unique optical properties and LSPR wavelengths that are sensitive to local refractive index changes. These nanoparticles can be synthesized in solution or fabricated as ordered arrays on solid supports (Khurana and Jaggi, 2021).

Engineered Nanostructures and Gratings: Beyond simple films or randomly dispersed nanoparticles, fabricating periodically arranged nanostructures (e.g., nanoholes, nanopillars, gratings) allows for advanced control over light-plasmon interactions. These structures can support surface lattice resonances or other enhanced plasmon modes that lead to sharper spectral features and improved sensitivity compared to conventional designs (Pandey and Sharma, 2021).

Antifouling Layers: To minimize non-specific adsorption from serum or plasma, which can obscure the signal from

target analytes and lead to false positives, the plasmonic surface is typically modified with an antifouling layer. Common strategies include using self-assembled monolayers (SAMs) of oligo(ethylene glycol) (OEG) thiols, or coating the surface with hydrophilic polymer brushes or hydrogels like carboxymethyl dextran or poly(ethylene glycol). These layers create a repulsive barrier to reduce background binding noise (Alves *et al.*, 2020).

4.2 Surface Functionalization Strategies

Immobilizing the biorecognition element, typically an antibody specific to the target adipokine, onto the functionalized plasmonic surface is crucial for achieving selective binding. The immobilization must maintain the biological activity of the recognition element and facilitate efficient binding kinetics. Optimal orientation of the antibody ensures maximal antigen-binding site accessibility, enhancing assay sensitivity and specificity. Additionally, surface chemistry modifications, such as using self-assembled monolayers or polymer coatings, can minimize non-specific adsorption while stabilizing antibody conformation for reproducible analyte capture (Shen, Rusling and Dixit, 2017).

Antibody Immobilization: Antibodies are the most common recognition molecules for protein adipokines due to their high affinity and specificity. Immobilization methods aim to create a stable, covalent attachment. The choice of immobilization strategy directly impacts assay reproducibility and detection limits, as improper attachment can lead to antibody denaturation or steric hindrance. Advances in site-specific conjugation techniques, such as engineered cysteine residues or enzymatic tagging, further refine antibody orientation and activity retention (Uludağ and Sezgintürk, 2022).

Thiol-Gold Chemistry: For gold surfaces, thiol chemistry is fundamental. Thiol-terminated molecules (like those in SAMs or certain linker molecules) spontaneously form strong covalent bonds with gold atoms, providing a versatile platform for subsequent attachment of biomolecules. This method ensures long-term stability under physiological conditions, critical for reusable biosensors in clinical settings. However, challenges like incomplete SAM formation or oxidation of thiol groups necessitate rigorous surface characterization to ensure consistent performance (Xue *et al.*, 2014).

NHS/EDC Coupling: Carbodiimide chemistry using N-hydroxysuccinimide (NHS) and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) is widely used to couple amine groups on antibodies to carboxyl groups presented by the modified sensor surface (e.g., a carboxymethylated dextran matrix or a SAM terminated with carboxylic acids). This results in stable amide bonds. The reaction's efficiency is highly pH-dependent, requiring precise buffer optimization to maximize coupling yield while minimizing hydrolysis of the NHS ester intermediate. Non-specific binding can occur if unreacted carboxyl groups remain, underscoring the need for post-coupling blocking agents like ethanolamine or BSA (Kamra *et al.*, 2016).

Oriented Immobilization: To maximize the number of functional binding sites available for the analyte, oriented immobilization strategies are often employed. These methods typically involve capturing the antibody via its non-antigen-binding region using immobilized Protein A or G, or utilizing streptavidin-biotin affinity where a biotinylated antibody

binds to an immobilized streptavidin layer. Oriented approaches enhance sensitivity by up to 10-fold compared to random covalent coupling, as demonstrated in adipokine detection assays. However, biotinylation requires careful control to avoid over-labeling, which can mask antigen-binding sites or induce aggregation (Sueda, 2022).

Microfluidic Integration: Delivering precisely controlled volumes of sample and reagents to the sensor surface is essential for reproducible and sensitive detection, especially in complex matrices like serum and for kinetic measurements. Microfluidic channels integrated over the sensor surface allow for efficient sample handling, reduced dead volume, laminar flow for controlled binding, and enable multiplexed analysis by defining distinct reaction areas on the chip. This is particularly important for performing sequential steps like baseline establishment, sample injection, regeneration, and running multiple assays on a single chip (Aleman *et al.*, 2021).

4.3 Signal Enhancement Techniques

Nanoparticle Amplification: A common and effective strategy involves using secondary detection antibodies conjugated to nanoparticles (e.g., gold nanoparticles, quantum dots). After the target adipokine binds to the capture layer, the nanoparticle-conjugated secondary antibody binds to the captured analyte. The relatively large size and mass of the nanoparticle significantly increases the total mass accumulated on the sensor surface, leading to a proportionally larger and more easily detected SPR angle shift or LSPR wavelength shift. This can dramatically lower the limit of detection (Tabasi and Falamaki, 2018).

Resonance Modulation: This involves optimizing the design of the plasmonic nanostructures to achieve sharper resonance peaks or enhanced field confinement, thereby increasing the sensor's inherent sensitivity to changes in the local dielectric environment upon binding. Examples include carefully tuning the dimensions of LSPR nanoparticles or fabricating plasmonic metamaterials. While these methods improve intrinsic performance, they are often complementary to biochemical amplification techniques (Chen *et al.*, 2018).

5. Multiplexed Adipokine Profiling with SPR and LSPR Platforms

The biological significance of adipokines in obesity lies not only in the absolute concentration of individual molecules but also in their complex interactions and relative ratios. Therefore, the ability to simultaneously detect and quantify multiple adipokines from a single sample (multiplexing) is crucial for comprehensive profiling and gaining deeper insights into the obese state and its metabolic consequences. Label-free SPR and LSPR biosensors, particularly in array formats, are well-suited for achieving multiplexed detection, offering significant advantages over conventional single-plex assays.

5.1 Principles of Multiplexing

Spatial Encoding: This is the most common principle for multiplexed protein detection using SPR and LSPR arrays. Different capture molecules (e.g., antibodies specific to different adipokines) are immobilized onto distinct, physically separated spots or regions on the sensor surface. Each spot serves as an independent sensing channel for a particular target analyte. When a multi-analyte sample flows over the array, each analyte binds only to its corresponding

capture molecule. The signal change (SPR angle shift or LSPR wavelength shift) from each spot is measured independently, allowing for the simultaneous quantification of multiple analytes from a single sample exposure (Qu *et al.*, 2020).

Spectral Discrimination: In some LSPR multiplexing approaches, nanoparticles with different optical properties (e.g., different shapes or sizes resulting in distinct LSPR peak wavelengths) functionalized with different biorecognition elements can be co-immobilized or mixed. Detection involves resolving the spectral shift of each nanoparticle population independently. While promising, spatial encoding is generally more straightforward for creating high-density multiplexed arrays for protein targets like adipokines (Nusz *et al.*, 2011).

Angle-Resolved Analysis (in SPRi): While angle-resolved analysis is the fundamental detection principle for SPR, in the context of multiplexing using SPR imaging (SPRi), the instrument typically performs angle scanning or fixed-angle measurement over the entire functionalized surface simultaneously. By imaging the reflectivity changes across the array at a specific angle or across a range of angles, the binding kinetics and equilibrium signals from each individual spot can be monitored in parallel, effectively utilizing angle-resolved detection across a spatially encoded array (Zeng *et al.*, 2017).

5.2 Platform Implementations

SPR Imaging for Simultaneous Cytokine Detection: SPR imaging (SPRi) is a powerful platform for multiplexing, specifically designed to monitor binding events across an array of capture spots on a planar SPR chip. Instead of using a single detector, SPRi systems utilize a CCD or CMOS camera to capture an image of the entire reflective surface. By measuring the changes in reflectivity or resonance angle/wavelength simultaneously for hundreds or even thousands of distinct spots, SPRi can profile large panels of analytes in parallel. SPRi has been successfully applied for the multiplexed detection of other protein biomarkers, including panels of cytokines, demonstrating its capability for high-throughput profiling in complex matrices like serum. This platform is highly suitable for developing multiplexed assays for panels of adipokines (Del Vecchio and Stahelin, 2016).

LSPR Nanostructure Arrays for Multiplexed Biomarker Panels: LSPR biosensing, especially when employing nanoparticle arrays fabricated on a substrate, is also highly amenable to multiplexing based on spatial encoding. Arrays of identical or different types of plasmonic nanoparticles (e.g., gold nanorods or nanodisks) are created on a surface, and distinct capture molecules are immobilized onto specific regions within the array. Detection involves using a microscope coupled with a spectrometer or camera to interrogate the LSPR spectrum or colorimetric changes of individual spots. This allows for the simultaneous monitoring of LSPR shifts from multiple sensing areas (Ruemmele *et al.*, 2013).

5.3 Analytical Limitations

Signal Overlap and Sensor Cross-talk: In densely packed arrays, signals from adjacent spots could potentially interfere, or non-specific binding might occur across spots (cross-talk), especially if the antifouling layer is compromised or the sample matrix is highly complex. Furthermore, some

adipokines exist in various isoforms or complexes, requiring highly specific antibodies to avoid false positives. There can also be cross-reactivity between different capture antibodies or between detection reagents in a sandwich assay format (Chu *et al.*, 2022).

Reproducibility: Achieving consistent performance across all sensing spots within a single array and between different sensor chips remains a challenge. Variability can arise from differences in surface functionalization efficiency, immobilization density of capture molecules, or variations in the plasmonic properties across the array. This variability impacts the reliability of quantitative measurements (Henseleit and Haustein, 2015).

Calibration Complexities: Unlike single-plex assays, multiplexed detection requires establishing reliable calibration curves for multiple analytes simultaneously in the same assay conditions and matrix. Matrix effects can vary between samples and potentially affect the binding efficiency or signal generation for different analytes on the same chip unevenly. Developing robust multi-analyte calibration standards in a relevant matrix is challenging (Chu *et al.*, 2022).

Matrix Interference: While antifouling layers help, complex matrices like serum can still cause significant non-specific binding or bulk refractive index changes that affect all spots simultaneously. This requires careful baseline correction, sample dilution, or optimized sample pre-treatment protocols to accurately quantify the signal arising specifically from target adipokine binding (Bae *et al.*, 2015).

Sensitivity Discrepancies: Different adipokines are present at vastly different concentration ranges in serum. Developing a single multiplexed assay that provides optimal sensitivity for both high-abundance (e.g., leptin, adiponectin) and low-abundance (e.g., TNF- α , IL-6) targets on the same chip can be challenging, often requiring signal amplification strategies tuned for the lowest concentration targets (Aćimović *et al.*, 2017).

6. Clinical and Research Applications in Obesity

The clinical and research significance of adipokines as biomarkers for obesity and its metabolic complications underscores the need for advanced analytical tools capable of providing sensitive, specific, and comprehensive profiles. Label-free SPR and LSPR biosensors are uniquely positioned to address this need, offering distinct advantages for studying adipokine dynamics in clinically relevant settings.

6.1 Clinical Studies: Adipokine Profiles in Obese vs. Lean Subjects; Correlation with Metabolic Indices

Characterizing the differences in adipokine profiles between obese and lean individuals, and correlating these profiles with key metabolic health indicators (e.g., insulin resistance, lipid profiles, inflammatory markers), is fundamental to understanding the pathogenesis of obesity-related diseases. While many existing studies rely on conventional immunoassays, the capabilities of SPR and LSPR offer new avenues for more detailed investigation (Bellassai *et al.*, 2019). SPR/LSPR platforms can be used to directly measure the binding affinity and kinetics of adipokines in complex matrices like serum, potentially revealing subtle differences in adipokine-receptor interactions or the presence of interfering factors in obese states that might not be evident from simple concentration measurements alone (Ouchi *et al.*, 2011). Furthermore, the multiplexing capability of SPRi and

LSPR arrays allows for simultaneous analysis of panels of adipokines, enabling the study of their combinatorial effects and the identification of specific adipokine signatures associated with different obesity phenotypes or metabolic complications. Although direct SPR/LSPR studies specifically focused on comparing comprehensive adipokine profiles in large cohorts of obese vs. lean subjects with detailed metabolic correlations are emerging, the demonstrated ability of these sensors to detect specific adipokines like leptin in serum highlights their potential for generating the quantitative data necessary for such comparative analyses (Rangel-Huerta, Pastor-Villaescusa and Gil, 2019).

6.2 Advantages over Immunoassays: Low Sample Volume, Dynamic Biomarker Tracking, Non-Invasive Monitoring

Low Sample Volume: SPR and LSPR microfluidic-integrated platforms typically require significantly smaller sample volumes (microliters) compared to the hundreds of microliters often needed for traditional assays, especially when multiplexing is performed. This is particularly advantageous for pediatric studies, longitudinal monitoring requiring frequent sampling, or when sample availability is limited. The reduced volume also minimizes sample depletion, preserving precious clinical specimens for downstream analyses or biobanking. Furthermore, integration with microfluidics enables automated, serial measurements from a single aliquot, enhancing efficiency in high-throughput screening workflows (Bellassai *et al.*, 2019). **Dynamic Biomarker Tracking (Real-Time & Kinetics):** The real-time nature of SPR and LSPR allows for continuous monitoring of binding events, providing insights into the kinetics of adipokine interactions beyond just endpoint concentrations. This dynamic information can be invaluable for studying how adipokine levels change rapidly in response to stimuli (e.g., meal intake, exercise) or for evaluating the functional activity of adipokines by observing their interaction with immobilized receptors (Del Vecchio and Stahelin, 2016). Kinetic profiling can identify transient interactions, such as rapid hormone-receptor binding, that correlate with metabolic responsiveness in obesity. This temporal resolution also aids in distinguishing between adipokine isoforms with similar affinities but divergent biological roles, refining diagnostic specificity (Douzi, 2017).

Potential for Less Invasive Monitoring: While current applications largely focus on serum or plasma, the high sensitivity potential (especially with amplification) and low volume requirement could pave the way for analyzing adipokines in less invasive biofluids like saliva or interstitial fluid in the future, reducing the burden of sample collection. For example, wearable SPR-based devices could enable real-time adipokine tracking in sweat, offering insights into metabolic fluctuations during daily activities (Nan *et al.*, 2023). Such advancements align with personalized medicine goals, allowing non-invasive, continuous monitoring of obesity-related biomarkers in ambulatory settings (Judák *et al.*, 2021).

6.3 Potential for Longitudinal Studies and Personalized Metabolic Profiling

The combination of multiplexing, low sample volume requirements, and potential for rapid analysis positions

SPR/LSPR biosensors as ideal tools for longitudinal studies in obesity research. Tracking changes in a panel of adipokines over extended periods in response to lifestyle interventions (diet, exercise), pharmacological treatments (e.g., anti-diabetic drugs, GLP-1 agonists), or surgical interventions (e.g., bariatric surgery) is crucial for evaluating efficacy and understanding disease progression. The ability to perform comprehensive adipokine profiling on small, serial blood samples non-invasively facilitates such studies (Na *et al.*, 2018).

Furthermore, the capacity to generate detailed, multi-analyte adipokine signatures for individual patients opens exciting possibilities for personalized metabolic profiling. Identifying specific patterns or ratios of adipokines could help stratify patients based on their metabolic risk, predict responsiveness to different treatments, or monitor individual disease trajectories. For instance, differential changes in leptin, adiponectin, and inflammatory adipokines could indicate distinct pathological pathways or responses to intervention in different individuals. SPR/LSPR platforms, by providing these multiplexed, quantitative insights, have the potential to move beyond population-level observations towards personalized approaches in the diagnosis, prognosis, and management of obesity and its related metabolic complications (Cifuentes *et al.*, 2021).

7.0 Current Challenges and Translational Barriers

Despite the significant advancements in SPR and LSPR biosensing technology and their demonstrated potential for label-free and multiplexed detection, translating these platforms from research laboratories to routine clinical practice for adipokine profiling in obesity faces several technical hurdles and broader translational barriers. Overcoming these challenges is crucial for the widespread adoption of photonic biosensors in clinical diagnostics and research.

7.1 Technical Limitations

Detection Limits: While sensitivity has improved significantly, detecting very low-abundance adipokines, particularly certain inflammatory cytokines that are present at picogram per milliliter concentrations in basal states, remains challenging without signal amplification techniques. Achieving the required sensitivity across the broad dynamic range of different adipokines in a multiplexed format is a key challenge (Unser *et al.*, 2015).

Matrix Complexity: Biological fluids like serum or plasma contain a high concentration of non-target molecules, including abundant proteins (e.g., albumin, immunoglobulins), lipids, salts, and other metabolites. This complex matrix can affect the bulk refractive index baseline, reduce the signal-to-noise ratio, and interfere with specific binding interactions, making accurate quantification difficult (Cortese *et al.*, 2020).

Biofouling: A persistent problem in biosensing with complex samples is non-specific binding, or biofouling, where matrix components adsorb onto the sensor surface. This can lead to false positive signals, mask the specific binding signal, reduce the active surface area for target capture, and impede sensor regeneration, ultimately decreasing sensor performance and lifetime. While antifouling layers help, achieving complete resistance to biofouling in undiluted or minimally diluted serum remains difficult (Qu *et al.*, 2020).

Stability: The long-term stability of the sensor surface,

including the plasmonic material, the linker chemistry, the antifouling layer, and the immobilized biorecognition element (e.g., antibody), is critical for practical applications. Sensor performance can degrade over time due to denaturation of the recognition molecule, degradation of surface chemistry, or irreversible fouling, affecting shelf life and reproducibility. Stability under various storage conditions and during repeated use cycles is essential for a clinically viable device (Topor, Puiu and Bala, 2023).

7.2 Batch-to-Batch Reproducibility and Standardization Issues

Batch-to-Batch Reproducibility: Variations can occur at multiple stages of sensor fabrication, including the quality and thickness of metal films, the size, shape, and arrangement of nanoparticles, the consistency of the antifouling layer, and the density and activity of immobilized biorecognition elements. These variations can lead to differences in sensor response and performance between different manufacturing batches, hindering quantitative analysis and comparison of results over time (Gaudreault *et al.*, 2021).

Standardization Issues: Establishing universal standards and reference materials for SPR/LSPR-based adipokine quantification is necessary for clinical implementation. This includes developing standard protocols for sample preparation, assay execution, data analysis, and reporting. Without robust standardization, comparing results obtained using different platforms, sensor designs, or even different batches of the same sensor technology becomes problematic, limiting their utility in multi-center studies or across different healthcare providers (Kumar *et al.*, 2023).

Addressing these technical limitations and translational barriers through continued innovation in materials science, surface chemistry, sensor design, instrumentation, and rigorous validation pipelines is essential for realizing the full potential of photonic biosensors for routine, multiplexed adipokine profiling in the fight against the global obesity epidemic.

8. Future Directions and Technological Horizons

The field of plasmonic biosensing for adipokine detection is advancing quickly, with research focused on improving sensitivity, integrating new technologies, and making these systems more accessible for clinical and research use. One promising direction is the development of hybrid systems that combine the strengths of both SPR and LSPR (Liu, Zhang and Fang, 2017). These approaches can offer both broad-range detection and high sensitivity at the nanoscale. Researchers are also exploring the use of plasmonic materials to enhance fluorescence signals. Although this method involves labeling, it can significantly improve detection of adipokines at very low concentrations, especially in complex biological samples.

Another important goal is the creation of portable and easy-to-use devices. These systems are being designed to work outside traditional laboratories by combining compact optical components with microfluidic channels that handle sample processing automatically. Although wearable biosensors for adipokines, such as those that analyze sweat or interstitial fluid, are still under development, improvements in sampling and signal detection are bringing this idea closer to reality (Almenara, Cimino and Cerniglia, 2022).

In the near future, multiplexed biosensors that can measure multiple adipokines at the same time will be critical for

gaining a more complete picture of metabolic health. When combined with lab-on-a-chip systems, these sensors can automate the entire testing process from sample input to final result. As the amount of data generated by these devices increases, artificial intelligence and machine learning will be needed to help interpret complex results and identify meaningful patterns related to obesity and related conditions (Chu *et al.*, 2022).

There is also growing interest in using alternative sample types that are easier and more comfortable to collect than blood. Saliva, sweat, and interstitial fluid offer non-invasive options for frequent or even continuous monitoring (Gürlek and Çolak, 2021). Although these fluids contain lower levels of adipokines and require more sensitive detection methods, they hold great potential for improving access to metabolic testing in everyday settings. Overall, these technological advancements point toward a future where adipokine monitoring is more accurate, more convenient, and better suited for personalized healthcare.

9. Conclusion

The global obesity epidemic demands advanced diagnostic tools to unravel metabolic and inflammatory dysregulation, with adipokines serving as pivotal biomarkers for understanding disease mechanisms and comorbidities. Traditional methods like ELISA, though valuable, are constrained by limited throughput, labeling requirements, and static measurements. Label-free SPR and LSPR biosensors emerge as transformative solutions, enabling real-time, multiplexed adipokine profiling with high sensitivity through optimized plasmonic nanostructures, as demonstrated in detecting leptin and adiponectin in serum. Challenges persist including ultra-low detection limits, matrix interference, and scalability necessitating interdisciplinary collaboration among material scientists, engineers, and clinicians to refine sensor stability, reproducibility, and clinical integration. Future advancements in hybrid photonic systems, AI-driven data analysis, and point-of-care platforms promise personalized obesity management, dynamic therapeutic monitoring, and deeper mechanistic insights. By bridging innovation with clinical translation, SPR/LSPR biosensors hold significant potential to revolutionize strategies against obesity and its global health burden.

10. References

1. Aćimović SS, Šipová H, Emilsson G, Dahlin AB, Antosiewicz TJ, Käll M. Superior LSPR substrates based on electromagnetic decoupling for on-a-chip high-throughput label-free biosensing. *Light Sci Appl.* 2017;6(8):e17042.
2. Agharazy Dormeny A, Abedini Sohi P, Kahrizi M. Design and simulation of a refractive index sensor based on SPR and LSPR using gold nanostructures. *Results Phys.* 2020;16:102869.
3. Aleman J, Kilic T, Mille LS, Shin SR, Zhang YS. Microfluidic integration of regeneratable electrochemical affinity-based biosensors for continual monitoring of organ-on-a-chip devices. *Nat Protoc.* 2021;16(5):2564-93.
4. Aljafary MA, Al-Suhaimi EA. Adiponectin system (rescue hormone): The missing link between metabolic and cardiovascular diseases. *Pharmaceutics.* 2022;14(7):1430.

5. Almenara CA, Cimino S, Cerniglia L. Sensor technology and intelligent systems in Anorexia Nervosa: Providing smarter healthcare delivery systems. *Biomed Res Int*. 2022;2022:1955056.
6. Alves P, Gomes LC, Vorobii M, Rodriguez-Emmenegger C, Mergulhão FJ. The potential advantages of using a poly(HPMA) brush in urinary catheters: effects on biofilm cells and architecture. *Colloids Surf B Biointerfaces*. 2020;191:110976.
7. Anal AK. *Bionanotechnology: Principles and Applications*. Boca Raton, FL: CRC Press; 2018.
8. Bae YM, Jin SO, Kim I, Shin KY, Heo D, Kang DG. Detection of biomarkers using LSPR substrate with gold nanoparticle array. *J Nanomater*. 2015;2015:302816.
9. Balzer AHA, Whitehurst CB. An analysis of the biotin-(strept)avidin system in immunoassays: Interference and mitigation strategies. *Curr Issues Mol Biol*. 2023;45(11):8733-54.
10. Bellassai N, D'Agata R, Jungbluth V, Spoto G. Surface plasmon resonance for biomarker detection: Advances in non-invasive cancer diagnosis. *Front Chem*. 2019;7:570.
11. Blüher M. Adipokines - removing road blocks to obesity and diabetes therapy. *Mol Metab*. 2014;3(3):230-40.
12. Butt MA, Kazanskiy NL, Khonina SN, Voronkov GS, Grakhova EP, Kutluyarov RV. A review on photonic sensing technologies: Status and outlook. *Biosensors*. 2023;13(5):568.
13. Chait A, den Hartigh LJ. Adipose tissue distribution, inflammation and its metabolic consequences, including diabetes and cardiovascular disease. *Front Cardiovasc Med*. 2020;7:22.
14. Chen J, Gan F, Wang Y, Li G. Plasmonic sensing and modulation based on Fano resonances. *Adv Opt Mater*. 2018;6(9):1701152.
15. Chiu NF. The current status and future promise of SPR biosensors. *Biosensors*. 2022;12(11):933.
16. Chu SS, Nguyen HA, Zhang J, Tabassum S, Cao H. Towards multiplexed and multimodal biosensor platforms in real-time monitoring of metabolic disorders. *Sensors*. 2022;22(14):5200.
17. Cifuentes L, Hurtado AM, Eckel-Passow J, Acosta A. Precision medicine for obesity. *Dig Dis Interv*. 2021;5(3):239-48.
18. Cortese M, Gigliobianco MR, Magnoni F, Censi R, Di Martino PD. Compensate for or minimize matrix effects? Strategies for overcoming matrix effects in liquid chromatography-mass spectrometry technique: A tutorial review. *Molecules*. 2020;25(13):3047.
19. D'Agata R, Bellassai N, Jungbluth V, Spoto G. Recent advances in antifouling materials for surface plasmon resonance biosensing in clinical diagnostics and food safety. *Polymers*. 2021;13(12):1929.
20. Das S, Devireddy R, Gartia MR. Surface plasmon resonance (SPR) sensor for cancer biomarker detection. *Biosensors*. 2023;13(3):396.
21. Del Vecchio K, Stahelin RV. Using surface Plasmon Resonance to quantitatively assess lipid-protein interactions. *Methods Mol Biol*. 2016;1376:141-53.
22. Douzi B. Protein-protein interactions: Surface plasmon resonance. *Methods Mol Biol*. 2017;1615:257-75.
23. El Hini SH, Ahmed ATZ, Hamed EMS, Mahmoud YZ, Eldin AMK, Abdelghany HM. Pivotal Role of Both TNF- α 238G/A and TCF7L2 C/T Gene Polymorphisms in Type 2 Diabetes. *Open Access Maced J Med Sci*. 2020;8(F):283-6.
24. Frühbeck G, Catalán V, Rodríguez A, Ramírez B, Becerril S, Salvador J, *et al*. Adiponectin-leptin ratio is a functional biomarker of adipose tissue inflammation. *Nutrients*. 2019;11(2):454.
25. Fuster JJ, Ouchi N, Gokce N, Walsh K. Obesity-induced changes in adipose tissue microenvironment and their impact on cardiovascular disease. *Circ Res*. 2016;118(11):1786-807.
26. Gaudreault J, Forest-Nault C, De Crescenzo G, Durocher Y, Henry O. On the use of surface plasmon resonance-based biosensors for advanced bioprocess monitoring. *Processes*. 2021;9(11):1996.
27. Gürlek B, Çolak S. Saliva resistin as a screening marker of gestational diabetes mellitus. *Gynecol Endocrinol*. 2021;37(4):324-7.
28. Hammond JL, Bhalla N, Rafiee SD, Estrela P. Localized surface plasmon resonance as a biosensing platform for developing countries. *Biosensors*. 2014;4(2):172-88.
29. Henseleit A, Haustein N. A tailored self-assembling monolayer for monitoring biomarkers in cell culture media using the liSPR system. *J Biochips Tissue Chips*. 2015;5(1):112.
30. Hoyos-Nogués M, Gil FJ, Mas-Moruno C. Antimicrobial peptides: Powerful biorecognition elements to detect bacteria in biosensing technologies. *Molecules*. 2018;23(7):1683.
31. Izquierdo AG, Crujeiras AB, Casanueva FF, Carreira MC. Leptin, obesity, and Leptin resistance: Where are we 25 years later? *Nutrients*. 2019;11(11):2704.
32. Janssen YJ, Frölich M, Deurenberg P, Roelfsema F. Serum leptin levels during recombinant human GH therapy in adults with GH deficiency. *Eur J Endocrinol*. 1997;137(6):650-4.
33. Judák P, Esposito S, Coppieters G, Van Eenoo P, Deventer K. Doping control analysis of small peptides: A decade of progress. *J Chromatogr B*. 2021;1173:122551.
34. Kabashin AV, Kravets VG, Grigorenko AN. Label-free optical biosensing: going beyond the limits. *Chem Soc Rev*. 2023;52(18):6554-85.
35. Kamra T, Chaudhary S, Xu C, Montelius L, Schnadt J, Ye L. Covalent immobilization of molecularly imprinted polymer nanoparticles on a gold surface using carbodiimide coupling for chemical sensing. *J Colloid Interface Sci*. 2016;461:1-8.
36. Khurana K, Jaggi N. Localized surface plasmonic properties of Au and Ag nanoparticles for sensors: A review. *Plasmonics*. 2021;16(4):981-99.
37. Kim JE, Kim JS, Jo MJ, Cho E, Ahn SY, Kwon YJ, *et al*. The roles and associated mechanisms of adipokines in development of metabolic syndrome. *Molecules*. 2022;27(2):334.
38. Kumar VR, Kampan NC, Abd Aziz NH, Teik CK, Shafiee MN, Menon PS. Recent advances in Surface Plasmon Resonance (SPR) technology for detecting Ovarian Cancer biomarkers. *Cancers*. 2023;15(23):5607.
39. Liu F, Zhang X, Fang X. Plasmonic plano-semicylindrical nanocavities with high-efficiency local-field confinement. *Sci Rep*. 2017;7:40071.
40. Li Y, Yang Q, Cai D, Guo H, Fang J, Cui H, *et al*. Resistin, a novel host defense peptide of innate immunity. *Front Immunol*. 2021;12:699807.
41. Mantovani RM, Rocha NP, Magalhães DM, Barbosa IG,

- Teixeira AL, Simões E Silva AC. Early changes in adipokines from overweight to obesity in children and adolescents. *J Pediatr*. 2016;92(6):624-30.
42. Nan M, Darmawan BA, Go G, Zheng S, Lee J, Kim S, *et al*. Wearable localized surface plasmon resonance-based biosensor with highly sensitive and direct detection of cortisol in human sweat. *Biosensors*. 2023;13(2):184.
 43. Na W, Kang M, Jang D, Shin S. Miniaturized surface plasmon resonance biosensor with vacuum-driven hydrodynamic focusing. *Sens Actuators B Chem*. 2018;254:64-71.
 44. Nguyen HH, Park J, Kang S, Kim M. Surface plasmon resonance: a versatile technique for biosensor applications. *Sensors*. 2015;15(5):10481-510.
 45. Ning Y, Hu J, Lu F. Aptamers used for biosensors and targeted therapy. *Biomed Pharmacother*. 2020;132:110902.
 46. Nusz GJ, Marinakos SM, Rangarajan S, Chilkoti A. Dual-order snapshot spectral imaging of plasmonic nanoparticles. *Appl Opt*. 2011;50(21):4198-206.
 47. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. *Nat Rev Immunol*. 2011;11(2):85-97.
 48. Özcan B, Sezginürk MK. Highly sensitive and single-use biosensing system based on a GP electrode for analysis of adiponectin, an obesity biomarker. *ACS Biomater Sci Eng*. 2021;7(8):3658-68.
 49. Pandey AK, Sharma AK. Advancements in grating nanostructure based plasmonic sensors in last two decades: A review. *IEEE Sens J*. 2021;21(11):12633-44.
 50. Qu JH, Dillen A, Saeys W, Lammertyn J, Spasic D. Advancements in SPR biosensing technology: An overview of recent trends in smart layers design, multiplexing concepts, continuous monitoring and *in vivo* sensing. *Anal Chim Acta*. 2020;1104:10-27.
 51. Rangel-Huerta OD, Pastor-Villaescusa B, Gil A. Are we close to defining a metabolomic signature of human obesity? A systematic review of metabolomics studies. *Metabolomics*. 2019;15(6):93.
 52. Ruemmele JA, Hall WP, Ruvuna LK, Van Duyne RP. A localized surface plasmon resonance imaging instrument for multiplexed biosensing. *Anal Chem*. 2013;85(9):4560-6.
 53. Samuel VR, Rao KJ. A review on label free biosensors. *Biosens Bioelectron X*. 2022;11:100216.
 54. Sang S, Wang Y, Feng Q, Wei Y, Ji J, Zhang W. Progress of new label-free techniques for biosensors: a review. *Crit Rev Biotechnol*. 2016;36(3):465-81.
 55. Sankiewicz A, Hermanowicz A, Grycz A, Łukaszewski Z, Gorodkiewicz E. An SPR imaging immunosensor for leptin determination in blood plasma. *Anal Methods*. 2021;13(5):642-6.
 56. Sethi JK, Hotamisligil GS. Metabolic Messengers: tumour necrosis factor. *Nat Metab*. 2021;3(10):1302-12.
 57. Shen M, Rusling J, Dixit CK. Site-selective orientated immobilization of antibodies and conjugates for immunodiagnosics development. *Methods*. 2017;116:95-111.
 58. Stenken JA, Poschenrieder AJ. Bioanalytical chemistry of cytokines--a review. *Anal Chim Acta*. 2015;853:95-115.
 59. Sueda S. Antibody immobilization for immunosensing. *Anal Sci*. 2022;38(1):1-2.
 60. Suni II. Substrate materials for biomolecular immobilization within electrochemical biosensors. *Biosensors*. 2021;11(7):239.
 61. Tabasi O, Falamaki C. Recent advancements in the methodologies applied for the sensitivity enhancement of surface plasmon resonance sensors. *Anal Methods*. 2018;10(32):3906-25.
 62. Takemura K. Surface plasmon resonance (SPR)- and localized SPR (LSPR)-based virus sensing systems: Optical vibration of nano- and micro-metallic materials for the development of next-generation virus detection technology. *Biosensors*. 2021;11(8):250.
 63. Topor CV, Puiu M, Bala C. Strategies for surface design in surface plasmon resonance (SPR) sensing. *Biosensors*. 2023;13(4):465.
 64. Torres S, Fabersani E, Marquez A, Gauffin-Cano P. Adipose tissue inflammation and metabolic syndrome. The proactive role of probiotics. *Eur J Nutr*. 2019;58(1):27-43.
 65. Uludağ İ, Sezginürk MK. A direct and simple immobilization route for immunosensors by CNBr activation for covalent attachment of anti-leptin: obesity diagnosis point of view. *3 Biotech*. 2022;12(1):33.
 66. Unser S, Bruzas I, He J, Sagie L. Localized surface plasmon resonance biosensing: Current challenges and approaches. *Sensors*. 2015;15(7):15684-716.
 67. Xue Y, Li X, Li H, Zhang W. Quantifying thiol-gold interactions towards the efficient strength control. *Nat Commun*. 2014;5:4348.
 68. Yadav A, Kataria MA, Saini V, Yadav A. Role of leptin and adiponectin in insulin resistance. *Clin Chim Acta*. 2013;417:80-4.
 69. Yesudasu V, Pradhan HS, Pandya RJ. Recent progress in surface plasmon resonance based sensors: A comprehensive review. *Heliyon*. 2021;7(3):e06321.
 70. Zanchetta G, Lanfranco R, Giavazzi F, Bellini T, Buscaglia M. Emerging applications of label-free optical biosensors. *Nanophotonics*. 2017;6(4):627-45.
 71. Zeng Y, Hu R, Wang L, Gu D, He J, Wu SY, *et al*. Recent advances in surface plasmon resonance imaging: detection speed, sensitivity, and portability. *Nanophotonics*. 2017;6(5):1017-30.
 72. Bitragunta SL. Empowering the Future: The Rise of Electric Vehicle Charging Hubs. *IJLRP-Int J Lead Res Publ*. 2024;5(11).