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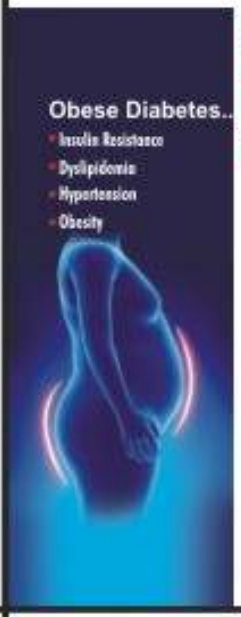

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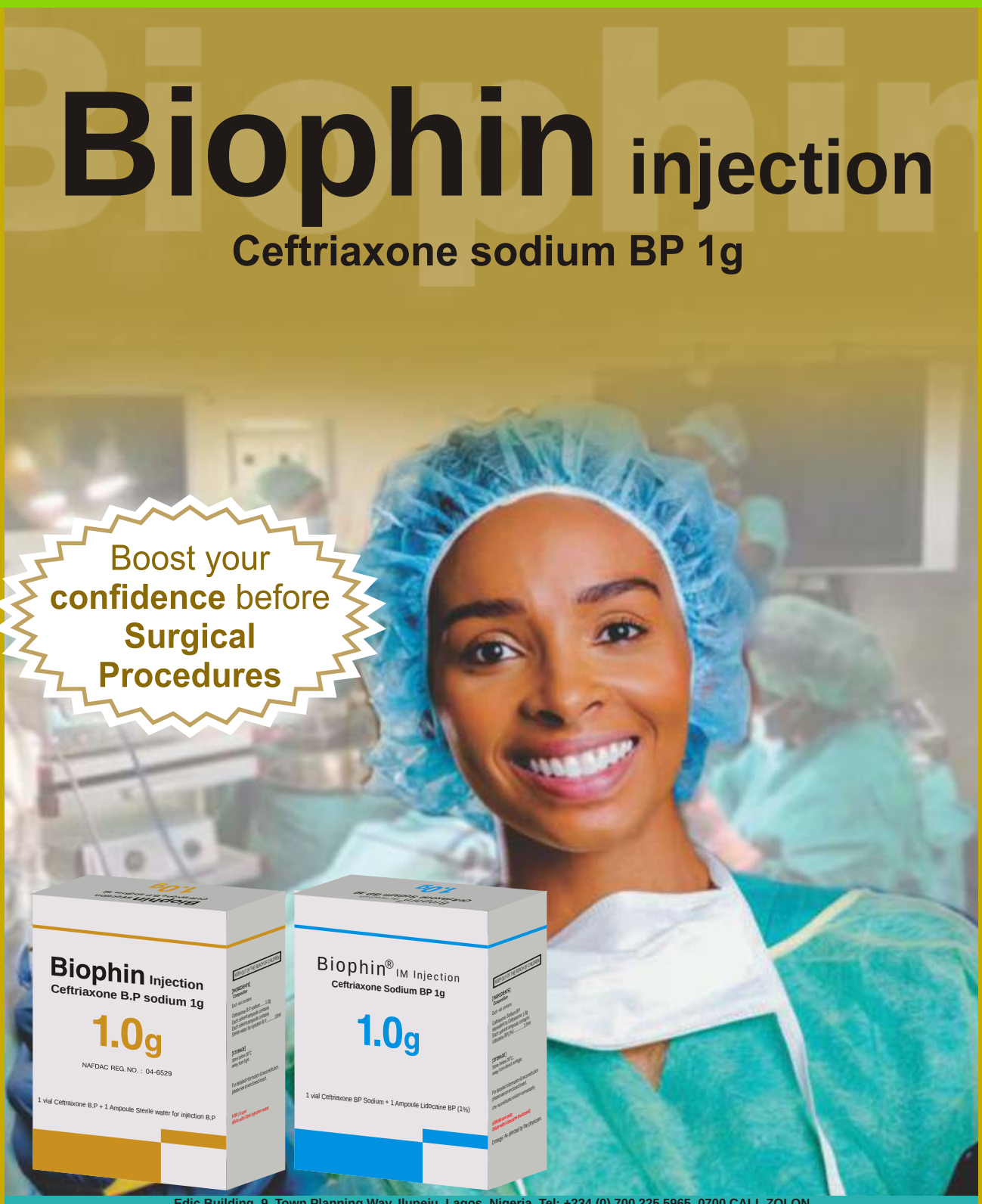

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Implementation of Obesity Science Into Clinical Practice: A Scientific Statement From the American Heart Association

Deepika Laddu, PhD, FAHA, Chair, Ian J. Neeland, MD, FAHA, Vice Chair, Mercedes Carnethon, PhD, FAHA, Fatima C. Stanford, MD, MPH, MPA, MBA, FAHA, Morgana Mongraw-Chaffin, PhD, FAHA, Bethany Barone Gibbs, PhD, FAHA, Chiadi E. Ndumele, MD, PhD, FAHA, Chris T. Longenecker, MD, FAHA, Misook L. Chung, PhD, RN, FAHA, and Goutham Rao, MD, FAHA on behalf of the American Heart Association Obesity Committee of the Council on Lifestyle and Cardiometabolic Health; Council on Epidemiology and Prevention; Council on Clinical Cardiology; Council on Hypertension; Council on the Kidney in Cardiovascular Disease; and Council on Cardiovascular and Stroke Nursing

Abstract

Obesity is a recognized public health epidemic with a prevalence that continues to increase dramatically in nearly all populations, impeding progress in reducing incidence rates of cardiovascular disease. Over the past decade, obesity science has evolved to improve knowledge of its multifactorial causes, identifying important biological causes and sociological determinants of obesity. Treatments for obesity have also continued to develop, with more evidence-based programs for lifestyle modification, new pharmacotherapies, and robust data to support bariatric surgery. Despite these advancements, there continues to be a substantial gap between the scientific evidence and the implementation of research into clinical practice for effective obesity management. Addressing barriers to obesity science implementation requires adopting feasible methodologies and targeting multiple levels (eg, clinician, community, system, policy) to facilitate the delivery of obesity-targeted therapies and maximize the effectiveness of guideline-driven care to at-need patient populations. This scientific statement (1) describes strategies shown to be effective or promising for enhancing translation and clinical application of obesity-based research; (2) identifies key gaps in the implementation of obesity science into clinical practice; and (3) provides guidance and resources for health care professionals, health care systems, and other stakeholders to promote broader implementation and uptake of obesity science for improved population-level obesity management. In addition, advances in implementation science that hold promise to bridge the know-do gap in obesity prevention and treatment are discussed. Last, this scientific statement highlights implications for health research policy and future research to improve patient care models and optimize the delivery and sustainability of equitable obesity-related care.

The prevalence of obesity in the United States and globally has been escalating for decades, with recent estimates that >40% of US adults are living with obesity.[1,2] The continued rise in obesity has inevitably slowed the decline in rates of cardiovascular disease (CVD) despite improvements in other population risk factors.[3] Moreover, forecasted trends in global obesity prevalence underscore the significant impact that obesity will continue to have on CVD incidence, especially among people of underrepresented races and ethnicities.[4–6] Over the past decade, significant progress made in obesity science has contributed to the discovery of knowledge cutting across the domains of basic, translational, and biobehavioral science; epidemiology; and clinical studies/trials. Treatment of obesity also continues to evolve, with more empirical evidence supporting the efficacy of lifestyle modification programs, new pharmacotherapies, and robust outcomes data for bariatric surgery.

Despite the ubiquity of these advancements, effective implementation of obesity science into routine clinical practice for prevention and treatment of obesity remains suboptimal. There are major gaps between our knowledge of the science of obesity and the clinical implementation of that science for ideal patient care. The lack of sufficient implementation exemplifies important gaps that exist between our biological and sociological under standing of obesity, interventions that target obesity (eg, lifestyle, pharmacological, and surgical), and the application of evidence-based research into clinical practice for

improved management of obesity.[7–9] These gaps are sustained by structural, societal, and cultural barriers that are pervasive in real-world clinical practice and require a redoubling of efforts and alternative strategies for resolution and advancement. Therefore, prioritizing the implementation of obesity science will be instrumental in informing evidence-based practice and consequently guiding the delivery and maintenance of contextually appropriate care to diverse, underrepresented populations with obesity.[10,11] Bridging the gap in obesity implementation science requires a multitargeted approach that addresses long-standing implementation challenges across various levels (eg, clinician, community, system, policy) and applies effective implementation strategies based on core frameworks[11,12] to advance the integration of novel, empirically supported obesity science into routine clinical care (Figure 1).

The purposes of this scientific statement are to (1) describe strategies shown to be effective or promising for enhancing the clinical application of obesity-based research; (2) identify key gaps in the implementation of obesity science into clinical practice; and (3) provide guidance and resources for clinical and community health care professionals, health care systems, and other stakeholders to facilitate improved population-level management of obesity. This scientific statement also discusses additional implications for policy, as well as future research to improve patient care models and optimize the delivery and sustainability of equitable obesity-related care.

Keywords: AHA Scientific Statements; evidence-based practice; implementation science; obesity; risk factors.

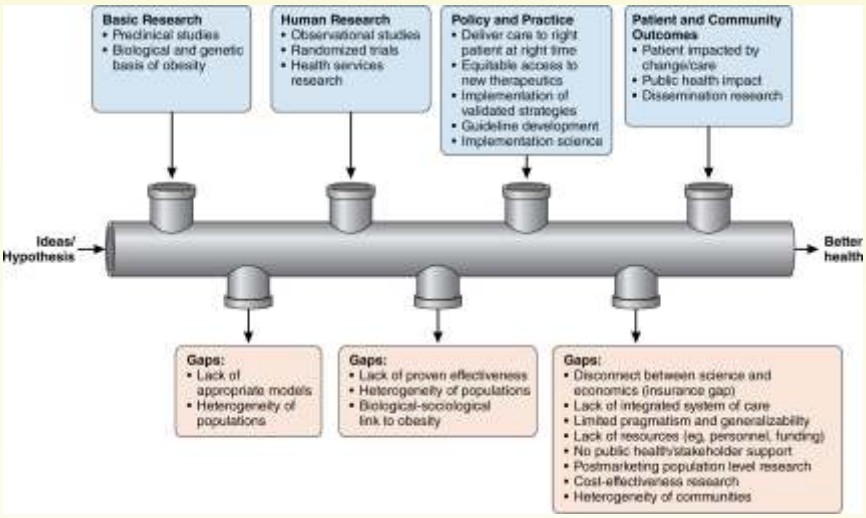

Figure 1. The implementation pipeline for obesity science. Scientific advancement in knowledge and treatment of obesity begins at the bench, where ideas and hypotheses are tested with basic research tools such as preclinical biological and genetic models of obesity. In this phase, lack of appropriate models and heterogeneity of populations limit the success and application of basic research and prevent advancement to human research. In the human research phase, observational studies, randomized trials, and health services research inform and confirm how knowledge from basic research can be applied to the human clinical setting. In this phase, new diagnostic tools and treatment strategies for obesity are tested for efficacy and safety. Barriers to implementation in this phase include lack of proven effectiveness for therapies, heterogeneity of populations studied/lack of generalizability, and a breakdown in the biological-sociological link to obesity. In the next phase of implementation, health care policy and practice become essential to deliver care to the right patient at the right time, provide equitable access to new therapeutics, and implement validated strategies and guideline recommendations to broad populations. Implementation science is used in this phase to assess, measure, and modify clinical approaches to increase the uptake and effectiveness of validated interventions. This implementation is furthered by patient and community outcomes, demonstrating that patients are positively affected by the change in care, with potential for additional public health impact through dissemination of the research to reach those in need. In this phase, there are often gaps between policy and implementation into practice, highlighted by limitations in systems of care, reimbursement for care, timely and equitable access to resources, and lack of demonstration of cost-effectiveness. Overcoming and narrowing these gaps between knowledge/science and clinical implementation can lead to better health for all patients living with obesity and better health outcomes.

METHODS FOR SUCCESSFUL IMPLEMENTATION OF OBESITY SCIENCE INTO CLINICAL PRACTICE

The successful implementation of obesity science into clinical practice requires a methodological framework that moves scientific knowledge from bench to bedside and addresses gaps in the implementation pipeline. Obesity science is well established, and emerging therapeutic options for obesity based on scientific discoveries have become increasingly prevalent in the past several years. Education on the complex origins and clinical consequences of obesity, a framework for the successful delivery of obesity care, and health policy interventions to enhance the provision of obesity care are examples of implementation priorities that are essential to the success of obesity science.

To address the growing obesity epidemic and successfully implement obesity science, healthcare professionals must first be equipped with the proper knowledge and implementation skills. Yet, numerous

studies have demonstrated that obesity education is lacking. For example, although the American Board of Medical Specialties certification examinations influence medical knowledge and practice for physicians throughout the United States, only 25% of the 24 general certification content outlines (ie, preparatory material for examinations) mention obesity. This gap indicates a need for translating the complexity of obesity science into practice with an increased emphasis on the diagnosis, prevention, and treatment of obesity.[13] In a comprehensive international systematic review on obesity education across varying levels of medical training, Mastrocola and colleagues[14] determined that there is a paucity of obesity education programs for medical students, residents, and fellow physicians in training programs throughout the world despite high obesity prevalence. Still, they note that these programs often improve outcomes when administered.

One increasingly successful method for improving healthcare professional education and subsequent implementation is the certification program in obesity medicine offered by the American Board of Obesity Medicine (ABOM). Studies show that physicians certified in obesity medicine tend to deliver more effective evidence-based care such as lifestyle and behavioral counseling, pharmacotherapy, and care for patients who undergo metabolic and bariatric surgery.[15] In a cross-sectional analysis of the ABOM-certified physicians, certified physicians' practices were likely to be concordant with published guidelines, including the American College of Cardiology/American Heart Association/The Obesity Society, American Association of Clinical Endocrinologists/American College of Endocrinology, and Obesity Medicine Association guidelines. However, although health care practitioners may be confident that ABOM-certified physicians will deliver evidence-based care, access to these physicians is often unavailable because of the high prevalence of obesity and the relative shortage of certified professionals. Although all states in the United States have at least 1 ABOM-certified adult physician, there are geographic disparities in physician availability relative to obesity prevalence, leading to widened health care disparities. This is even more pronounced in the pediatric population, with fewer ABOM-certified physicians.

The next step in implementation requires a framework for the successful delivery of obesity medicine care. The Society for Behavioral Medicine has an evidence-based model for primary care obesity management based on the 5As counseling frame- work (assess, advise, agree, assist, and arrange),[16] of which can be used to promote the implementation of obesity treatments in clinical practice settings (Figure 2). Two recent American Heart Association statements provide a comprehensive summary of how to implement the 5A model for health behavior change in primary care and community-based settings for CVD prevention and risk management.[17,18] There is a particular focus on guiding primary healthcare professional efforts to offer or refer patients for behavioral counseling beyond what can be done during the brief, episodic office visits.[17] Best-practice approaches for enhancing the adoption and implementation of behavior change programs in clinical or community-based health care settings, including the use of team-based care, reimbursement and referral models, and practical national resources, are described in detail.[17,19] Although more studies are needed on the effectiveness of health care professional–delivered behavior counseling interventions on the maintenance of behavioral outcomes, promoting a healthy lifestyle and assisting patients in achieving health behavior goals presents a feasible strategy that health care professionals in clinical and community-linked settings can use to proactively maximize impact on obesity care and reduce the burden of subsequent CVD risk at every visit.[18] It is important to note that building solid, sustainable clinic-community linkages is necessary to facilitate the implementation of obesity/weight management programs. Indeed, increasing clinician education and self-efficacy in obesity science, along with the workforce of specialized ABOM-certified diplomates, while building straight forward treatment work-flows that are evidence based with expanded and adequate clinician reimburse- ment also appears to be the logical next step to the successful implementation of obesity science into clinical practice.[20]

For example, beginning in 2014, the National Academy of Medicine established the Roundtable on Obesity Solutions and has convened workshops and related activities to address key issues related to obesity prevention, evaluation, and treatment. These have included assessing training needs and defining competencies.[21] The roundtable's work is a valuable resource in filling important gaps in knowledge and skills among health care professionals.

Health policy interventions that can enhance the provision of obesity care are emerging globally. One example is Life's Essential 8, the key measures for improving and maintaining cardiovascular health as defined by the American Heart Association.[22] The goal of Life's Essential 8 is to link science to

Figure 2. The 5A (assess, advise, agree, assist, and arrange) model for implementing obesity treatment in primary care.

implementation. Many of the Life's Essential 8 health behaviors and habits affect body weight, and the Life's Essential 8 advisory contains important methods for implementing cardiovascular health assessment and longitudinal monitoring, as well as potential data sources and tools to promote widespread adoption in policy, public health, clinical, institutional, and community settings.

IDENTIFYING GAPS IN OBESITY SCIENCE: CLINICIAN KNOWLEDGE, COMFORT, AND SENSITIVITY; PATIENT AVOIDANCE; CONNECTION TO RESOURCES; AND COST-EFFECTIVENESS

A critical gap in implementing obesity science into practice is the central focus on ascertaining a particular body weight. Obesity, as traditionally defined by body mass index (BMI), is remarkably heterogeneous, and the use of the BMI alone leads to confusion about when and how to initiate targeted obesity interventions. It is well known that BMI cannot distinguish between lean and fat mass and that it fails to discriminate between adipose tissue depots in different anatomic regions. For example, a BMI-centric approach has spawned a debate about metabolically healthy obesity, referring to populations with lower cardiovascular risk due to lower visceral abdominal adiposity and higher levels of cardiorespiratory fitness despite an elevated BMI.[23,24] Moreover, the debate about the potential dangers of weight loss and the concept of the obesity paradox—whereby patients with symptomatic CVD (eg, heart failure) who maintain higher body weight (overweight or class I obesity) experience improved survival—has led to controversies, particularly in cardiovascular medicine, and skepticism of the merits in prescribing weight loss interventions to patients with existing cardiac conditions.[24,25] These weight-centric approaches to obesity management, rather than a focus on obesity-related complications and adverse health outcomes, can be confusing and may discourage some clinicians from even considering obesity management interventions. Therefore, it is clear that we need better tools to assess the degree of obesity and its relationship to associated health risks. Furthermore, over-reliance on BMI may paradoxically hinder efforts by clinicians in many settings to address obesity. The net effect is that the vast majority of patients whom clinicians encounter may benefit from weight management. Therefore, BMI adds little useful information for most clinicians in terms of how to prioritize care for obesity based on the risk of obesity-related conditions. There is evidence that waist circumference may be useful in this regard; however, implementing routine measurements and actionable steps to address waist circumference in the context of BMI remains a challenge in clinical practice.

Implementation Gaps in Lifestyle Interventions

In a recent study, few health care practitioners (16%) could identify evidence-based lifestyle treatments for obesity, and there was a high level of heterogeneity by practice type.[26] This included low levels of working knowledge about diet and nutrition specialists (ie, when to refer and identification of barriers to specialist referral), intensive behavioral therapy, and physical activity. This gap in recognition may help explain the low rates of referrals to clinical weight management programs and other weight reduction systems for those who are eligible. Further barriers include a lack of clinician comfort in initiating and conducting discussions about obesity with patients; hesitancy to reduce trust or offend patients who may be seen, incorrectly or not, as wanting to avoid these interactions; assumptions about patient interest in weight management strategies and access to them; and structural issues such as poor coverage or low levels of reimbursement for obesity-related care.

Not surprisingly, 23% of patients never speak to a clinician about their weight or lifestyle interventions for weight management. When discussions did occur, almost 60% of respondents reported that clinicians never asked for permission before discussing sensitive issues related to obesity, and only about half (52%) thought that their clinician understood the challenges of overweight or obesity.[27] Thirty percent of respondents reported that their clinician did not discuss resources for weight management. Last, >15% of patients reported not seeking care to avoid being weighed or having discussions about weight, with a higher prevalence for those with more severe obesity.[27] This is a major gap in implementation, given that there is clear evidence that intensive lifestyle therapy is considerably more effective than brief advice, and general educational information is provided far more often by physicians than connection or referral to classes, programs, or tangible resources for lifestyle change.[19,27]

Clinicians need to adopt effective and sensitive ways to initiate discussions about weight. As part of a 2017 roundtable workshop,[21] Rao describes the "opening the door" approach to initiating discussions, which seeks permission to initiate discussions in a direct but sensitive way that allows the engagement of patients in further obesity-related discussions. One way to open the door is the following[21]: "I am concerned about your weight. It puts you at risk for several conditions such as diabetes. Is this something that concerns you as well? Is this something you would like to discuss and work on together?"

Alternatively, patients can be empowered to ask their clinicians about weight. Patient empowerment is an important, emerging concept in the engagement and delivery of healthcare.[28] Patients can be encouraged to ask questions about a wide range of issues of importance to them related to their care.[29] Prompts (sent, for example, by an electronic patient portal) such as "Don't forget to ask your doctor about your weight" or encouraging the question "I'm concerned about my weight and would like your help in achieving and maintaining a healthy weight" are easy ways for patients and clinicians to begin discussions. Many of the barriers to receiving obesity care are exacerbated by socioeconomic and racial or ethnic inequities. Despite a greater interest in weight management conversations and opportunities,[27] underrepresented racial and ethnic groups and those with public insurance are less likely to be referred to weight management programs or have them covered by insurance.[30] Furthermore, there is a significant contribution of psychiatric /psychological factors in terms of both contributing to obesity and creating barriers to engaging in appropriate therapies that are not adequately addressed in current care models.

Implementation Gaps in Pharmacotherapies for Treating Obesity

Newer pharmacotherapies for obesity treatment demonstrate impressive effectiveness in real-world settings that approximates their efficacy in clinical trials. The 2 pharmacotherapies approved most recently by the US Food and Drug Administration (FDA) for long-term weight management are high-dose semaglutide and tirzepatide, which are both associated with an average weight loss of >10% at 6 months in clinical environments, greater than weight loss achieved from other FDA-approved antiobesity medications (AOMs).[31,31a] However, obesity pharmacotherapies continue to be dramatically underprescribed. Although >50% of adults meet the eligibility criteria for obesity pharmacotherapies, a striking minority of adults trying to lose weight are receiving these agents.[32,33] These prescribing patterns for obesity pharmacotherapies stand in stark contrast to those for diabetes and hypertension, conditions that are common consequences of obesity.

The reasons for the low use rates of obesity pharmacotherapies are likely related principally to (1) knowledge gaps among clinicians, (2) concerns about the safety of obesity pharmacotherapies, and (3) perhaps most importantly, coverage limitations. A survey of health professionals demonstrated that only 15% of clinicians were familiar with the guideline-directed indications for prescribing obesity pharmacotherapies.[26] A report from the Government Accountability Office identified limited clinician education and experience related to the provision of obesity pharmacotherapies as a critical barrier to the appropriate use of these medications.[33] The low use of obesity pharmacotherapies is also linked to widespread concerns about their potential harms. These concerns likely reflect a legacy effect of the relatively high side effects of older sympathomimetic and combination obesity medications relative to those seen with newer glucagon-like peptide-1 receptor agonists and dual glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide receptor agonist agents. A 2018 statement from the US Preventive Services Task Force describing the potential harm of obesity pharmacotherapies compared with lifestyle modification may have a powerful influence on clinicians.

Perceptions about the safety of obesity pharmacotherapies.[34] Perhaps the most significant barrier to the

greater use of obesity pharmacotherapies is limited coverage and high out-of-pocket costs for these medications. A 2016 analysis of health insurance plans within the market place exchanges demonstrated that only 11% of the plans had some coverage for obesity pharmacotherapies.[35] Additionally, although only 7 state Medicaid plans provided coverage for obesity pharmacotherapies, historically, Medicare Part D has explicitly excluded them. Given the high cost of these agents, these coverage limitations have contributed significantly to the undertreatment of excess weight, particularly in high-risk, under re- presented, and historically excluded populations with the highest burden of obesity and its associated comorbidities.

The recent approval by the FDA to expand the indication of the AOM semaglutide to reduce the risk of cardiovascular death, heart attack, and stroke in adults with CVD and either obesity or overweight based on the results of the SELECT (Semaglutide Effects on Heart Disease and Stroke in Patients With Overweight or Obesity) trial[35a] is the first step in a potentially major transformation shifting the coverage conversation away from obesity treatment for the goal of weight management to obesity treatment to reduce the risk of resulting adverse clinical consequences. Building on this shift, the Centers for Medicare & Medicaid Services recently issued guidance to Medicare Part D plans stating that AOMs that receive FDA approval for an additional medically accepted indication (eg, CVD) can be considered a Part D drug for that specific use. State Medicaid programs for low-income populations, who are disproportionately affected by obesity and CVD, will also be required to cover FDA-approved AOMs for this same population. However, states may still require step therapy with other medications or treatments before authorization, posing potential delays in access. This news marks transformational progress in policy toward expanding access to AOMs for high-risk, high-need patients for the prevention of adverse cardiovascular events. Nevertheless, ongoing challenges remain, as supplies of glucagon-like peptide-1 agonists, in particular, have been scarce, further limiting their use.

Implementation Gaps in Metabolic and Bariatric Surgery

Bariatric surgery has long been considered the last-line therapy for severe obesity that cannot be managed through lifestyle changes or pharmacotherapies alone. Since bariatric surgery was introduced in the 1950s, the procedures have become safer and more effective.[36] In an umbrella review of meta-analyses, patients who underwent bariatric surgery had lower risks for incident CVD, multiple other obesity-associated conditions (eg, type 2 diabetes, hypertension), and adverse pregnancy outcomes, including gestational hypertension and diabetes.[37] Among patients with preexisting type 2 diabetes or CVD who underwent bariatric surgery, glycemic parameters and measures of cardiac structure and function improved.[38,39] As surgical expertise has grown, eligibility has expanded to include adults with type 2 diabetes and a BMI between 30 and 35 kg/m^2,[40] as well as adolescents with severe obesity and at least 1 major comorbidity.[41] These safety advances and health benefits offer clinicians and patients another option to treat severe obesity.

The critical challenge facing the field is ensuring that the populations with the greatest needs can access bariatric surgery. A significant barrier to the implementation of bariatric surgery, despite established disparities in the prevalence of severe obesity, is that adolescents and adults who identify as Black or Hispanic/Latino and those who have fewer social and economic resources are far less likely to undergo surgery.[40,42,43] Although structural factors that unfairly limit access to surgery account for some of the inequities, additional reasons include the perception and reality that the social supports needed for surgery to be successful are absent in underresourced populations with the greatest needs. Another gap in implementation may relate to the complexity of bariatric surgery that requires patients to have high levels of health literacy to enact the behavioral modifications necessary for favorable long-term (ie, 2-4 years) weight loss and maintenance.[44] Furthermore, widespread availability of high-volume centers is lacking, and as with any procedure, higher bariatric surgical volumes are associated with better outcomes.[45] High-volume bariatric surgery centers are more likely to be in major metropolitan areas and academic medical centers, which are the places that are less likely to treat patients with severe obesity and have fewer socioeconomic resources. In addition, although most private and public insurance companies cover the cost of the procedure and there are no differences in the effectiveness of therapy based on insurance status,[46] patients with public insurance may face additional socioeconomic barriers to follow-up care, including the time and expense required to travel to and from those visits and resulting lost wages while attending appointments. Last, legacy effects related to the social stigma of surgery, safety concerns due to historically higher complication rates, and the multiple requirements to even qualify for bariatric surgery (eg, visits with psychologists, cardiologists, dieticians, and others to meet criteria) contribute to the implementation gap between science and practice.

Cost-Effectiveness of Obesity Therapies and Its Impact on Implementation

Studies demonstrate that despite significant public health efforts to address obesity, rates of obesity are not declining, and the poorer outcomes among individuals with obesity during the COVID-19 pandemic further highlight the need for successful methods for implementing obesity science into clinical practice.[30] An important consideration that can stimulate or stall the implementation of scientific advancements in new treatments for obesity is cost-effectiveness. Among obesity treatments, bariatric surgery procedures consistently demonstrate cost savings, for example, reduced medical costs and expenditures.[47] The cost-effectiveness of nonsurgical obesity treatment (behavioral and pharmacotherapy) has been demonstrated, although the findings are less consistent. This is due in part to lower degrees of weight loss and the challenges in quantifying the multifactorial and likely long-term or lagged benefits from these therapies.[48,49] Studying the cost-effectiveness of obesity prevention is even more challenging.[50] Still, this lack of definitive cost-effectiveness data likely contributes to the low uptake of obesity science implementation in clinical settings. The engagement of stakeholders, community partners, and health economists to help prospective design measurement of program costs and benefits in obesity science research is one strategy that could address this gap.[51]

The limited availability of cost-effectiveness and health outcomes data for obesity treatment relates to additional challenges in clinician reimbursement and patient costs for obesity treatment, which are significant obesity science implementation barriers. A recent qualitative study concluded that primary care clinicians believed that addressing obesity is an essential part of their job and that many find it feasible and rewarding. Yet, a lack of adequate reimbursement emerged as a primary barrier to these clinicians implementing obesity counseling in their practice.[52] For example, policies in which patients must have a BMI $\geq$30 kg/m^2 or clinical pre-diabetes before obesity services are reimbursable can impede and frustrate clinicians and patients who seek to prevent obesity or maintain obesity treatment successes. Furthermore, the limited availability of hard outcomes data (eg, cardiovascular outcomes, mortality) impedes convincing payers to reimburse treatments for obesity. On the other hand, outcomes trials cannot be reasonably conducted in lower-risk populations with obesity (i.e., younger people, those with no/minimal prevalent comorbidities) because of low event rates, high costs, and prolonged follow-up. Moreover, the lack of consensus on appropriate clinical parameters or quality benchmarks to define obesity-related outcomes (given its heterogeneity)[24] that qualify for reimbursement further adds to clinician frustrations and thwarts the implementation of obesity management programs.[23,53] For example, although cardiovascular outcomes and mortality are important, weight loss by itself may lead to more immediate improvements in quality of life and well-being that are not captured in outcomes used by payers to decide coverage. In addition, inadequate reimbursement for evidence-based behavioral treatments by non- physicians (eg, dieticians and psychologists) further limits the application of evidence-based behavioral treatments for obesity.[20] Bariatric surgery is covered by Medicaid, but reimbursement has declined dramatically over the past decade, disincentivizing the provision of this effective treatment.[54] Although questions about the cost-effectiveness and long-term outcomes of many obesity-related treatments remain, evidence for benefit is gradually emerging, and there is a gap between this body of evidence and the willingness of payers to cover treatments.[50,53] This leads to a situation in which clinicians are not incentivized to provide obesity management services, resulting in the further widening of the implementation gap.

FOCUS ON RESOURCES TO FACILITATE IMPROVED POPULATION-LEVEL OBESITY MANAGEMENT

Despite advancements in under - standing the causes and mechanisms that contribute to obesity, ongoing gaps in implementing evidence-based obesity science have impeded efforts to improve the quality, effectiveness, scalability, and equitability of successful obesity strategies into clinical practice. The following sections summarize existing and promising opportunities to address key implementation gaps and enable progress in the translation of obesity science into clinical practice for greater prevention, treatment, and control of this epidemic (Table).

Will Technology Help Address Gaps in Care?

Technology solutions to bridge the know-do gap in obesity prevention and treatment are promising, with emerging evidence to support multiple implementation strategies,[55,56] including in many low- and middle-income countries that have seen the rapid adoption of digital technologies.[57] Mobile health solutions, including popular weight

loss applications (apps), are implementing various evidence-based behavior change techniques.[58] However, the literature needs to be improved by better reporting of implementation strategies.[59] Challenges with awareness, access, and engagement persist, especially in historically excluded groups who experience a high prevalence of obesity and disparities in care. One modeling study, for example, suggested that 75% awareness, 75% downloading, and 75% engagement with notifications may be required to achieve significant changes in physical activity and obesity prevalence among African American women in Washington, DC.[60] Leveraging the increased access to telemedicine is another future opportunity to improve access to obesity specialists and treatments,[56] especially in rural areas where the distance to the clinic is a major barrier.[61] Increasing the use of inexpensive obesity-related health indicators such as routine measurement of the waist circumference as part of the standard vital signs or measurement of the supine sagittal abdominal diameter using sliding-beam caliper to estimate visceral adipose tissue burden[62,63] clinically may shift the focus of obesity treatment from weight/BMI to risk-based markers. An even simpler tool for weight loss is regular (i.e., weekly) self-weighing. Self-weighing as part of weight management programs has been shown to improve weight loss.[64] Wireless scales allow remote monitoring of weight and transmission of weight data to clinicians, peers involved in group programs, and others to provide feedback, accountability, and support.[65]

Table. Implementation Gaps in Translating Obesity Science Into Clinical Practice and New Opportunities (Table view)

Implementation gap	Existing and emerging opportunities
Clinician knowledge about diet and nutrition, physical activity recommendations, intensive behavioral therapy, pharmacological therapies, and bariatric surgeries	Increase clinician training; facilitate referral to obesity medicine specialists
Clinician comfort to discuss weight management and sensitivity to weight stigma/bias	Increase clinician training and diversity; facilitate referral to obesity medicine specialists; engage community health workers for underrepresented racial and ethnic groups
Cost/insurance coverage/lack of access for pharmacological, bariatric, and behavioral therapies	The Centers for Medicare & Medicaid Services now recognize obesity as a target for treatment to improve cardiovascular health outcomes Increase reimbursement for all obesity management options across all payer types, and increase support for community resources
Patient avoidance and stigma	Make available clinician sensitivity training to reduce weight bias; use telemedicine to enhance adherence to visits; make clinical spaces safe and comfortable for all patients
Perceptions of safety and rates of adverse effects	Offer clinician training and education in obesity medicine; facilitate referral to obesity medicine specialists
Connection to resources	Pair clinical recommendations directly with tangible community resources; enhance use of technology such as digital tools and mobile health solutions
Uncertain cost-effectiveness	Enhance cost-effectiveness and implementation research; engage stakeholders, community partners, and health economists to help design cost-effectiveness programs
Lack of social and community support	Provide treatment approaches focused on couples, families, or households; offer connection to patient and community groups that provides resources and support

Barriers to Commercial and Community Resources Must Be Addressed

In addition to a multilevel need to improve referrals and equitable access to clinical weight management programs and other treatment options, future implementation work needs to emphasize connections to programs outside clinical settings. Some commercial weight loss programs can be effective, but access is often limited by cost, distance, and patient perceptions of safety and belonging. Nonprofit community programs like the YMCA or Take Off Pounds Sensibly[66]

may be more acceptable and affordable than medical or commercial options. These programs offer tangible and structured support for weight loss or maintenance and often serve as a link to social support as well. Strategies to inform patients and clinicians about which programs are available in their area and which might be appropriate according to different patient factors may enhance equitable reach and quality of health services delivery but need to be developed and tested at scale.

Targeting the Patient's Broader Social Support Network to Promote Broader, More Comprehensive Implementation of Obesity-Focused Strategies

Although much of the evidence available for successful weight loss and maintenance programs comes from individual-level clinical trials, there is increasing acknowledgment that lifestyle changes may be more likely to succeed if implemented at the couple, family, or household level.[67–69] The importance of social support for success is well recognized, even for pharmacological and surgical treatments that are inherently implemented at the individual level. Behaviors and environments that contribute to obesity are often shared among those with close social connections, and unsurprisingly, attempts to modify behavior without influencing other sociological factors that contribute to obesity in the first place are often unsuccessful. Despite this growing understanding, widespread implementation of successful programs is rare, and accessibility and acceptability in underrepresented racial and ethnic populations that commonly impede implementation of weight-modifying programs are often overlooked.[70] Although clinicians care for individual patients with obesity, patients are part of families and communities. A patient's social environment has a great deal of influence on their weight, and clinicians need to recognize and assess this broader context, as well as for programs to consider sociological factors. Here is an example: A community-based program must take into consideration what food sources are available at a reasonable cost to participants in the vicinity to be practical and effective.

More Evaluation Is Needed to Support Health Policy Change, Implementation, and Scalability

Health policy changes are essential to increase the provision of available evidence-based strategies such as behavioral therapy, pharmacotherapy, and bariatric surgery to the large population of individuals with or at risk for obesity who critically need these services.[30] Efforts to provide evidence-based coverage for the treatment of obesity are often inhibited by current legislation in the US Congress. Despite recent important advancements in obesity pharmacotherapy coverage by Medicare Part D for individuals with existing CVD to reduce adverse cardiovascular events, further action is needed. Efforts led by the Treat and Reduce Obesity Act to expand Medicare coverage, including screening and treatment of obesity for a broader range of health care clinicians, as well as providing coverage for FDA-approved medications for long-term weight management, have stalled at the federal level over the past decade.[71] This underscores the persistent challenges in achieving universal coverage for AOMs, despite notable progress in addressing specific patient populations, such as those with existing CVD who are at the highest risk for adverse cardiovascular outcomes. Several professional societies and stakeholders are currently engaged in efforts to lobby for health policy changes to make obesity treatment more accessible and affordable for patients with obesity at high risk for developing CVD.

Further evaluation is needed to support policy implementation and scalability. Evidence on implementation costs and cost-effectiveness will be integral to policy implementation efforts and systemic change. Because they can be more rapidly scaled with fewer human resources, digital interventions may prove particularly cost-effective, as shown in a recent analysis of adolescent obesity interventions.[72] This is particularly true for resource-constrained health systems worldwide, where health departments will continue to focus on "best buys" to address an epidemic of noncommunicable diseases.[73,74] Lastly, forming a shared resource library accessible to all clinicians engaged in obesity care might help clinicians connect patients to resources and offer connections that might be the best fit for each patient. Establishing such resources and evaluating their effectiveness is only one example of a future goal for implementing obesity science into clinical practice. Given the widespread increase in obesity prevalence worldwide, attention to global implementation strategies, each with its unique geographic challenges, will be important to address the implementation gap of obesity science in practice worldwide in the future.

CONCLUSIONS

The science of obesity is a relatively young field, gaining traction in the 1970s when the prevalence of obesity among men and women of

all ethnic groups, ages, and educational and socioeconomic levels started to increase.[75] Despite decades of advancement in our scientific understanding of the pathophysiology underlying obesity and its potential treatment, a substantial gap between that knowledge and the successful implementation of obesity science to treat obesity within clinical practice remains. It is equally important to recognize that the lack of sufficient implementation of evidence-informed science into practice, albeit largely evidenced in adults, is magnified in the pediatric and adolescent populations, wherein identifying and managing overweight or obesity is vital to preventing the development of long-term obesity and its sequalae.[76] Determining when and how to implement obesity-targeted therapies for maximum effectiveness remains challenging. Identification of barriers to implementing guideline-driven care and prompt discussions about solutions are needed to ensure that patients in greatest need have access to appropriate therapies. To reach and successfully affect these populations in need, clinicians may consider how the social determinants of health, including insurance type, health literacy, access to health-promoting resources, and social support influence the likelihood of successful treatment. Addressing the barriers to the successful implementation of obesity science into practice requires investment in methodologies proven to narrow the know-do gap that includes education about the complex origins and clinical consequences of obesity, a framework for the delivery of obesity care, and health policy interventions that are essential to the success of applying obesity science to the individual patient. Health care systems can contribute to the success of implementation by coordinating care teams into fewer visits to reduce the burden on patients who would find themselves coming for multiple visits and scheduling with numerous specialists. Comprehensive care teams that include various health care professionals, in addition to social workers and social services personnel, are essential to addressing nonmedical barriers to successful implementation. Public policy should align with implementation efforts to further support the research and evaluation needed to drive policy implementation and scalability. Evidence for implementation costs and cost-effectiveness will be integral to prioritizing policy implementation efforts and systemic change. Funding to promote and sustain such research is vital to the success and reach of these endeavors. Last, there is an urgent need for better education and training in implementing science in obesity medicine. Building obesity care around these principles requires substantial financial input and engagement from multiple stakeholders. Still, the rewards of lower mortality, long-term health care cost savings, and improved quality of life warrant the investment.

Conflict of interest statement

The American Heart Association makes every effort to avoid any actual or potential conflicts of interest that may arise as a result of an outside relationship or a personal, professional, or business interest of a member of the writing panel. Specifically, all members of the writing group are required to complete and submit a Disclosure Questionnaire showing all such relationships that might be perceived as real or potential conflicts of interest.

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Step 2

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Step 3

"Severe Anaemia Contributes to 53% of Malaria-Related Deaths"[3]

In Malaria Treatment

Reference

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** **DHP:** Dihydroartemisinin-Piperaquine Phosphate combination.*
***AA:** Artesunate-Amodiaquine combination.*
***AL:** Artemether-Lumefantrine combination.*

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Allelic frequency of *msp2* and *glurp* genes in *Plasmodium falciparum* isolates from Awka, Anambra, Nigeria.

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ABSTRACT

Introduction

The genetic diversity of *Plasmodium falciparum* correlates with its pathogenicity, therefore the design of evidence-based intervention strategies to eradicate malaria requires genetic diversity surveillance. This study characterised the allelic frequencies and genetic diversity of *P. falciparum* parasites isolated from Awka, Nigeria.

Materials and Methods.

Genomic DNA was extracted from 177 *P. falciparum* isolates, and the polymorphic regions of the *msp2* and *glurp* genes were genotyped by nested polymerase chain reaction (PCR).

Results.

Two *msp2* alleles (3D7 and FC27) were analysed. The 3D7 (93.55%) *msp2* allelic family was predominant in *msp2* positive isolates. Polyclonal *msp2* infection was observed in 24 (38.71%) isolates. Twenty-one distinct *msp2* alleles were detected, with fragment sizes ranging from 200 bp to 1200 bp. The 300 bp allelic fragment (26.83%) was predominant for the 3D7 allele, while the 400 bp allelic fragment (29.54%) was predominant for the FC27 allele. The multiplicity of infection (MoI) in *msp2* was 2.03, and the expected Heterozygosity (He) was 0.34. 69 isolates (38.98%) were positive for the RII repeat region of the *glurp* gene. For the *glurp* gene, nine alleles were detected for fragment sizes ranging from 200 bp to 1150 bp, and the most prevalent allelic fragment was 200 bp (19%). The MoI and He for the glurp gene were 0.45 and 0.98, respectively.

Conclusions.

The high level of polyclonal infections with *P. falciparum* parasites observed in this study indicates extensive genetic diversity in the study area. The data provide essential baseline information that can be implemented in developing malaria control strategies and elimination in the study area and Nigeria

INTRODUCTION

Nigeria has the highest burden of malaria globally. It is a major public health concern in Nigeria with about 200,000 deaths from the disease annually, the primary victims being children <5 yrs and pregnant women [1]. *Plasmodium falciparum* is the deadliest and most prevalent plasmodium species associated with malaria infection in sub-Saharan Africa [1,2]. The extensive genetic diversity of *P. falciparum* strains poses a challenge to efforts to eradicate malaria. It possibly contributes to malaria pathology by suppressing acquired immunity and prompting the emergence of drug resistance and insecticide resistance variants [3,4,5]. Therefore, genomic surveillance of the parasite is essential to develop an effective control strategy to eradicate malaria in Nigeria.

An established method to survey the parasite is by targeted genotyping of merozoite surface

protein (*msp-2*) and glutamate-rich protein (*glurp*) genes as they contain polymorphic regions that are used as markers for defining genetic variation in *P. falciparum* malaria and determining the multiplicity of infection (MoI) [4,6,7]. The *msp-2* glycoprotein is an asexual blood stage antigen that consists of 5 polymorphic blocks, with block 3 being the most polymorphic. The two main allelic families of *msp-2*, FC27 and 3D7, are based on the polymorphic regions of the central repeat sequences [8,9]. The *glurp* protein is an antigen expressed in both the pre-erythrocytic and erythrocytic stages of the parasite, as well as on the surface of newly released merozoites. This antigen consists of three regions, with the immunodominant C-terminal repetitive region (R2) being the most polymorphic [10]. The *msp-2* and *glurp* proteins are also considered promising candidate antigens for the development of a malaria vaccine as they are targeted by cytophilic antibodies and are associated with natural immune protection against clinical malaria [11,12].

Understanding the genetic diversity of *P. falciparum* in different geographical regions of Nigeria is essential for developing new and effective malaria control interventions. Currently, there is limited information on the genetic diversity and multiplicity of *P. falciparum* infection in southeast Nigeria.

This study aimed to evaluate the allelic frequency of *msp2* and *glurp* genes in *P. falciparum* parasites isolated in Awka, an urban city in southeast Nigeria. This research will contribute information on disease pathogenesis and immunity acquisition in Nigeria. It also provides information that is beneficial for the development of an effective malaria vaccine.

MATERIALS AND METHODS

This survey was conducted to assess the allelic frequency of *P. falciparum msp2* and *glurp* genes. A total of 179 participants, aged 6 months to 68 years, were recruited between February 2019 and January 2020. Participants were included if they presented with malaria-related symptoms (e.g., fever, chills, headache) at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka, Anambra State, Nigeria. The recruitment was based on clinical suspicion of malaria, and participants were screened using a rapid diagnostic test (RDT) for malaria before enrolment. Written informed consent was obtained from all participants or their legal guardians before inclusion in the study.

Study site and population

The study was conducted in Awka, Anambra State, Nigeria, a region with a tropical climate characterised by a wet season from April to October and a dry season from November to March. The area experiences an average annual rainfall of 1,200 mm and a temperature range of 25–32°C. Malaria transmission in this region is perennial, with peaks during the rainy season. Control measures include the use of insecticide-treated nets (ITNs) and intermittent preventive treatment for pregnant women (IPTp).

Participants were recruited from outpatient clinics, with inclusion criteria as follows: Age between 4 months and 60 years, presentation with clinical signs and symptoms suggestive of malaria and positive RDT for malaria before study enrolment. Exclusion criteria included participants on malaria treatment within the last two weeks or those unwilling to provide informed consent.

Sample collection, preparation and parasite detection

Venous blood samples (2 mL) were collected into EDTA tubes from each participant. RDT was performed using the SD Bioline Malaria Ag Pf/Pan (South Korea), which detects *P. falciparum* and non-falciparum species. Samples positive by RDT were preserved at - 20°C until genomic DNA extraction. DNA extraction was performed using the Quick DNA Miniprep kit (Zymo Research, USA) following the manufacturer's protocol.

Molecular genotyping of *P. falciparum msp2* and *glurp* genes

The polymorphic regions of the *msp2* and *glurp* genes were amplified in a nested PCR reaction. The primary PCR conditions for both the *msp2* and *glurp* genes were an initial denaturation step of 95 °C for 5 min followed by 30 cycles of 95 °C for 1 min, 54 °C for 1 min, 72 °C for 1 min, and a final extension of 72 °C for 5 min. The nested PCR parameters for the *glurp* gene were identical to the primary reaction; only the annealing temperature was adjusted to 59 °C. For the *msp2* gene, the nested PCR conditions were initial denaturation at 94°C for 5 min, followed by 30 cycles at 94 °C for 10 s, 57 °C for 30 s, and 72 °C for 40 s. The final cycle had a prolonged extension at 72 °C for 3 min. The primers targeting the RII region of *glurp* [13] and the 3D7 and FC27 regions of *msp2* [14] are shown in Table 1. The PCR products were separated on a 1.5% agarose gel, stained with ethidium bromide, and visualised using a UV transilluminator (Vilber, France).

Heterozygosity and multiplicity of infection

The expected heterozygosity index

(He) was calculated using the formula:

$$He = \left[\frac{n}{n-1}\right][1 - ?Pi^2]$$

Where n = number of isolates analysed and Pi = the frequency of the i^{th} allele in the population. The multiplicity of infection (MoI) was calculated by dividing the total number of fragments detected in an antigenic marker by the number of samples positive for that same marker. Samples where only one genotype was detected per allelic family were monoclonal, while samples with two or more genotypes per allelic family were polyclonal *P. falciparum* infections.

Data analysis

The Heterozygosity index (He) and multiplicity of in fection (MoI) were calculated. The presence of polyclonal infections was determined by the detection of multiple alleles within the same sample.

Ethical approval

The Chukwuemeka Odumegwu Ojukwu University Teaching Hospital's Ethics Review Board granted ethical approval for the study. (COOUTH/CMAC/ETH.C/Vol.1/0035). Informed consent was obtained from the parent or legal guardian of each child before being included in the study.

RESULTS

The study population consisted of 53.03% female and 46.97% male patients aged 6 months to 68 years. The mean age of participants was 18.67 ± 0.32 yrs. A total of 179 blood samples were analysed for Plasmodium spp. using a malaria RDT kit, which confirmed the presence of *P. falciparum* in 177 (99%) samples

Table 1.

Different sequences of the primers used to amplify *msp2* and *glurp* genes of *P. falciparum* isolates.

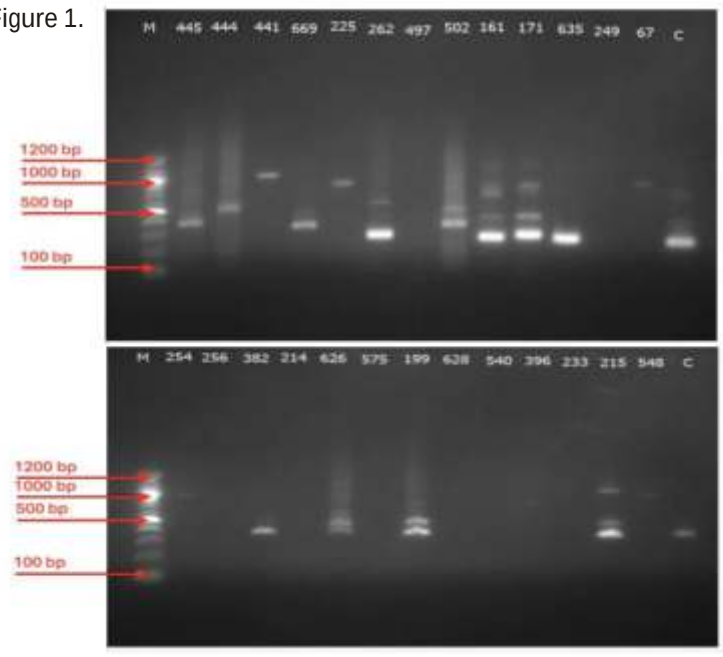
Gene	Primers
msp2 (N1)	F: 5'-ATGAAGGTAATTAAAACATTGTCTATTATA-3'
	R: 5'-TTATATGAATATGGCAAAAGATAAAACAAG-3'
FC27 family (N2)	F: 5'-GCAAATGAAGGTTCTAATACTAATAG-3'
	R: 5'-GCTTTGGGTCCTTCTTCAGTTGATTC-3'
3D7 family (N2)	F: 5'-GCAGAAAGTAAGCCTCTACTGGTGCT-3'
	F: 5'-GATTGTTTCGGCATTATTATGA-3'
glurp (N1)	F: 5'-TGAATTTGAAGATGTTCACACTGAAC-3'
	R1: 5'-ACATGCAAGTGTTGATCCTGAAG-3'
(N2)	F: 5'-TGTTCACACTGAACAATTAGATTTAGATCA-3'
	R2: 5'-TGTAGGTACCACGGGTTCTTGTGG-3'

Table 2.

Prevalence of *msp2* and *glurp* genes.

Markers	Frequency (%)	Allele size (bp)	Total No of Alleles	Distinct alleles
FC27	28 (45.16%)	250 -1200	44	10
3D7	58 (93.55%)	200 -1100	82	11
FC27 + 3D7	24 (38.71%)			
Glurp	69 (38.98%)	200-1150	79	9

Figure 1.



Representative Gel images of samples showing different alleles for 3D7 (top) and FC27 (bottom).

Genetic diversity of *P. falciparum* infection

PCR amplification was successful in 35.02% (62/177) of the isolates for the *msp2* gene and 38.98% (69/177) of the isolates for the *glurp* gene (Table 2). For *msp2*, the Fc27 allele had a frequency of 45.16% (28/62), while the predominant 3D7 allele had a frequency of 93.55% (58/62). Monoclonal infections were identified in 38 isolates (61.29%), with 34 isolates (54.84%) positive for the 3D7 allele and 4 isolates (6.45%) positive for the FC27 allele. Polyclonal infections (FC27+IC3D7) were detected in 24 isolates (38.71%). The RII repeat region of the *glurp* gene was detected in all 69 isolates. Representative gel pictures are presented in Figures 1.

Allelic frequency of *msp2* and *glurp* genes

The allelic genotyping data revealed the polymorphic nature of *P. falciparum* parasites in Awka. In the *msp2* and *glurp* genes, different allelic types were identified. Alleles of *msp2* and *glurp* were successful in 69 isolates. Nine alleles with fragment sizes ranging from 200 to 1150 bp were identified. The most prevalent allelic fragment was 200 bp (45.24%), while both the 1050 bp and 1150 bp allelic fragments were the least prevalent (2.38%) (Figure 2)

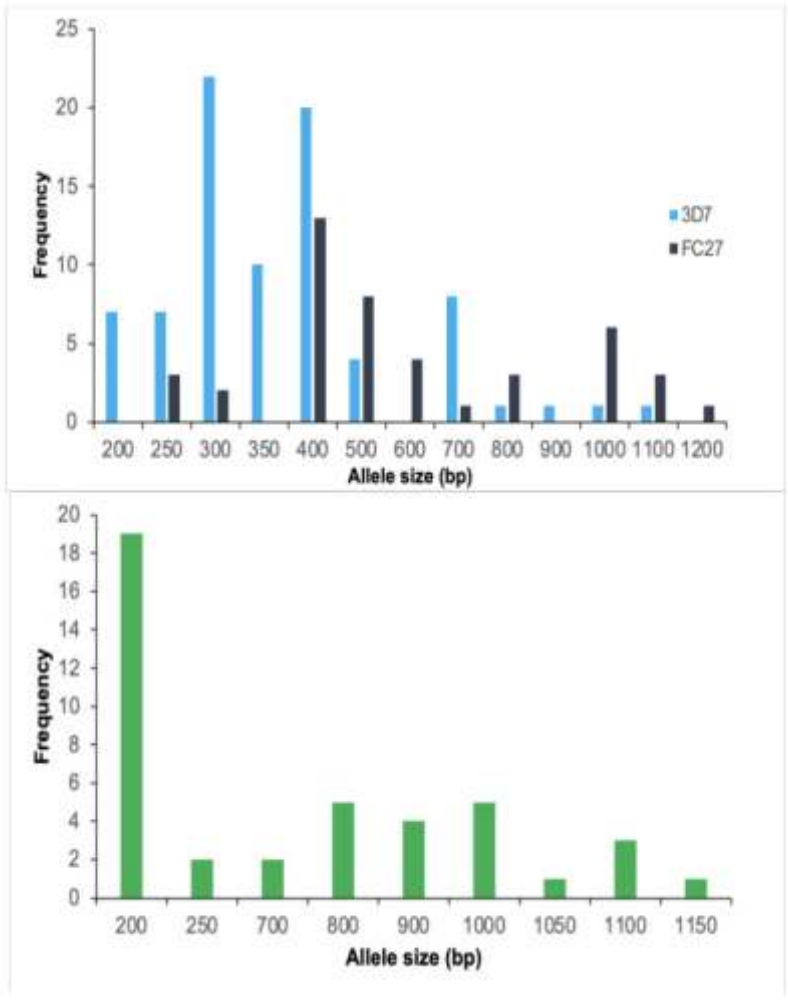
Multiplicity of infection (MoI)

The MoI for the *msp2* gene (MoI = 2.03) was high, as was the heterozygosity (He = 0.34). The MoI and He for the *glurp* gene were 0.45 and 0.98, respectively.

DISCUSSION

Nigeria is a malaria-endemic country, and an increase in the genetic diversity

Figure 2.



Prevalence of *P. falciparum* 3D7 and FC27 *msp2* alleles (top) and *glurp* alleles (bottom).

of *P. falciparum* strains could lead to more complex infections and the emergence of more virulent or drug-resistant variants, endangering efforts to eradicate the disease [15]. Genetic diversity and polymorphism are key in the acquisition of anti-malaria parasite immunity [16,17]. Therefore, determining the frequency of Plasmodium genotypes in different geographical locations would facilitate the development of effective control strategies. Polymorphic markers in *P. falciparum* isolates were used to examine the genetic diversity and complexity of parasite populations in patients with symptomatic malaria at the Chukwuemeka Odumegwu Ojukwu Teaching Hospital, Awka, Nigeria.

In this study, allele-specific genotyping of *msp2* in *P. falciparum* isolates reveals high allelic diversity in Awka, Nigeria. The high malaria transmission rate, the incidence of mixed illnesses, and the subsequent exposure of locals to mosquito bites may be responsible for this trend in the research area. For *msp2,* the 3D7 alleles were predominant, with a 93.54% occurrence. This data is consistent with studies from Kaduna [18], Ibadan [19], Anambra [7], and north-central Nigeria [20,21] that reported a high prevalence of the 3D7 family, in symptomatic patients, but the

results contrast with a study by Ojurongbe et al. [22] in Osogbo, Nigeria, which reported the FC27 allele as the predominant allele. The result is in agreement with other studies conducted in sub-Saharan Africa [23,24], South America [16], and Asia [17,25]. The results suggest that the frequency of 3D7 alleles strongly correlates with symptomatic malaria. The result is conflicting as the 3D7 allele is associated with asymptomatic malaria infections, and it is thought to offer protection against clinical disease [26]. The contrasting observation from various studies indicates a need to understand the influence of human genetic factors on the antigenicity of *msp2* alleles as the variations observed in different studies may be attributed to immune selection pressures.

Among the *msp2* positive *P. falciparum* isolates, polyclonal infections were observed in 38.71% of participants. Complex infections marked by multiclonality impact drug efficacy, severity of disease and the population diversity of the parasite [4]. Polyclonal infections are associated with low levels of protective malaria antibodies, increased prevalence of drug-resistant parasites and possibility of recrudescence [27,28]. More importantly, malaria vaccine efficacy studies on the *msp2* gene have shown that vaccination with only one *msp2* variant induces an allele-specific response. In one study involving a vaccine that comprised the 3D7 allelic family, vaccinated patients showed an increase in morbidity associated with the FC27 alleles [29]. Subsequent studies have shown that chimeric vaccines [30] and vaccines containing both *msp2* allelic families [31] induce a strain-transcending immune response. This necessitates that the malaria vaccine design incorporates both allelic variants of the *msp2* gene to account for the increase in polyclonal infections observed in this study.

The high genetic diversity for *msp2* is consistent with observations in malaria-endemic regions [4]. The high genetic diversity may correlate with the rate of polyclonal infections and the intensity of malaria transmission in the region [4]. The multiplicity of *P. falciparum* infections (MoI) for *msp2* reported in this study is similar to studies from Nnewi [7] and Ibadan [32] in Nigeria. However, other studies from southwest Nigeria [33,34,35] and Pahang, Malaysia [36] reported a lower MoI, while a study on children living close to a lake in Taabo, Côte d'Ivoire, reported a higher MoI. The multiplicity of infection (MoI), i.e., the number of different *P. falciparum* strains co-infecting a single host in many malaria-endemic areas is a common feature and has been reported to vary with age, parasite density, immune status, epidemiological settings, and transmission intensity [37]. The MoI observed in this study could indicate high transmission levels of the parasite.

The RII region of the *glurp* was also shown to have a high prevalence in the study population, with a 38.98% occurrence. The study by Ullah et al. [6] showed a higher prevalence of 70% in Pakistan, consistent with another study conducted in south western Nigeria [32]. Furthermore, a study in Osogbo, Nigeria, reported that the genetic diversity of the R2 polymorphic region of the glurp gene remained diverse despite the implementation of the artemisinin-based combination therapy ACT therapy in the study area [38]. The *glurp* gene is an antigen of *P. falciparum* that is highly conserved, present in all stages of the malaria parasite and associated with clinical immunity. These attributes make it a promising biomarker for diagnosis and the development of vaccines against malaria.

CONCLUSIONS

The present study shows that there is a high level of polyclonal *P. falciparum* infections in the population. The *P. falciparum* parasites harbour multiple gene alleles with high MoI. This indicates the extensive genetic diversity of *P. falciparum* infection in the study area. The data provides important baseline information to guide malaria control and elimination strategies in the study area and across Nigeria.

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COMPETING INTERESTS

The author declares no competing interests.

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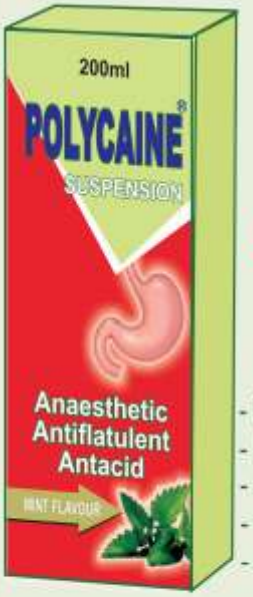

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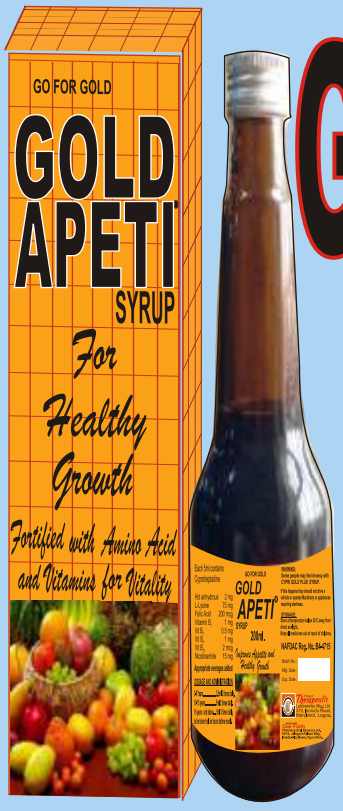

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Irritable Bowel Syndrome: A Hallmark of Psychological Distress In Women?

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Abstract

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterized by abdominal pain, bloating, and altered bowel habits. Women are disproportionately affected by IBS due to a complex interplay between genetic, environ-mental, and psychosocial factors, along with a crucial role of the gut–brain axis in modulating both bowel function and pain perception. Evidence suggests a strong association between psychological distress and IBS symptoms. Women with IBS report higher levels of psychological distress compared to men, and sex is a biological variable that shapes several aspects of the mechanisms, epidemiology, and clinical manife- stations of IBS. This paper explores the bidirectional relationship between psychological factors and IBS with a focus on women. Stress, anxiety, depression, and childhood trauma contribute to IBS symptoms, and societal and biological factors unique to women may exacerbate this condition. Strategies for integrated care approaches and gender-specific treatment strategies are needed to improve patient outcomes and quality of life.

Keywords: irritable bowel syndrome, psychological distress, women, anxiety, depression

1. Introduction

Irritable bowel syndrome (IBS) is a prevalent condition affecting 10– 20% of the global population. It is considered a common functional gastrointestinal disorder characterized by chronic or recurrent abdominal pain associated with altered bowel habits, such as diarrhea (IBS-D), constipation (IBS-C), or a mix of both (IBS-M) [1]. Unlike structural gastro- intestinal disorders, IBS lacks detectable anatomical abnormalities or inflammatory markers, leading to its classification as a disorder of gut– brain interaction. The characteristic presentation of IBS is the presence of abdominal pain, typically relieved or exacerbated by defecation, along-side irregular bowel patterns. The Rome IV criteria, a standardized diagnostic framework, are widely used to identify IBS based on symptomatology [2]. IBS is strongly linked to disturbances in the gut– brain axis, which encompasses bidirectional communication between the central and enteric nervous systems, modulated by microbial, hormonal, and immune pathways [3]. Enhanced sensitivity to gastrointestinal stimuli, referred to as visceral hypersensitivity, is frequently observed in patients with IBS and contributes to symptom severity [4]. Dysbiosis, or an imbalance in gut microbial composition, is implicated in the pathogenesis of IBS. Alterations in microbiota can influence intestinal permeability, immune activation, and neuroendocrine signaling [5].

Women are 1.5 to 3 times more susceptible than men to IBS, suggesting a potential link between gender-specific factors and the disorder [6]. The mechanisms of irritable bowel syndrome (IBS) involve both central and peripheral mechanisms that alter several bowel functions. These dysfunctions are associated with motor, sensory, immune, barrier, and intraluminal perturbations [7]. While there is evidence that these mechanisms are altered in both females and males, sex represents a biological variable that impacts several aspects aspects of the mechanisms, epidemiology, manifestations, and exacerbation of IBS [8].

The fact that women exhibit a higher prevalence of IBS compared to men is documented by a female-to-male ratio of approximately 2:1 [6]. This sex disparity extends to symptom severity, with women often reporting more intense abdominal pain, bloating, and diarrhea. Further- more, women with IBS are more likely to experience a higher burden of psychological comorbidities, including anxiety and depression. Several factors may contribute to these sex-based differences. Hormonal fluctuations, particularly variations in estrogen and progesterone levels, may influence gut motility, visceral sensitivity, and immune function in women. Sex-specific differences in gut microbiota composition and function may also play a crucial role. Additionally, women may experience higher levels of stress and anxiety, which can exacerbate IBS symptoms through complex neuroendocrine and immune pathways [7,8].

The disorder has a significant impact on quality of life, often leading to psychological distress, reduced productivity, and increased health-care utilization [9]. Workers with IBS report greater occupational stressors and work productivity impairments and suffer from psychological distress, a low quality of life, and medical and

economic problems [10].

Research indicates that women with IBS report higher levels of psychological distress compared to their male counterparts [11]. This gender disparity in IBS and psychological distress may be attributed to various factors, including hormonal fluctuations, social and cultural stressors, and gender-specific coping mechanisms.

A systematic review examined the prevalence of anxiety and depression in patients with IBS and found that these psychological conditions are significantly more common in patients with IBS than in the general population [12]. Stress can exacerbate IBS symptoms, leading to a vicious cycle of worsening gastrointestinal and psychological symptoms. Additionally, the presence of PD in patients with IBS has been linked to poorer treatment outcomes and a reduced quality of life [12].

A genetic predisposition is recognized in IBS, and a heritable component of IBS has been demonstrated in twin and family studies, but whether these genetic factors differently predispose individuals to various IBS forms and/or subtypes remains unclear. It has been hypothesized that IBS is a multifactorial, polygenic complex genetic disorder, and a combination of genetic factors and environmental factors lead to alterations in gastrointestinal sensation and motor function that ultimately result in symptom manifestation [13]. A study identified and confirmed six genetic suscepti- bility loci for IBS. Implicated genes included NCAM1, CADM2, PHF2/FAM120A, DOCK9, CKAP2/TPTE2P3, and BAG6. The first four are associated with mood and anxiety disorders, are expressed in the nervous system, or both [14]. A study associated variants at the locus 9q31.2 with the risk of IBS in women. Since this region has been linked to a series of human conditions that are under the influence of hormonal stimuli, especially those involving the mechanisms of action of sex hormones, authors speculate that this result may provide additional rationale for investigating the role of sex hormones and autonomic dysfunction in IBS [15].

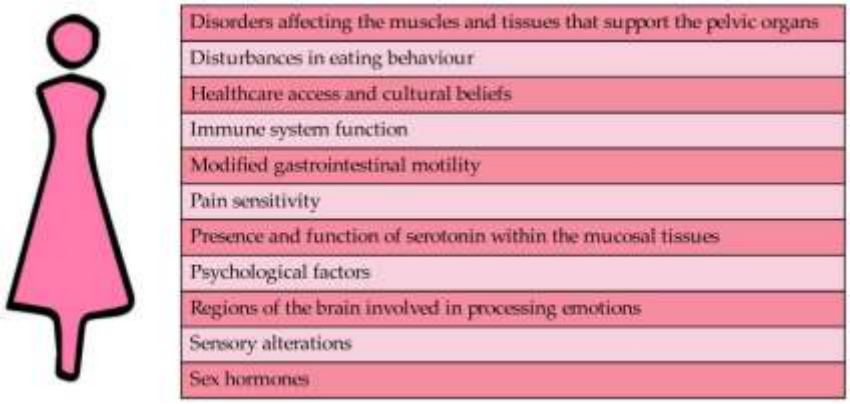
Figure 1 presents a comprehensive overview of the multifaceted factors that may contribute to this gender disparity. As illustrated in the figure, hormonal fluctuations and altered gut–brain interactions are among the key biological factors that may play a role. Additionally, psychological factors such as stress and anxiety, as well as social factors such as healthcare access and cultural beliefs, may also contribute to the higher prevalence of IBS in women. Understanding the complex interplay of these factors is essential for developing effective prevention and treatment strategies for IBS in women.

This paper reviews current research to investigate whether IBS may serve as a hallmark of psychological distress in women. A comprehensive literature search was conducted in the PubMed, CINAHL, EMBASE, and PsycINFO databases up to December 2024. The following keywords were utilized: “irritable bowel syndrome”, “gastrointestinal symptoms”, “psychological distress”, “women”, “anxiety”, and “depression”. To ensure the relevance and quality of the included studies, specific inclusion and exclusion criteria were applied. Studies were included if they explored the psychological aspects of IBS regarding women’s mental health. Clinical trials, observational studies, and systematic reviews were prioritized, while non-peer-reviewed articles, conference abstracts, case reports, and single-patient studies were excluded. Only peer-reviewed articles written in English were considered. Studies that were not published in English or that focused on pediatric populations were excluded from the research. Additional articles were identified through a manual search of the reference lists of the retrieved publications.

2. Pathophysiology of IBS

The pathophysiology of IBS is multifactorial and involves a complex interplay of various mechanisms. The key factors include visceral hypersensitivity, abnormal gut motility, and dysfunction of the autonomic nervous system. These mechanisms make the bowel susceptible to both exogenous and endogenous factors, such as gut flora alterations, psychosocial stressors, and dietary components [8].

IBS is classified as a functional disorder, meaning no identifiable structural abnormalities explain the symptoms. Central to its pathophysiology are disturbances in the gut–brain axis, a bidirectional communication system involving the central and enteric nervous systems. In particular, the pathophysiology of IBS can be considered multifactorial and is still incompletely understood, implicating neuroenteric communication through dysregulated signaling within the gut–brain axis, involving neurotransmitters like serotonin [16] and immune activation, and through low-grade inflammation and immune dysregulation, noted in subsets of patients with IBS, particularly following infections [17].



Sex differences in IBS: key contributing factors. This figure highlights the key factors contributing to the observed sex differences in IBS. It depicts the influences of hormones, immune function, psychosocial factors, and differences in gut physiology.

Among the key players in these processes are ion channels, which are integral membrane proteins regulating ion flow across cellular membranes. The role of these channels is increasingly recognized in the pathogenesis of IBS. Ion channels regulate diverse physiological processes, such as neurotransmission, muscle contraction, and secretion in the gastrointestinal tract. These channels are expressed in enteric neurons, smooth muscle cells, and epithelial cells. The dysfunction of ion channels disrupts homeostasis, contributing to symptoms of IBS. The transient receptor potential (TRP) channel family consists of nonselective cation channels activated by a variety of stimuli, including mechanical stress, temperature, and chemical signals. Several members of this family have been involved in IBS. TRPV1 is a heat-sensitive channel activated by capsaicin and protons. The upregulation of TRPV1 in colonic afferent nerves has been observed in patients with IBS, correlating with enhanced visceral hypersensitivity. Its activation contributes to abdominal pain by lowering the pain threshold in the gut [18]. TRPA1 is activated by irritants and oxidative stress and is linked to neurogenic inflammation and visceral hypersensitivity in IBS. Studies indicate that TRPA1 activation results in the release of pro-inflammatory neuropeptides, exacerbating pain perception [19]. TRPV4 plays a role in mechanosensation. The dysregulation of TRPV4 has been implicated in visceral hypersensitivity seen in IBS, particularly in pain exacerbated by colonic distension [20]. Voltage-gated ion channels contribute to the excitability of enteric neurons and muscle cells. Mutations in the SCN5A gene, encoding the Voltage-Gated Sodium Channel NaV1.5, are linked to IBS, especially in subtypes with constipation-predominant symptoms. These mutations affect the excitability of enteric neurons and GI motility [21]. Calcium channels modulate neurotransmitter release in the enteric nervous system. The dysregulation of these channels alters neuronal signaling, contributing to visceral hypersensitivity [22]. Chloride channels, such as CFTR (Cystic Fibrosis Transmembrane Conductance Regulator), regulate fluid secretion in the gut. Dysfunctional chloride transport can lead to diarrhea or constipation, common symptoms in IBS [23]. Mechanosensitive channels, including Piezo channels, detect mechanical stress in the gut. The abnormal activation of these channels may enhance pain sensitivity and alter motility, which are hallmark features of IBS [24].

Psychological factors seem to play a pivotal role: anxiety, depression, and stress are highly comorbid with IBS, exacerbating its clinical presentation and perpetuating gut–brain communication disturbances [25]. In particular, women with IBS often exhibit heightened visceral hypersensitivity, dysregulated gut motility, and altered microbiota composition, which may interact with psychological distress to perpetuate symptoms [16].

Recent studies have highlighted the role of immune activation in the development of IBS [26]. Auto- antibodies targeting the enteric nervous system have been identified in some patients with IBS, suggesting an autoimmune component to the disorder [26]. Additionally, genetic factors and polymorphisms in human DNA may contribute to susceptibility to IBS [8]. Understanding these pathophysiological mechanisms is crucial for developing targeted treatments that address the underlying causes of IBS rather than just managing symptoms [27].

3. Gut–Brain Axis and IBS

The gut–brain axis plays a crucial role in the regulation of gastrointestinal functions. Chronic stress has been widely recognized as a trigger and exacerbating factor of irritable bowel syndrome. Figure 2 illustrates a conceptual framework outlining the potential mechanisms underlying the development of IBS. Adverse experiences during early life may predispose individuals to a dysregulated HPA axis response to subsequent stressors. Chronic activation of the HPA axis can lead to neurochemical imbalances, particularly in brain regions involved in pain processing, and consequently enhance pain sensitivity. Furthermore, bidirectional communication between the brain and gut can be disrupted, leading to alterations in gut motility, increased intestinal permeability, and dysbiosis. These gastrointestinal disturbances, in conjunction with central sensitization, contribute to the development and persistence of visceral hypersensitivity and IBS symptoms.

The gut–brain axis involves complex interactions between the gastrointestinal tract, central nervous system, autonomic nervous system, and immune signaling pathways. Dysregulation in this axis is thought to play a central role in the etiology of IBS. Neurotransmitters such as serotonin and dopamine, which influence both mood and gastrointestinal function, are frequently dysregulated in patients with IBS. Women with IBS demonstrate unique patterns of gut–brain communication, potentially linked to hormonal variations and psychosocial stressors [28].

Recent research has expanded the understanding of the gut–brain axis by highlighting the role of the vagus nerve, gut microbiota, and inflammatory mediators in IBS. The vagus nerve serves as a critical communication highway between the gut and brain, with alterations in vagal tone correlating with increased symptom severity and psychological distress [29]. Additionally, microbial metabolites such as short-chain fatty acids (SCFAs) influence neurochemical signaling and can modulate mood and gut motility, with disruptions in these pathways being linked to IBS [30].

Chronic low-grade inflammation has also emerged as a significant contributor to gut–brain axis dysfunction in IBS. Elevated levels of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), have been observed in subsets of patients with IBS, suggesting an immune component to the disorder [31]. This inflammatory signaling can impact the integrity of the blood–brain barrier and lead to alterations in central nervous system functioning, further exacerbating symptoms [32].

Advances in neuroimaging have provided further insights into the gut–brain axis in IBS. Functional MRI studies have revealed altered connectivity in brain regions responsible for pain processing, emotional regulation, and interoception, such as the anterior cingulate cortex, amygdala, and insula. These findings underscore the importance of integrated approaches to address both central and peripheral mechanisms in IBS management [25].

4. Microbiota Alterations in IBS

The gut microbiota, a diverse ecosystem of microorganisms residing in the gastrointestinal tract, plays a critical role in maintaining gut health and modulating the gut–brain axis. Dysbiosis, or microbial imbalance, has been consistently observed in patients with IBS. Studies report reduced microbial diversity and alterations in the abundance of key bacterial species, such as reductions in *Lactobacillus* and *Bifidobacterium* populations and an increase in pathogenic strains like *Escherichia coli* [33].

Emerging research highlights the role of microbial metabolites, such as short-chain fatty acids (SCFAs) and tryptophan derivatives, in influencing gut motility, intestinal barrier integrity, and neurochemical signaling. SCFAs, produced through the fermentation of dietary fibers by gut bacteria, have anti-inflammatory properties and are critical for maintaining gut health. Disruptions in SCFA production have been implicated in the pathophysiology of IBS [30].

Recent advances in metagenomics and metabolomics have provided deeper insights into the functional alterations of the gut microbiome in IBS. For example, studies using 16S rRNA sequencing have identified specific microbial signatures associated with diarrhea-predominant (IBS-D) and constipation-predominant (IBS-C) subtypes. These findings suggest that tailored microbiome-targeted therapies can offer a promising avenue for treatment [34]. Probiotics and prebiotics have gained attention for their potential to restore microbial balance and alleviate IBS symptoms. Clinical trials have demonstrated that multi-strain probiotics, including *Lactobacillus* and *Bifidobacterium* species, can reduce bloating, abdominal pain, and stool irregularities [1]. However, the efficacy of these interventions varies, highlighting the need for personalized approaches based on individual microbiome profiles.

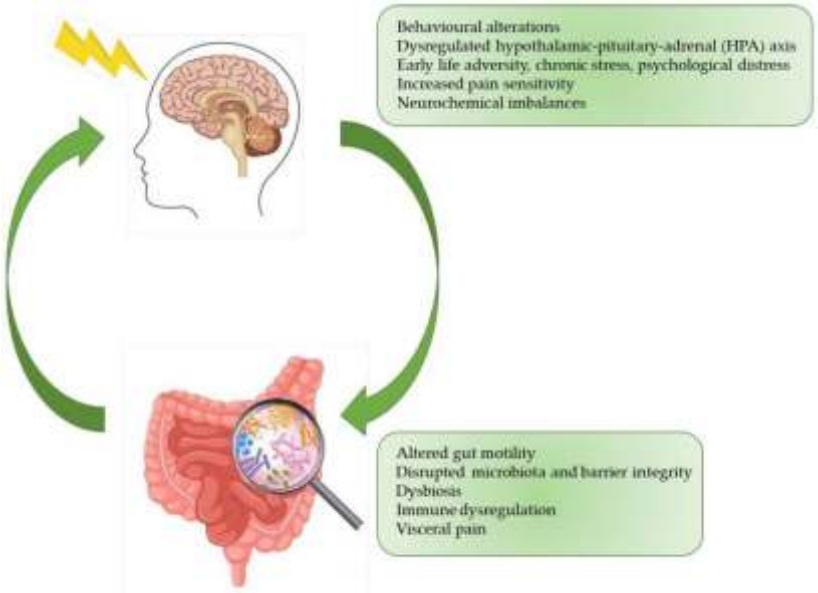
The gut virome and fungal communities are also gaining recognition as contributors to the pathogenesis of IBS. Preliminary studies suggest that bacteriophages and fungal species, such as *Candida*, may interact with bacterial popula- tions to influence gut health and symptomatology. These findings underscore the complexity of the gut ecosystem and its relevance to IBS [35]. In addition, there are potentially distinct sex-related differences in the mucosal microbiomes of patients with IBS, supporting the importance of studying sex-specific mechanisms in the pathophysiology of IBS [36,37]. In women with IBS, lower abundances of *Coriobacteriaceae* and *Shuttleworthia* and increased abundances of *Bacteroidales*, *Christensenellaceae Christensenella*, *Anaerovorax*, *Mogibacterium*, and *Psuedobutyrivibrio* species have been observed compared to men with IBS [38].

Overall, microbiome research underscores the importance of gut microbial composition and functionality in IBS. Although there are clinical trials that have made good progress, more standardized, more generalized, larger-scale, and multi-omics clinical studies are missing. Future research integrating multi-omics approaches may unlock novel therapeutic targets, paving the way for microbiome-based precision medicine in IBS management.

5. The Role of Stress in IBS

Stress is a well-established trigger for IBS symptoms. The activation of the HPA axis during acute or chronic stress leads to elevated levels of cortisol and other stress mediators, which influence gut motility, permeability, and immune activation [39]. Women with IBS often report heightened symptom severity during stressful life events, such as bereavement, work challenges, or

Figure 2.



Bidirectional interactions between the brain and gut in IBS. This figure illustrates the complex bidirectional communication pathways between the brain and the gut in IBS. It emphasizes the role of this gut–brain axis in the development and maintenance of IBS symptoms.

caregiving responsibilities [40]. Hormonal influences further compound the effects of stress in women. Fluctuations in estrogen and progesterone levels, particularly during the menstrual cycle, can modulate the stress response and exacerbate gastrointestinal symptoms. Significant associations between endometriosis and IBS, and a linear relationship between acyclic pelvic pain severity and the odds of IBS, have also been observed [41,42]. There is still debate about a possible association between polycystic ovary syndrome (POCS) and IBS; it is clear that several common potential pathways may directly and indirectly contribute to the interaction between IBS and PCOS, including an alteration in sex hormones or the gut–brain axis, the dysregulation of neurotransmitters and inflammatory factors, metabolic or reproductive disturbances, and psychological, environmental, and lifestyle factors [43].

Pregnant and postpartum patients with IBS have higher odds of psychological comorbidities in addition to medical comorbidities, such as migraines, connective tissue diseases, and autoimmune diseases [34].

Menopause—characterized by a decline in estrogen levels—can also lead to changes in gut function and symptom severity [44]. These hormonal dynamics underscore the importance of considering gender-specific factors in the management of IBS.

It has been outlined that women with IBS not only show greater psychological distress, but also lower body appreciation, higher body dissatisfaction, and higher self-criticism than controls. Body appreciation and self-criticism sequentially mediate the link between IBS status and both depression and anxiety. IBS is associated with reduced body appreciation, which in turn is linked to heightened self-criticism, thereby leading to elevated psychological distress. In other words, particularly in women, IBS negatively impacts body image appreciation, fostering self-critical judgments that exacerbate psychological symptoms [45].

The impact of disease on sexuality and intimacy is one of the main concerns of patients with IBD. The prevalence of sexual dysfunction in patients with IBD has been reported to be 45–60% among women and 15–25% in men, with the most frequently associated factor being depression. Disease characteristics or disease activities are not associated with sexual dysfunction. The quality of sex life is a major component of quality of life in patients with IBD. In addition, in these patients, sexual dysfunction has a significant influence on the overall quality of life and is linked with poorer family functioning [46].

Additionally, societal pressures and higher rates of gender-based violence and discrimination may increase psychological distress in women, further aggravating IBS. Research has shown that women with IBS report higher levels of catastrophizing (a tendency to anticipate the worst outcomes) and reduced coping strategies compared to men [47]. Societal expectations and gender-specific stressors may contribute to the higher prevalence of IBS in women. Women are more likely to face caregiving responsibilities, workplace inequalities, and experiences of harassment or abuse. These stressors can increase their vulnerability to both psychological distress and IBS [6].

6. Psychological Distress and IBS

Psychological factors play a significant role in IBS. Studies revealed that up to 60% of patients with IBS also suffer from psychiatric comorbidities such as anxiety or depression [48]. Stress has been shown to exacerbate IBS symptoms by activating the hypothalamic –pituitary –adrenal (HPA) axis and increasing intestinal permeability. Chronic psychological distress can lead to the sustained activation of these pathways, contributing to the chronicity of IBS.

Previous studies have shown that IBS seems to be tightly linked to psychiatric disorders, more frequently anxiety disorders and depression. While psychiatric disorders are the most common comorbidity of IBS, it is often difficult to determine the temporal sequence of these conditions. It was found that major depressive disorder increases the risk of IBS, and IBS also increases the risk of MDD, demonstrating a significant bidirectional association between these conditions [49]. Psychiatric disorders and, in particular, mood disorders may play an important role in the development and persistence of IBS.

During pregnancy, maternal stress, signaled through elevated cortisol, can influence the fetus's development and potentially the mother's health. This interaction may have implications for the development of IBS and postpartum depression in the mother, and it may have potential health effects on the child. Maternal prenatal stress acts on the HPA axis, consequently increasing circulating cortisol levels, which in turn can affect the maternal gut microbiota. Maternal cortisol crosses the placenta, thus increasing cortisol-circulating levels in the fetus. This leads to the dysregulation of the HPA axis, conditioning the gut microbiota, microbial metabolites, and intestinal permeability in the fetus. Microbial metabolites, such as short-chain fatty acids (which also regulate the development of the fetal enteric nervous system), can induce epigenetic changes and modulate a range of diseases. Implicit epigenetic stress information from the fetal enteric nervous system can be conveyed to the fetal central nervous system through the bidirectional microbiota–gut–brain axis, leading to perturbed functional connectivity among various brain networks and the dysregulation of affective and pain processes. This means that elevated fetal cortisol can influence HPA axis development in the fetus. This "programming" may predispose the child to stress-related disorders, including IBS and anxiety, later in life. On the other hand, elevated fetal cortisol may feed back into the maternal system, exacerbating the mother's stress response and potentially contributing to IBS symptoms. In addition, the immune modulation required to maintain pregnancy may transiently suppress inflammation but can rebound postpartum, potentially exacerbating IBS symptoms in the mother [50,51].

Anxiety and depression are highly prevalent in IBS populations, with these psychiatric comorbidities amplifying symptom severity and reducing the quality of life [52]. These conditions contribute to heightened visceral hypersensitivity and dysregulation in brain–gut signaling. Studies suggest that patients with IBS in comorbidity with depression or anxiety present alterations in gut microbiota composition and cause immune, endocrine, and serotonergic system alterations relevant to the common pathophysiology of these comorbid conditions [53]. Neuroimaging studies have shown abnormal activity in the anterior cingulate cortex, insula, and amygdala—regions critical for emotional regulation and pain processing—in patients with IBS, suggesting a neurobiological basis for these associations [16]. The anterior cingulate cortex, a region involved in pain processing, has been investigated regarding its role in the regulation of visceral sensitivity and mental disorders. A crucial involvement of gamma-aminobutyric acid-producing (GABAergic) neurons' movement to the lateral hypothalamus has been found to modulate anxiety-like behaviors, intestinal motility alterations, and visceral hypersen- sitivity [54].

Women with IBS are disproportionately affected by these psychological factors. Studies have shown that women are more likely to report higher levels of anxiety and depression compared to men, potentially due to societal pressures, hormonal fluctuations, and a greater tendency to internalize stress [55]. This internalization can perpetuate the cycle of psychological distress and exacerbate IBS symptoms. Emerging evidence suggests that early-life stressors, such as childhood trauma or adverse experiences, may predispose individuals to IBS by altering brain–gut axis development and increasing vulnerability to stress-related disorders [56]. In addition, although the likelihood of developing IBS due to adverse childhood experiences is similar for women and men, the higher prevalence of adverse childhood experiences and anxiety in women may contribute to the female predominance of IBS [57].

In a recent study, it was shown that a significant increase in 5-HT expression due to neonatal maternal separation in rodent models leads to alterations in intestinal structure and function; inhibiting 5-HT reversed these observed effects. Excess 5-HT in mice with early-life stress increased intestinal neural network density and promoted excitatory motor neuron expression. In particular, 5-HT activated the Wnt signaling pathway (a family of proteins that play critical roles in embryonic development and adult tissue homeostasis) through the 5-HT4 receptor, promoting neurogenesis within the intestinal nervous system. These findings confirm the regulatory role of 5-HT in the enteric nervous system and contribute to providing potential insights for the development of novel therapies for gastrointestinal disorders [58]. Interestingly, maternal separation resulted in increased visceral hypersensitivity while showing a trend for a sex-dependent increase in negative valence behavior in adulthood. Four clusters representing distinct pathophysiological domains reminiscent of the behavioral consequences of early-life stress have been identified (resilient, pain, immobile, and comorbid). These results suggest that the gut microbiota in early life shows sex-dependent alterations in each cluster that precede particular behavioral phenotypes in adulthood and might demonstrate the fact that stress-induced gut microbiota alterations appear in early life and contribute to the sex-specific susceptibility to specific gut–brain phenotypes in adulthood [59].

Addressing these underlying factors through psychological therapies may provide long-term relief for affected individuals.

In summary, women with IBS often report higher levels of psychological distress compared to men with the same condition. This disparity arises from a complex interplay of biological, hormonal, psychosocial, and cultural factors, which can intensify the experience and perception of IBS symptoms in women. Fluctuations in estrogen and progesterone during the menstrual cycle can increase visceral sensitivity, alter gut motility, and exacerbate IBS symptoms. This can lead to stronger emotional and psychological responses to IBS symptoms in women. Hormonal differences may enhance the activity of the hypothalamic –pituitary–adrenal (HPA) axis in women, leading to greater reactivity to stress and subsequent psychological distress. Hormonal changes during perimenopause and menopause can amplify symptoms of anxiety and depression, which are often comorbid with IBS [60].

Studies suggest that women with IBS have greater visceral hypersensitivity compared to men, meaning they perceive gastrointestinal pain and discomfort more acutely. This heightened pain perception can contribute to higher levels of anxiety, depression, and psychological distress. This increased sensitivity may result from differences in the gut–brain axis, which governs the bidirectional communication between the gut and the central nervous system [61].

Women may face unique stressors, such as caregiving responsibilities, societal expectations, and gender-based inequalities. These chronic stressors can exacerbate psychological distress and IBS symptoms. Women are more likely than men to have experienced trauma, including sexual or emotional abuse. Such experiences are strongly associated with IBS and psychological conditions like PTSD, anxiety, and depression [62].

Cultural norms and taboos about discussing bowel habits may lead women to feel embarrassed or isolated, increasing their psychological distress. Societal expectations for women to maintain a certain image or perform caregiving roles may make it harder for women to prioritize their health, exacerbating stress and frustration. In addition, women are more likely to internalize stress and emotions, which can worsen the psychological impact of chronic illnesses like IBS. Women with IBS are more likely to experience comorbid anxiety, depression, and somatization disorders than men. Psychological factors, such as catastrophizing factors, are more commonly reported in women and are

associated with increased IBS symptom severity and distress. All of these conditions can amplify the perception of IBS symptoms and lead to a more profound emotional toll [63].

There is often a tendency for the underdiagnosis or misdiagnosis of IBS in women. Women's symptoms are sometimes dismissed or attributed solely to psychological causes, leading to feelings of invali-dation and frustration. In addition, women are more likely than men to have their IBS attributed to stress or emotional issues, potentially down- playing their physiological concerns. This imbalance can increase distress and hinder effective treatment [64].

7. Integrated Interventions for Psychological Distress in Patients with IBS

The effective management of IBS requires addressing both the gastrointestinal and psychological aspects of the condition.

Due to the limitations of conventional treatments, and considering that treatments are not devoid of side effects and may not be cost-effective, there is a growing interest in complementary and alternative medicine approaches for symptom management. Herbal remedies have been extensively utilized in managing IBS symptoms. Peppermint oil, known for its antispasmodic properties, has demonstrated efficacy in reducing abdominal pain and overall IBS symptoms. A systematic review concluded that soluble fiber, such as psyllium, can improve constipation and global IBS symptoms [65]. Probiotics, which help maintain gut flora balance, have shown promise in alleviating IBS symptoms. However, evidence supporting the use of specific strains is limited [66].

Traditional Chinese medicine, including acupuncture and herbal formulations, has been employed in treating IBS. While some studies suggest benefits, the overall quality of evidence is limited, necessitating further rigorous research [67]. Mind–body therapies are also gaining recognition in IBS management. Gut-directed hypnotherapy seems to be a beneficial therapeutic option for certain patients [68].

Psychological therapies have shown significant efficacy in mitigating the impact of psychological distress on IBS. Cognitive behavioral therapy (CBT) and gut-directed hypnotherapy have been particularly effective in breaking the cycle of stress and symptom exacerbation. These interventions target mal adaptive thought patterns, stress responses, and emotional regulation, offering relief to patients with significant psychological comorbidities [69]. Mindfulness-based stress reduction and acceptance – commitment therapy also offer promising results, helping patients manage their emotional responses to symptoms and improving their overall well-being [70].

Physical exercise through the modulation of gut microbiota may alleviate IBS symptoms. In particular, aerobic exercise (running, swimming, and cycling) enhances the diversity and abundance of beneficial gut bacteria (such as *Lactobacillus* and *Bifidobacterium*). These bacteria produce short-chain fatty acids that are anti-inflammatory and support gut barrier integrity. Studies on patients with IBS participating in aerobic exercise programs have demonstrated significant improvements in the composition and diversity of gut microbiota, together with an alleviation of symptoms like abdominal pain and bloating. Additionally, exercise seems to positively influence mental health by reducing stress and improving mood, which can further relieve IBS symptoms via the gut–brain axis [71,72].

Patients with IBS often attribute the onset or worsening of gastro-intestinal symptoms to food intake. In a Swedish study conducted on a population of predominantly women with IBS, subjects who consumed fewer carbohydrates, coffee, and dairy products and more fats, lactose-free dairy products, and nuts and seeds were compared with controls. In patients, symptom severity and gastrointestinal-specific anxiety were associated with reduced energy and increased carbohydrate intake, lower diet diversity, and worse diet quality. Poor diet quality was associated with a younger age, more severe IBS symptoms, anxiety, and depression [73].

Dietary modifications, such as the low-FODMAP diet, are commonly recommended for IBS management. FODMAP (Fermentable Oligosac-charides, Disaccharides, Mono-saccharides, and Polyols) is an acronym for a certain class of carbohydrates (fermentable short-chain carbohydrates) which are more difficult to digest. The low-FODMAP diet temporarily restricts these carbohydrates to relieve uncomfortable symptoms and give the digestive system a rest, helping restore a healthy balance of gut flora [74]. A gluten-free diet and the Mediterranean diet have also demonstrated efficacy in improving IBS symptoms. It is important to carefully consider age or gastro-intestinal, oncological, or cardiovascular comorbidities when prescribing specific diets to patients with IBS [75].

The efficacy of acupuncture has recently been demonstrated as a non-drug alternative therapy for visceral hyperalgesia-induced IBS: acupuncture can block the excessive stimulation of abnormal pain signals in the brain and spinal cord; it can also stimulate glial cells to block visceral hypersensitivity pain perception and cognition. Further-more, acupuncture can regulate the emotional components of IBS by targeting hypothalamic–pituitary–adrenal axis-related hormones and neurotransmitters via relevant brain nuclei [76].

Pharmacological treatments include antispasmodics, probiotics, and serotonergic agents. The first-line treatment of constipation is the use of laxatives, with secretagogues being used when laxatives are ineffective. Anti-diarrheal drugs should be used as a first-line treatment for diarrhea, with second-line drugs including 5-hydroxytryptamine-3 antagonists, eluxadoline, or rifaximin, where available. The first-line treatment of abdominal pain should be the use of antispasmodics, with gut–brain neuromodulators being prescribed as a second-line treatment. Low-dose tricyclic antidepressants, such as amitriptyline, are preferred [77].

Women may benefit from tailored interventions that address hormonal influences on gut function [78]; for example, post-menopausal women with IBD who underwent hormone replacement therapy showed an improvement in their disease [79].

Vagus nerve stimulation may reduce constipation and abdominal pain and can open possibilities for responding to patient expectations [29,80].

Targeting ion channels offers promising therapeutic avenues for IBS. Antagonists of TRPV1 and TRPA1 have shown potential in reducing visceral pain. Similarly, modulators of voltage-gated sodium and calcium channels may help normalize neuronal excitability and motility. Emerging therapies aiming to correct chloride and mechanosensitive channel dysfunction are under investigation [81].

Some researchers have proposed a latent class analysis, a method of mathematical modeling, to show that patients with IBS can be classified into seven unique clusters based on a combination of gastrointestinal symptoms, abdominal pain, extraintestinal symptoms, and psychological comorbidity. These clusters can be used to predict the prognosis of IBS (e.g., symptom severity), healthcare use (e.g., consultation behavioral, prescriptions, and costs), and impact (e.g., quality of life, work and productivity, activities of daily living, and income). These clusters can also be used to increase the personalization of IBS treatment that better recognizes the heterogenous nature of the condition [9]. Of note, fecal microbiota transplantation holds promise as a microbiota-modulating treatment for major depressive disorder [82].

A multidisciplinary approach involving gastroenterologists, psychologists, dietitians, and gynecologists can provide comprehensive care for women with IBS. Addressing the interplay of psychological, hormonal, and gastrointestinal factors can lead to improved outcomes and quality of life.

8. Conclusions

Evidence underscores a significant interplay between psychological distress and IBS, particularly in women. While IBS is not solely a psychological condition, its strong association with stress, anxiety, and depression highlights the need for integrated care approaches. In women, the bidirectional relationship between psychological factors and IBS is shaped by hormonal fluctuations, heightened visceral sensitivity, and unique psychosocial stressors.

Recognizing IBS as a potential marker of psychological distress can help healthcare providers develop more effective, gender-specific treatment strategies to improve the quality of life in women affected by this condition. Integrated care approaches should address the multifaceted nature of the condition and recognize the unique challenges faced by women. Including gynecologists can help address the interplay between IBS symptoms and hormonal changes. Psychological interventions, such as CBT and gut-directed hypnotherapy, can help manage psychological distress and reduce symptom severity. Integrating these therapies with pharmacological and dietary interventions—such as low-FODMAP diets and serotonergic agents, considering women's specific nutritional needs, particularly during life stages like pregnancy or menopause—can provide a holistic approach to IBS management. For women whose IBS symptoms worsen during menstruation or menopause, hormonal therapy or oral contraceptives may help stabilize hormone levels and alleviate symptoms. Stress reduction techniques may help women manage physical and emotional stress, improving their overall well-being.

Integrated care includes trauma-informed approaches, recognizing that women with IBS may have a history of trauma, such as abuse, which influences their symptoms.

Gender-specific policies and resources ensure that women have access to multidisciplinary care, flexible scheduling, and supportive services like childcare, which can reduce barriers to seeking treatment. For example, telemedicine options and online support groups also provide accessible platforms for ongoing care and peer support.

Healthcare providers trained in gender-sensitive communication are more likely to validate women's experiences and avoid dismissing their symptoms as purely psychological. Building trust through empathetic care encourages women to share details about their symptoms and psychosocial stressors, leading to better diagnostic accuracy and treatment adherence.

Future research should continue to explore the interplay between psychological factors, gender-specific influences, and gastrointestinal symptoms to optimize treatment strategies.

Author Contributions

Conceptualization, G.M. and M.M.; methodology, G.M., G.T. and M.M.; resources, R.P., E.G. and G.T.; data curation, R.P., E.G. and G.T.; writing—original draft preparation, G.M. and M.M.; writing—review and editing, G.M. and M.M.; supervision, G.M., A.G. and M.M. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

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Novel proteomic signatures may indicate MRI-assessed intrahepatic fat state and changes: The DIRECT PLUS clinical trial

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Abstract

Background and Aims:

We demonstrated in the randomized 18-month DIRECT PLUS trial (n = 294) that a Mediterranean (MED) diet, supplemented with polyphenol-rich Mankai duckweed, green tea, and walnuts and restricted in red /processed meat, caused substantial intrahepatic fat (IHF%) loss compared with 2 other healthy diets, reducing NAFLD by half, regardless of similar weight loss. Here, we investigated the baseline proteomic profile associated with IHF% and the changes in proteomics associated with IHF% changes induced by lifestyle intervention.

Approach and Results:

We calculated IHF% by proton magnetic resonance spectroscopy (normal IHF% <5% and abnormal IHF% ≥5%). We assayed baseline and 18-month samples for 95 proteomic biomarkers. Participants (age = 51.3 ± 10.8 y; 89% men; and body mass index = 31.3 ± 3.9 kg/m^2) had an 89.8% 18-month retention rate; 83% had eligible follow-up proteomics measurements, and 78% had follow-up proton magnetic resonance spectroscopy. At baseline, 39 candidate proteins were significantly associated with IHF% (false discovery rate <0.05), mostly related to immune function pathways (eg, hydroxyacid oxidase 1). An IHF% prediction based on the DIRECT PLUS by combined model (R^2 = 0.47, root mean square error = 1.05) successfully predicted IHF% (R^2 = 0.53) during testing and was stronger than separately inputting proteins/ traditional markers (R^2 = 0.43/0.44). The 18-month lifestyle intervention induced changes in 18 of the 39 candidate proteins, which were significantly associated with IHF% change, with proteins related to metabolism, extracellular matrix remodeling, and immune function pathways. Thrombospondin-2 protein change was higher in the green-MED compared to the MED group, beyond weight and IHF% loss (p = 0.01). Protein principal component analysis revealed differences in the third principal component time distinct interactions across abnormal/ normal IHF% trajectory combinations; (p < 0.05 for all).

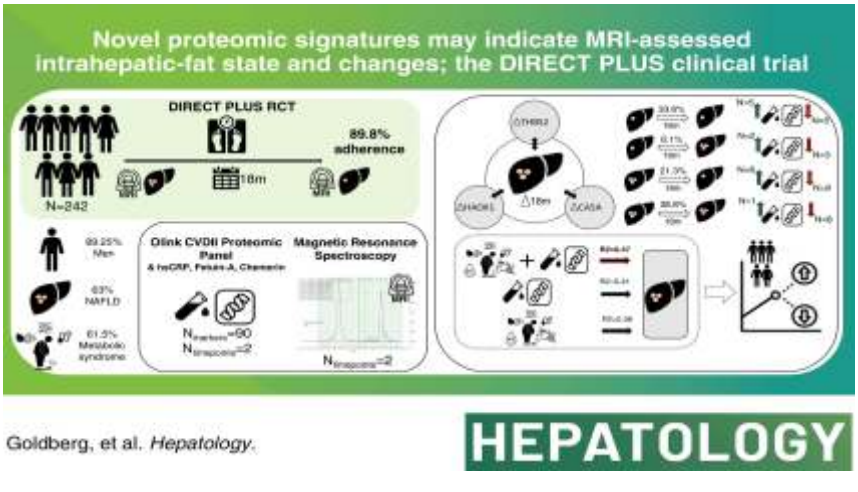
Conclusions:

Our findings suggest novel proteomic signatures that may indicate MRI-assessed IHF state and changes during lifestyle intervention. Specifically, carbonic anhydrase 5A, hydroxyacid oxidase [1], and thrombospondin-2 protein changes are independently associated with IHF% change, and thrombospondin-2 protein change is greater in the green-MED/high polyphenols diet.

INTRODUCTION

Proteomics has the potential to provide extensive insights regarding an individual's health status and the likelihood of developing certain diseases,[1] while large-scale protein scanning has become available to identify biomarkers related to disease states[2] and specifically in the context of NAFLD,[3] which is highly prevalent,[4] and can develop from hepatic steatosis to NASH, fibrosis, and cirrhosis.[5,6]

Hepatic steatosis, the first stage of NAFLD, is defined as intrahepatic fat (IHF) content exceeding 5%,[7] is associated with an increased risk of metabolic syndrome (Met syn), type 2 diabetes (T2D), cardiovascular and



kidney disease, gut dysbiosis, liver and other cancers.[8–16] IHF% accumulation is typically asymptomatic, but several biomarkers and noninvasive prediction tools for NAFLD diagnosis and staging are being used and investigated, as it is necessary to monitor the initiation and evolution of the disease. [17–24] An encouragement for combining omics with traditional biomarkers has recently emerged to improve diagnostic performance.[17,23,24] In NAFLD, omics have been explored in different study settings, including cohorts, therapeutic interventions, and following bariatric surgeries.[25–27]

Recently, we reported in the 18-month DIRECT PLUS trial among 294 participants, with an NAFLD prevalence of 62% at baseline, that a green-Mediterranean (green-MED) diet, amplified with green plant –based proteins/polyphenols such as Mankai, green tea, and walnuts and restricted in red/processed meat can increase IHF% loss more than other healthy nutritional strategies and reduce NAFLD by half.[16] Here, we aimed to identify, in the DIRECT PLUS lifestyle intervention trial, whether novel proteomic signatures may indicate an MRI-assessed IHF state and changes.

METHODS

Study population

This was a secondary analysis of the 18-month DIRECT PLUS randomized controlled trial. The DIRECT PLUS randomized controlled trial included 294 participants (age >30 y) with abdominal obesity (waist circumference: men >102 cm, women >88 cm) or dyslipidemia (TG >150 mg/ dL and HDL cholesterol ≤40 mg/dL for men, ≤50 mg/dL for women) who were recruited from an isolated workplace (Nuclear Research Center Negev). The participants were randomized to 1 of 3 lifestyle interventions: healthy dietary guidelines (HDGs), the Mediterranean (MED), or the green-MED diet, all combined with physical activity, where a monitored lunch was provided. Both MED diets were similarly hypocaloric and included 28 g/d of walnuts. The green-MED group further consumed green tea (3–4 cups/d) and a *Wolffia globosa* (Mankai) green plant shake.

IHF acquisition and quantification

The participants underwent MRI at baseline and the end of the intervention using proton magnetic resonance spectroscopy, as detailed.[16] Briefly, localized, single-voxel proton spectra were acquired using a whole-body 3-Tesla MRI scanner (Philips Ingenia). Data were analyzed using the Mnova software (Mestrelab Research) by an experienced physicist blinded to the intervention groups. The total hepatic fat fraction in the image was determined as the ratio between the sum of the area under all fat peaks divided by the sum of the area under all fat and water peaks.

We determined distinct trajectories of IHF% based on the 18-month change: (i) *normal-IHF% trajectory* included subjects with IHF% <5% at the baseline and 18-month timepoints; (ii) *normal-to-abnormal-IHF% trajectory* included subjects with IHF% <5% at the baseline and IHF% ≥5% at the 18-month timepoints; (iii) *abnormal-to-normal-IHF% trajectory* included subjects with IHF% ≥5% at the baseline and IHF% <5% at the 18-month time- points; and (iv) *abnormal-IHF% trajectory* included subjects with IHF% ≥5% at the baseline and 18-month time points.

Proteomic panel and classification

The protein panel was assessed using the proteomics platform of the Olink CARDIOVASCULAR II panel. The Olink technique employs proximity extension assay technology[28] in a homogenous 96-well plate format. It incorporates 92 oligonucleotide-labeled antibody probe pairs per panel that bind their target proteins in serum. A PCR reporter sequence was formed, amplified, and quantified by real-time PCR following a proximity-dependent DNA polymerization event. Internal and external controls are used for data normalization and quality control. Intra- and inter-coefficient of variance (CV)% were based on control samples (pooled plasma samples) included on each plate. The average intra-assay CV% was 6% CV, and the inter-assay CV was 17%. This platform provides normalized protein expression data on a log2 scale. Further information regarding the Olink analysis can be found in the Supplemental Methods (https://links.lww.com/HEP/I360).

Three other proteins, high-sensitivity C-reactive protein, Chemerin, and Fetuin-A, were also added to the proteomic analyses on a log2 scale.

Based on prior literature (Supplemental Table S1, https://links.lww.com/HEP/I361), we classified 95 proteins according to the following pathway-related groups: metabolism, blood coagulation, blood pressure regulation, endothelial dysfunction, extracellular matrix remodeling, heart functioning, and immune function (Supplemental Table S1, https://links.lww.com/HEP/I361).

Data cleaning

We removed 5 proteins with more than 5% missing data (BNP, ITGB1BP2, SERPINA12, STK4, and PARP1) (Supplemental Figure S1, https://links.lww.com/HEP/I362), considering the study design, including sample size and experimental variables. We used the remaining protein data for the baseline (N=242) and 18-month change analysis (N=184, participants with complete data at baseline and 18 mo).

Statistical analysis

The present study aimed to investigate the proteomic profile associated with IHF% in the DIRECT PLUS intervention trial. Baseline characteristics are presented as sex-specific IHF% tertiles. Continuous variables are presented as mean ± SD. The significance of trends between tertiles was assessed using the Kendal-Tau test. We used Spearman correlations to quantify the associations between protein levels and IHF%. The false discovery rate (FDR) was applied to correct for multiple testing, with FDR <0.05 as the significance threshold. The partial correlations were adjusted for age and sex and, in a subsequent analysis for age, sex, and weight. For the 18-month change analysis, we

adjusted for age and sex and, in a subsequent analysis, for age, sex, baseline weight, and weight loss. ANOVA and ANCOVA were performed to examine the changes in thrombospondin-2 (THBS2), hydroxyacid oxidase 1 (HAOX1), and carbonic anhydrase 5A (CA5A) proteins between the 3 intervention groups (HDG, MED, and green-MED), further adjusted for weight loss and IHF% loss by ANCOVA. The change of markers and IHF was calculated as the devision between 18-months time-point values and values at baseline, unless otherwise stated. Tukey multiple comparisons of means analysis was used as a post-hoc analysis. We performed principal component analysis (PCA) and Linear Mixed Effects Model to examine the differences in the proteomic panel of distinct trajectories of IHF%, including preintervention and postintervention time points. Also, a volcano plot was used to present the differences in proteomics between different IHF% trajectories. Paired t-test or Wilcoxon signed-rank tests were used to compare 18-month changes in proteomic markers from baseline. The prediction of IHF% at baseline was performed by elastic net regression analysis where 10-fold cross-validation was used, with a multivariate linear regression analysis performed to assess the prediction of known variables taken from the literature.[21] Baseline data from the DIRECT PLUS was used as a training set. The DIRECT PLUS data at 18 months were used as a test set. We used the CENTRAL trial baseline data[29] as a validation set. The CENTRAL trial has proteomic measurements for 212 individuals by Olink platform with the additional proteins (high-sensitivity C-reactive protein, Chemerin, and Fetuin-A), with IHF% measured by the mDIXON approach as described.[30] All variables were transformed into a log2 scale except sex and age. Statistical analysis was performed using R (Version 4.2.0). Statistical significance was set at a 2-sided alpha value of 0.05.

RESULTS

Baseline characteristics

Participants' baseline characteristics incorporates 242 subjects (mean age= 51.3±10.8 y; 89.25% men; mean body mass index=31.3±3.9 kg/m^2; mean of IHF% men=10.4±8.3 or women = 10±12.2). Among the 242 participants at the baseline, 149 (61.5%) individuals with Met syn (assessed based on Huang PL,[31] Dis Model Mech. 2009), 24 (10%) with T2D (fasting plasma glucose levels ≥126 mg/dL or hemoglobin A1c levels ≥6.5% or if regularly treated with oral antihyperglycemic medications or exogenous insulin), and 153 (63%) exhibiting NAFLD. Thirty-five (14%) individuals were using antihypertensive medications, 15 (6%) were prescribed antiplatelets, 28 (12%) were undergoing lipid-lowering therapy, 4 (2%) were receiving insulin treatment, and 14 (6%) were administered oral glycemic control medications.

Further characteristics at the baseline are shown in sex-specific IHF% tertiles (Table 1). Age, cholesterol, and LDL cholesterol levels were similar across sex-specific IHF% tertiles. Individuals with a greater IHF% had significantly higher body mass index and waist circumference, markers of glucose metabolism, and inflammatory markers (p of trend <0.05 for all). Sex-specific IHF% tertiles stratified by body mass index are presented in Supplemental Table S2, https://links.lww.com/HEP/I362. Baseline characteristics across DIRECT PLUS intervention groups among 242 and 184 participants are presented in Supplemental Table S3, https://links.lww.com/ HEP/I362.

Protein classification pathways and cross-sectional associations of IHF% and proteomic panel

Baseline correlation analysis revealed that 34 proteins were significantly associated with IHF%. After adjustments for weight, overall, 35 proteins were significantly associated with IHF% at baseline. Several proteins changed following the adjustments; the correlations of LEP and TNFRSF11A with IHF% were attenuated, while ADAM_TS13, MMP12, and hOSCAR were found to correlate with IHF% (FDR <0.05). After adjustments for age and sex, 39 candidate proteins were significantly associated with IHF% at the baseline (Figure 1A; FDR values are shown in Supplemental Table S4, https://links.lww.com/HEP/I363). After adjustments for age, sex, and weight, 36 proteins were significantly associated with IHF% at the baseline overall. Several proteins changed following the adjustments; the correlations of TNFRSF11A, TRAIL_R2, and SLAMF7 with IHF% were attenuated, while ADAM_TS13 was found to correlate with IHF% (Figure 1B; FDR values are shown in Supplemental Table S4, https://links.lww.com/ HEP/I363).

A literature-based classification of the 39 proteins correlated with IHF% beyond age and sex. The proteins correlated with IHF% beyond age, sex, and weight were related to distinct pathways; 6 proteins related to metabolism, 1 protein related to blood coagulation, 2 proteins related to blood pressure regulation, 3 proteins related to endothelial dysfunction, 7 proteins related to extracellular matrix remodeling, 1 protein related to heart functioning, and 16 proteins related to immune function (Figure 1B).

Prediction of IHF% at the baseline by the proteomic panel and traditional variables

We constructed 3 models and used a published model by Kotronen et al[21] to examine whether proteomics only, known variables from the literature, or a combination of the proteomic panel with the literature variables would enhance the prediction of IHF% (Table 2). Model 1 included only the proteomic panel (90 proteomics) with an R^2 value of 0.41 and root mean square error (RMSE) of 1.11. Model 2 consisted of both proteomics and known variables (90 proteomics + 5 literature-known variables; aspartate transaminase, aspartate transaminase /alanine transaminase ratio, fasting insulin levels, and the presence of T2D and Met syn, input as forced covariates). Both models were performed with elastic net regression. Model 3

TABLE 1 - Baseline characteristics of the DIRECT PLUS participants across sex-specific intrahepatic fat % tertilesa

	Low IHF% tertile (n=81)	Intermediate IHF% tertile (n=81)	High IHF% tertile (n=80)	P of trend[b]
IHF% men, %	2.90±1.27	7.96±2.17	20.30±5.42	
IHF% women, %	1.66±0.89	4.14±0.85	23.59±11.92	
Age, y	52±12	52±11	50±10	0.3
Weight, kg	89±9	91±13	100±16	<0.001
BMI, kg/m^2	29.9±2.8	30.9±3.1	33.0±4.6	<0.001
WC, cm	106±7	109±8	114±12	<0.001
Systolic BP, mm Hg	126±12	131±15	133±13	0.001
Diastolic BP, mm Hg	79±10	81±11	82±10	0.049
Fasting glucose, mg/dL	99±14	100±15	105±16	0.019
Insulin, µU/mL	10±5	14±6	20±10	<0.001
HOMA-IR	2.57±1.35	3.47±1.43	5.24±2.90	<0.001
HbA1c, %	5.37±0.48	5.43±0.46	5.59±0.72	0.018
Cholesterol, mg/dL	185±35	195±32	191±31	0.5
Triglycerides, mg/dL	123±61	153±61	174±74	<0.001
HDL, mg/dL	50±12	47±12	42±9	<0.001
LDL, mg/dL	120±32	129±31	126±30	0.3
ALT, U/L	29±12	31±12	45±21	<0.001
AST, U/L	25±8	24±6	29±9	0.001
ALKP, mg/dL	69±18	76±19	76±21	0.021
Leptin, ng/mL	12±9	14±13	17±13	<0.001
hsCRP, mg/L	2.49±1.99	2.90±1.78	3.86±2.29	<0.001
IL6, pg/mL	3.20±1.79	3.51±1.58	4.03±1.91	<0.001
Fetuin A, µg/mL	322±89	348±92	359±100	0.02
Chemerin, ng/mL	186±36	209±34	223±51	<0.001
FGF21, pg/mL	172±170	195±130	234±121	<0.001

Note: N=242. Values are presented as mean±SD.
Bold values represent significance level below 0.05.
[a]Sex-specific tertiles: Low tertile men ≤4.97%; women ≤2.64%; Intermediate tertile men: 5.05%–13.1%; women: 2.79%–5.17%; High tertile men ≥13.11%; women ≥7.49%.
[b]P of trend was analyzed using Kendall Tau test.
Abbreviations: ALKP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; BP, blood pressure; BMI, body mass index; HbA1c, hemoglobin A1c; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; hsCRP, high-sensitivity C-reactive protein; IHF, intrahepatic fat; IL6, interleukin-6; WC, waist circumference.

(multivariate linear model) included 5 variables as predictors of IHF%. A 10-fold cross-validation was performed for all 3 models (Table 2).

The values of R^2 were calculated for all subjects at the baseline timepoint of the DIRECT PLUS trial. This overall R^2 value describes the values received from all 10 models trained in the 10-fold cross-validation for each of the 3 models. The R^2 value for model 1 was higher than that of model 3 (0.41 vs. 0.36), while the joint prediction model (model 2) had the highest R^2 value compared to the other 2 models (0.47).

Furthermore, we used the coefficients of the published variables by multivariate linear regression

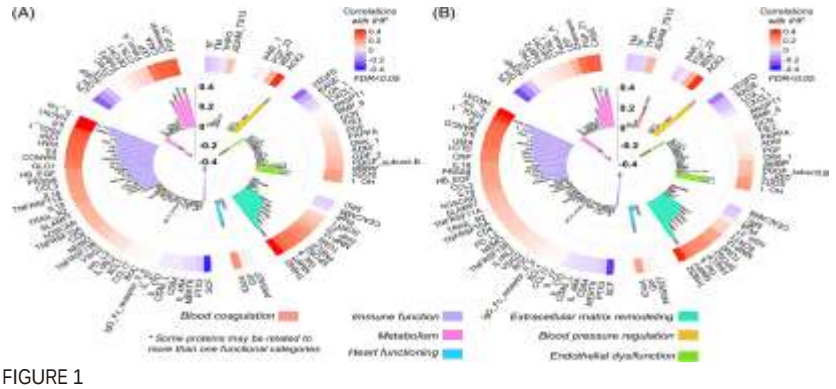


FIGURE 1

(A, B) Cross-sectional baseline correlations between the proteomic panel and IHF. (A). Circular heatmap: Spearman partial correlation test adjusted for age and sex between baseline IHF% and proteomic panel, FDR <0.05 (n=242). Circular bar plot: Significant correlations between IHF% and the proteomic panel classifiedby literature-based related pathways, FDR <0.05 (n=242). (B) Circular heatmap: Spearman partial correlation test adjusted for age, sex, and baseline weight between baseline IHF% and proteomic panel, FDR <0.05 (n=242). Circular bar plot: Significant partial correlations between IHF% and the proteomic panel classified by literature-based related pathways, FDR <0.05 (n=242). Abbreviation:IHF, intrahepatic fat.

TABLE 2 - Intrahepatic fat % prediction by the proteomic panel and traditional variables at baseline

	Model information	R^{2a}	RMSE
Model 1: 10-fold CV: proteomics panel only	90 proteomics N=238	0.41	1.11
Model 2: 10-fold CV: proteomics panel + forced covariates: AST, AST/ALT ratio, insulin, type 2 diabetes (T2D), and metabolic syndrome (Met syn)	90 proteomics + 5 variables, N=238	0.47	1.05
Model 3[b]: 10-fold CV: Linear regression: AST, AST/ALT ratio, insulin, T2D, Met syn as predictors	5 variables, N=238	0.36	1.15
Model 4: Validating published coefficients (variables of model 3[b]) on the DIRECT PLUS data	5 variables, N=238	0.34	1.21

Note: The equations for the 3 best models are presented below (the best model was evaluated by the highest R2 value from all 10-fold cross-validations in each model).
Model 1 equation includes only the proteomic panel (presented by R2=0.55, RMSE=1.11, alpha=0.7) including 41 variables not including intercept.
IHF% (%)=log2(- 8.949105975 + 0.020982581 × CRP + 0.124064859 × Fetuin A + 0.257878897 × Chemerin + 0.105294496 × ACE2 + 0.003910084 × ADM- 0.140516871 × AGRP + 0.171705645 × AMBP + 0.093995263 × BOC + 0.036709717 × CA5A + 0.098918898 × CCL3 - 0.115518974 × CTSL1 - 0.126018384 × CXCL1 + 0.182515064 × FGF21 + 0.462583347 × FS - 0.080465904 × GH + 0.253853845 × GLO1 - 0.039272049 × GT + 0.079075909 × HAOX1 + 0.217665607 × HB EGF + 0.110859333 × IDUA + 0.246152831 × IL1ra - 0.217431847 × IL27 - 0.265710678 × IL 4RA + 0.119777214 × IL6 + 0.150230258 × KIM1 - 0.158423769 × LPL + 0.342213205 × MARCO + 0.038349936 × PD L2 + 0.074397322 × PDGF subunit B + 0.698262345 × PSGL 1 - 0.053068183 × PTX3 - 0.161383714 × RAGE - 0.409735013 × SCF + 0.089245470 × SLAMF7 - 0.155720892 × SRC + 0.102552593 × TGM2 + 0.272236204 × THBS2 - 0.012744240 × THPO - 0.112077212 × TIE2- 0.038506027 × TNFRSF10A - 0.341484947 × VEGFD).

Model 2 equation includes the proteomic panel combined with traditional variables (presented by R2=0.73, RMSE=0.85, alpha=0.8), including 22 variables, not including intercept.

Model 2 equation (alpha=0.8) of the combined model which yielded the best prediction:

IHF% (%)=log2(- 9.519305 + 0.4320752×AST - 0.9110219 ×AST/ALT ratio + 0.4769744 × Insulin + 0.2635675 × T2D + 0.2085045 ×Met syn + 0.05490866 × CRP + 0.06058877 × Chemerin – 0.007101739 × AGRP + 0.1558187 × FGF21+ 0.3634254 × FS -0.01474900 × GH + 0.2265951 × GLO1 + 0.08827305 × IL6 + 0.001913739 × KIM1 - 0.00002970062 × LOX1 - 0.06997135 × LPL + 0.2034404 × MARCO + 0.4838826 × PSGL1 - 0.1823542 × SCF + 0.04138332 × SLAMF7 - 0.01380251 × SRC - 0.1199580 × VEGFD).

Model 3 equation includes only the traditional variables (presented by R2=0.69, RMSE=0.91 including 5 variables not including intercept: IHF% (%)=log2(- 2.2965238 + 0.4618139 × AST - 0.9570679 × AST/ALT ratio + 0.6211023 × Insulin + 0.3963679 × T2D + 0.4161643 × Met syn).
[a]The values of R2 are for all subjects (N=238).

[b]Based on Kotronen et al.[21].

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; CV, cross-validation; IHF, intrahepatic fat; Met syn, metabolic syndrome; RMSE, root mean square error.

analysis for the prediction of IHF% (model 4) and found that a model trained with the published variables on our data (model 3) had a higher R^2 value than the one published previously (0.36 vs. 0.34).

Following the 10-fold cross-validations on the training set, we chose the model with the highest R^2 value from all the 10 models created for each model (1, 2, and 3) and presented the equations for the 3 best models in Table 2. Further supplementary sheet shows all the chosen proteins and their coefficients from the 10-fold cross-validation for each elastic net model in Supple- mental Table S5, https://links.lww.com/HEP/I364.

Next, we tested and validated the models presented in Table 2 on different data points from the DIRECT PLUS and CENTRAL trials. The models with the highest R^2 value from the 10-fold cross-validation in the training set

were used for prediction on the validation sets (best R^2 value for model 1: R^2=0.55, RMSE=1.11, alpha=0.7; best for model 2: R^2=0.73, RMSE=0.85, alpha=0.8; and model 3: R^2=0.69, RMSE=0.91).

Extrapolating diabetes and Met syn status for the CENTRAL participants resulted in a sharp decrease in observations; as such, validation of models 2 and 3 on CENTRAL was not possible. We validated model 1 at the baseline timepoint of the CENTRAL trial for proteomics-only prediction model (model 1), which showed an R^2 value of 0.43 (Figure 2A). In addition, we tested models 1, 2, and 3 at the 18-month timepoint of the DIRECT PLUS trial, which showed an R^2 value of 0.43 for proteomic-only model (Figure 2B), 0.44 for the traditional markers only (Figure 2C), and 0.53 for the joint model (Figure 2D).

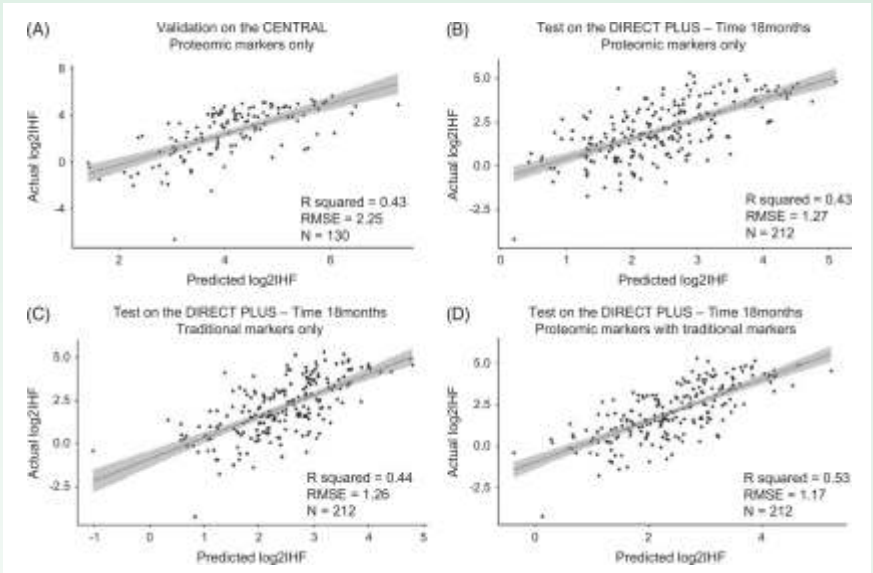


FIGURE 2

(A–D) Prediction results on the DIRECT PLUS test set and the CENTRAL validation set. (A) Model 1 evaluated the baseline timepoint of the CENTRAL trial (n=130) as a validation set, including proteomics only. (B) Model 1 evaluated the 18-month timepoint of the DIRECT PLUS trial (n=212) as a test set, including proteomics only. (C) Model 3 evaluated the 18-month timepoint of the DIRECT PLUS trial (n=212) as a test set, including proteomics and traditional markers (AST, ALT/AST ratio, insulin, type 2 diabetes, and metabolic syndrome status). (D) Model 2 evaluated the 8-month timepoint of the DIRECT PLUS trial (n=212) as a test set, including proteomics and traditional markers (AST, ALT/AST ratio, insulin, type 2 diabetes, and metabolic syndrome status). Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; IHF, intrahepatic fat; RMSE, root mean square error.

The dynamics of the proteomic panel with IHF

For 39 candidate proteins significantly correlated with IHF% after adjustments for age and sex at the baseline, we calculated the associations between the 18-month change of these proteins and the 18-month change of IHF%. We found 18 proteins significantly associated with IHF% change (FDR <0.05) after adjustments for age and sex, as listed in Figure 3A: 5 proteins related to metabolism, 1 protein related to blood pressure regulation, 2 proteins related to endothelial dysfunction, 3 proteins related to extracellular matrix remodeling, and 7 proteins related to immune function (Figure 3A). After adjustment for age, sex, baseline weight, and weight change, 3 proteins (CA5A, THBS2, and HAOX1) remained significantly associated with IHF% change (p<0.05) (Figure 3B).

We further investigated CA5A, HAOX1, and THBS2 changes between the 3 intervention groups (HDG, MED, and green-MED). Unadjusted analyses showed significant differences between the green-MED and the MED/HDG groups. For THBS2, green-MED versus MED, p=0.005; green-MED versus HDG, p=0.02; MED versus HDG, p=0.79. For CA5A, green-MED versus MED, p=0.018; green-MED versus HDG, p=0.05; MED versus HDG, p=0.84. For HAOX1, green-MED versus MED, p=0.04; green-MED versus HDG, p=0.04; MED versus HDG, p=0.99.

For all proteins, significant within-group differences were evident for the green-MED group (p<0.05 for all) but not for the MED and HDG groups (p>0.05 for both) for CA5A and THBS2. For HAOX1, significant within-group differences were evident also for the HDG group (p<0.05). Following weight loss and IHF% loss adjustment, significantly greater decrease was observed for THBS2 in the green-MED group compared to the MED group (p=0.01 between groups) (Figure 3C). No significant change was observed between the MED and HDG groups (p=0.5) and between the green-MED group compared to the HDG group (p=0.1). No statistical differences were found between groups for CA5A (Figure 3D) (green-MED versus MED, p=0.1; green-MED versus HDG, p=0.6; MED versus HDG, p=0.5). No statistical differences were found between groups for HAOX1 (Figure 3E) (green-MED versus MED, p=0.32; green-MED versus HDG, p=0.75; MED versus HDG, p=0.72).

We further explored these proteins by (i) examining mediation of the proteins for the association between the intervention groups and IHF% change and (ii) assessing the interaction between each protein and the intervention groups in a model predicting 18-month changes in IHF%. No significant results were observed for any of these analyses.

IHF trajectories and proteomic panel differences

Next, we examined the distinct trajectories of IHF% and their proteomic panel (Figure 4A): the normal-IHF% trajectory (n=71), the normal-to-abnormal-IHF% trajectory (n=13), the abnormal-to-normal-IHF% trajectory (n=45), and the abnormal-IHF% trajectory (n=82). We examined the differences in proteomics in each trajectory at the baseline and 18-month timepoints (Figure 4B).

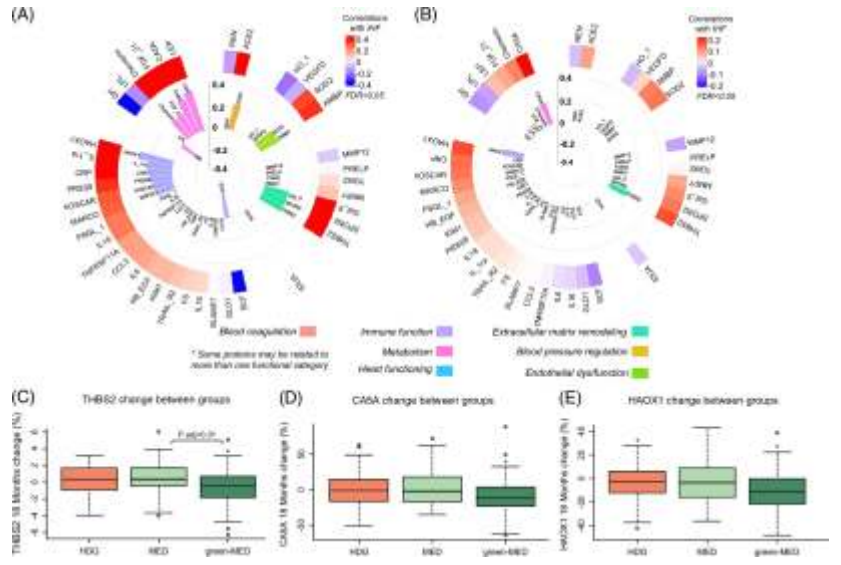
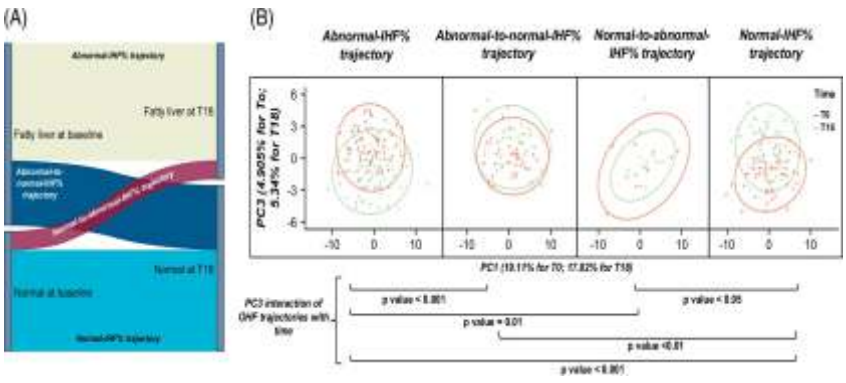


FIGURE 3

(A–E) Correlations between the change of the candidate proteomics and IHF change induced by intervention and proteins' changes between intervention groups. (A) Circular heatmap: Spearman correlation test between change of the candidate proteomics and IHF% change following the intervention (n=184). Circular bar plot: Significant partial correlation test adjusted for age and sex between the change of the candidate proteomics and IHF% change classified by literature-based related pathways, FDR<0.05 (n=184). (B) Circular heatmap: Spearman partial correlation test adjusted for age, sex, baseline weight, and weight loss between the change of the candidate proteomics and IHF% change following the intervention (n=184). Circular bar plot: Significant partial correlations adjusted for weight loss between change of the candidate proteomics and IHF% change classified by literature-based related pathways, FDR <0.05 (n=184). (C) THBS2 18-month change (%) between intervention groups adjusted for weight loss and IHF% loss (n=184). (D) CA5A 18-month change (%) between intervention groups adjusted for weight loss and IHF% loss (n=184). (E) HAOX1 18-month change (%) between intervention groups adjusted for weight loss and IHF% loss (n=184). Abbreviations: CA5A, carbonic anhydrase 5A; HAOX1, hydroxyacid oxidase 1; HDG, healthy dietary guideline; IHF, intrahepatic fat; MED, Mediterranean; THBS2, thrombospondin-2. P.adj: adjusted p value between groups, *p value<0.05 within groups (paired t test).



(A, B) Distinct IHF trajectories during the DIRECT PLUS trial and their proteomics panel. (A) IHF% dynamics during the DIRECT PLUS trial sorted by groups of the normal-IHF% trajectory included subjects with IHF% <5% at the baseline and 18-month (T18) timepoints (n=71), the normal-to-abnormal-IHF% trajectory included subjects with IHF% <5% at the baseline and IHF% ≥5% at the 18-month timepoint (n=13), the abnormal-to-normal-IHF% trajectory included subjects with IHF% ≥5% at the baseline and IHF% <5% at the 18-month timepoint (n=45), and the abnormal-IHF% trajectory included subjects with IHF% ≥5% at the baseline and 18-month timepoints (n=82) (overall n=211). (B) PCA analysis for proteomics in distinct IHF% subgroups, including baseline (T0) and 18-month timepoints (T18). PC1 and PC3 are presented with n=154 for the abnormal-IHF% group, n=86 for the abnormal-to-normal-IHF% group, n=23 for the normal-to-abnormal-IHF% group, and n=127 for the normal-IHF% group. Abbreviations: IHF, intrahepatic fat; PCA, principal component analysis.

Therefore, we conducted PCA analysis (derived from PCA across IHF% trajectories for the baseline and 18 months separately before combining both timepoints to 1 merged dataset) and discovered that the third principal component (PC3) accounted for some of the variability in the proteins (4.905% for baseline and 5.34% for 18 months) and was significantly different between distinct subgroups.

The normal-IHF% trajectory was significantly different from the abnormal-to-normal-IHF% and abnormal-IHF% groups (p<0.001), while the difference was not significant with the normal-to-abnormal-IHF% trajectory (p=0.16). The abnormal-IHF% trajectory differed significantly from the abnormal-to-normal-IHF% (p=0.03) and normal-to-abnormal-IHF% (p=0.002) groups. The normal-to-abnormal-IHF% trajectory was not significantly different from the abnormal-to-normal-IHF% trajectory (p=0.10). The results also suggest that when taking the time interaction with groups into account, the normal-IHF% trajectory was significantly different from abnormal-to-normal-IHF% (*p*<0.01), abnormal-IHF% groups (*p*<0.001), and normal-to-abnormal-IHF% trajectory (p<0.05). The abnormal-IHF% trajectory was significantly different from the abnormal-to-normal-IHF% (p<0.001) and normal-to-abnormal-IHF% (p=0.01) groups. The normal-to-abnormal-IHF% trajectory was not significantly different from the abnormal-to-normal-IHF% trajectory (p=0.61).

To further distinguish between proteomics changes related to distinct IHF% trajectories, we investigated the 18-month changes in proteins correlated with PC3 (correlation higher than 0.1) as it differed significantly between the IHF% trajectories and found 34 correlated proteomics with the third component. We used a volcano plot to explore from a comprehensive outlook. We focused on the 34 proteins correlated with PC3 and examined whether their 18-month changes would show different patterns among individuals showing different IHF% trajectories. We also assessed the percentage of protein change relative to the baseline protein levels and

found that distinct IHF% trajectories differed in their proteomic signatures (Figure 5). PC1, PC2, and PC3 across IHF trajectories were examined in Supplemental Figure S2, https://links.lww.com/HEP/I362. The results showed that only PC3 had significant differences between IHF% trajectories. A post-hoc analysis showed that there are significant differences between the abnormal-IHF% and abnormal-to-normal-IHF% (p=0.02) and between the normal-IHF% and abnormal-to-normal-IHF% trajectories (p=0.02). Results for others were insignificant. We also explored 18-month change analysis across IHF% trajectory groups for each protein (Supplemental Table S6, https://links.lww.com/HEP/I362). Furthermore, proportions of intervention in the distinct IHF% subgroups are elaborated in Supplemental Results S1, https://links.lww.com/HEP/I362.

DISCUSSION

In the current study, we found that the state and the changes in IHF% are associated with distinct proteomic biomarkers in various protein-related pathways. We further demonstrated that these combinations of proteins with traditional variables may enhance IHF% prediction. Also, we showed 3 protein changes: CA5A, HAOX1, and THBS2 that were associated with IHF% change following adjustments for age, sex, weight at baseline, and weight loss, and that THBS2 protein change was greater in the green-MED group, as compared to the MED group following adjustments for weight loss and IHF% loss. Moreover, we found that IHF% trajectory groups differed by protein changes across time, with each trajectory involving various proteins. Our findings suggest novel proteomic signatures that may indicate MRI-assessed IHF state and changes during lifestyle intervention.

This study has several limitations, including the high proportion of men participating, which limits our ability to extrapolate our results to women. Participants were abdominally obese or with dyslipidemia, with a high proportion of NAFLD at the baseline, which further limits generalizability. Also, a direct causal effect of the change in proteomics on IHF% and vice versa cannot be assessed, only the causal effects of the dietary interventions. Furthermore, for simplification, the literature-based classifications of proteins distinguish each protein into 1 related pathway and do not represent all related pathways; in practice, each protein may be involved in multiple pathways. The strengths of this study include a high degree of compliance with the trial intervention, a relatively large sample size, and long-term intervention. We used an accurate imaging technique for IHF% quantification,[32] which allowed us both continuous measurement and distinct trajectory group stratification of IHF%.

Our study explored various proteins correlated with IHF% at baseline after adjustments for age, sex, and weight. We revealed that most proteins were related to immune function pathways, with others related to extracellular matrix remodeling, metabolism, endothelial dysfunction, blood pressure regulation, blood coagulation, and heart functioning pathways. However, related pathways should be interpreted with caution due to the typical involvement of most proteins in several pathways in parallel. Some of the proteins were novel biomarkers to our knowledge in relation to their association with IHF% (eg, SCF, HAOX1, and PRELP). We also validated other proteins previously found to be associated with IHF% (eg, high-sensitivity C-reactive protein[33] and Chemerin[34]). In addition, we showed that the change in 3 proteins was directly correlated with IHF% change: CA5, THBS2, and HAOX1. Some varieties of carbonic anhydrases (Cas), which regulate acid-base balance,[35] were associated with the development of NAFLD.[36] CA5 is expressed in mitochondria and involved in various metabolic pathways, including the urea cycle, gluconeogenesis, and insulin secretion.[35,37] CA5A, a specific isozyme of CA5, is distributed in the liver and was identified as a potential target for treating NAFLD and NASH in a metabolite-target-disease network analysis.[36] Here, we show that the change of CA5 positively correlated with the change of IHF% following the 18-month lifestyle intervention

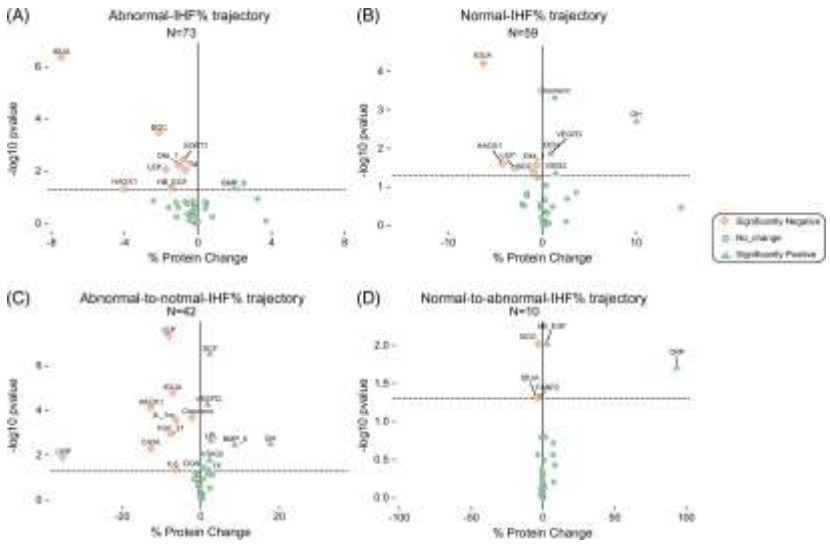


FIGURE 5

(A–D) Proteomic signatures stratified by IHF% trajectories. 18-month changes in protein among individuals within the (A) abnormal-IHF% trajectory (N=73), (B) normal-IHF% trajectory (N=59). © Abnormal-to-normal-IHF% trajectory (N=42), (D) normal-to-abnormal-IHF% trajectory (N=10). % protein change was calculated relative to the baseline protein's expression. The statistical significance of the protein's change (T18/T0) was analyzed using the Wilcoxon signed-rank test. The rhombus shape represents significant proteins with a negative % change. The triangular shape represents significant proteins with a positive % change. The circle shape represents nonsignificant proteins. Abbreviation: IHF, intrahepatic fat.

following adjustments for age and sex; this association persisted after further adjustment for weight at baseline and weight loss. THBS2, a protein involved in cell-extracellular matrix interactions, is a possible predictor of NAFLD severity.[38–40] We found that the change of THBS2 positively correlated with the change of IHF% following adjustments for age and sex, and beyond baseline weight and weight loss as well. HAOX1 is a peroxisomal liver enzyme. HAOX1 negatively regulates the inflammatory response of liver macrophages in alcoholic liver disease through the NF-kB pathway and the role of NF-kB in chronic liver diseases such as steatohepatitis, fibrosis, and cancer has been widely reported.[41] Here we found that HAOX1 change is positively associated with the change of IHF% after adjustments for age, sex, and further baseline weight and weight loss.

Further analyses adjusted for weight loss and IHF% loss showed that CA5A and HAOX1 protein change did not differ between intervention groups, suggesting a possible role of weight and IHF% on these proteins. In addition, the change of THBS2 protein was significantly higher in the green-MED group than in the MED group following 18 months of intervention. This may indicate that the green-MED diet had an impact on THBS2 protein levels beyond weight loss and IHF% loss. These results may encourage further exploration of potential mechanisms by which IHF% changes, and specifically, how the green-MED diet might induce this change through THBS2 protein.

While the cost factor of MRI versus blood metabolic panels and proteomics assays might be a consideration, it is important to note that a comprehensive understanding of IHF% changes requires a multifaceted approach. While liver MRI assesses IHF% percentage, it does not offer insights into the underlying metabolic processes. Proteomics and metabolomics provide a robust framework as metabolite concentrations are directly regulated by proteins and enzymes in their metabolic pathways. Thus, using protein markers may enhance our ability to explore and predict pathophysiology and mechanistic pathways in a more holistic manner. It is important to acknowledge that using cardiometabolic proteins may introduce a broader spectrum of information beyond IHF%-specific markers. In this study, we also used known traditional markers linked to IHF% to explore broader markers, including more specific ones. Prediction models incorporating proteomics alongside traditional measures could provide a more comprehensive assessment, especially in scenarios where frequent MRIs might not be feasible. Furthermore, the recognition of novel omics biomarkers is expanding as the interest in precision nutrition strategies rises with the encouragement of omics combinations for diagnostic performance improvement.[17,23,24] Thus, exploring proteomic biomarkers may help find new markers, which, combined with existing clinical measurements, could improve the prediction of IHF%. A previous publication[42] created a serum protein diagnostic model for NAFLD composed of 20 protein peaks, with a sensitivity of 89% and a specificity of 83%. One recent study[27] used serum protein scanning to identify signatures corresponding to the key components of liver biopsy in NAFLD and could also detect changes induced by therapeutic interventions. Additional studies showed proteome profiling holds great potential in generating novel insights into disease mechanisms and discovering new biomarkers for different stages in NAFLD.[3,25,26]

In this study, we combined variables from the previously published liver fat percentage prediction model[21] with our proteomic panel to examine whether combining proteomics with traditional variables (aspartate transaminase, aspartate transaminase/alanine transaminase ratio, fasting insulin levels, and the presence of T2D and Met syn) would improve the prediction of IHF%. Those variables were previously found to be associated with NAFLD.[43–45] Furthermore, Met syn and T2D are closely related comorbidities associated with NAFLD,[13,14] which are also evident in the recent dispute regarding renaming NAFLD into metabolic-associated fatty liver disease.[46] We found that the combined model yielded the best prediction as the addition of omics, specifically proteomics, increased the performance of the predictive models.

The PCA analysis of the protein panel suggests that the third principal component plays a role in the difference between groups with distinct IHF% trajectories while considering the interaction with time, though the power is limited. Further exploration of the proteomics correlated with PC3 revealed several combinations of proteins that might change differently in each IHF% trajectory. For instance, while IDUA significant protein change was consistently involved in all IHF% trajectories, several other proteins' significant change presence changed by different trajectories (eg, BOC, LEP, and Chemerin). In addition, this exploratory analysis enables a comprehensive outlook on a set of proteins simultaneously. For example, the abnormal IHF% trajectory includes significant 18-month changes in IDUA, BOC, HAOX1, LEP, SORT1, DKK_1, TM, HB_EGF, and BMP_6 while the normal-IHF% trajectory includes IDUA, BOC, HAOX1, LEP, DKK_1, Chemerin, GH, VEGFD, DCN, and VSIG2. Thus, we show that IDUA, BOC, HAOX1, LEP, and DKK_1 overlap between both trajectories, but there is a difference in the other mentioned proteins for each IHF% trajectory. This might imply that a portion of the proteomic panel change is linked with the trajectory of IHF%, indicating there might be a specific signature for different IHF% trajectories. However, the results for the normal-to-abnormal-IHF% trajectory should be interpreted with caution due to the low number of observations entered in the analysis.

By uncovering the relationship between IHF% and specific proteomic biomarkers, we pave the way for several practical benefits. First, we showed that integrating proteomic biomarkers with traditional variables offers the potential for improved prediction of IHF% in our study. This result may benefit along with other integrated prediction tools for future advancements in generating more accurate and noninvasive IHF%

and NAFLD monitoring tools. Second, identifying specific proteins associated with IHF% changes offers the prospect of developing biomarker-based monitoring strategies which could assist in regular patient assessments, provide early indications of IHF% alterations, and enable timely adjustments in treatment plans. Third, discovering proteins such as CA5A, HAOX1, and THBS2 associated with IHF% changes suggests potential therapeutic targets. These markers could be explored further to develop novel therapeutic interventions targeting IHF% regulation. Lastly, understanding the relationship between IHF% trajectories and distinct protein changes will possibly allow for personalized treatment approaches, which may enable clinicians to develop targeted interventions and therapies specific to an individual's response.

In conclusion, our findings enhance our understanding of the interplay between IHF% and proteomic biomarkers. Combining proteomics with traditional variables may yield a better prediction of IHF%, and distinct IHF% trajectories may differ in their proteomic signatures. The discovery of these markers improves patient monitoring, the detection of novel therapeutic mediators, personalized treatment approaches, and prognosis.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

AUTHOR CONTRIBUTIONS

Iris Shai conceptualized the DIRECT PLUS. Anat Yaskolka Meir, Ehud Rinott, Gal Tsaban, Hila Zelicha, and Alon Kaplan performed the data collection. Dana T. Goldberg and Anat Yaskolka Meir performed the statistical analysis, reviewed the literature, and drafted the manuscript. Anat Yaskolka Meir, Ehud Rinott, Gal Tsaban, Hila Zelicha, Alon Kaplan, Philip Rosen, and Ilan Shelef supervised the MRI acquisition. Anat Yaskolka Meir and Philip Rosen quantified the liver fat percentage. Nora Klöting performed the proteomic measurements. Uta Ceglarek and Berend Iserman performed laboratory analyses. All authors contributed to the interpretation of data and reviewed this work's language and intellectual content. Dana T. Goldberg, Anat Yaskolka Meir, Ohad Etzion, Meir J. Stampfer, Matthias Blüher, Frank B. Hu, Meir J. Stampfer, and Iris Shai revised the final draft of the study and approved the final version.

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CONFLICTS OF INTEREST

Uta Ceglarek received grants from Roche. Matthias Blüher consults, advises and is on the speakers' bureau for Lilly. He consults and is on the speakers' bureau for Amgen, Boehringer-Ingelheim, Novo Nordisk, and Sanofi. He is on the speakers' bureau for AstraZeneca, Bayer, and Novartis. The remaining authors have no conflicts to report.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Medical Ethics Board and Institutional Review Board at Soroka University Medical Centre, Be'er Sheva, Israel (SOR-0280-16). Participants provided written informed consent and received no compensation.

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Abbreviations:

CA5A, carbonic anhydrase 5A; CV, coefficient of variance; FDR, false discovery rate; green-MED, green-Mediterranean; HAOX1, hydroxyacid oxidase 1; HbA1c, hemoglobin A1; HDG, healthy dietary guidelines; IHF%, intrahepatic fat; MED, Mediterranean; Met syn, metabolic syndrome; PCA, principal component analysis; RMSE, root mean square error; T2D, type 2 diabetes; THBS2, thrombospondin-2.

Dana T. Goldberg and Anat Yaskolka Meir contributed equally.

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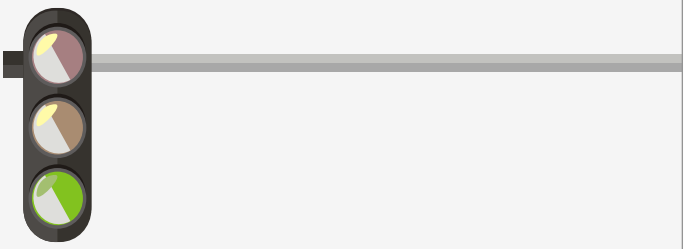

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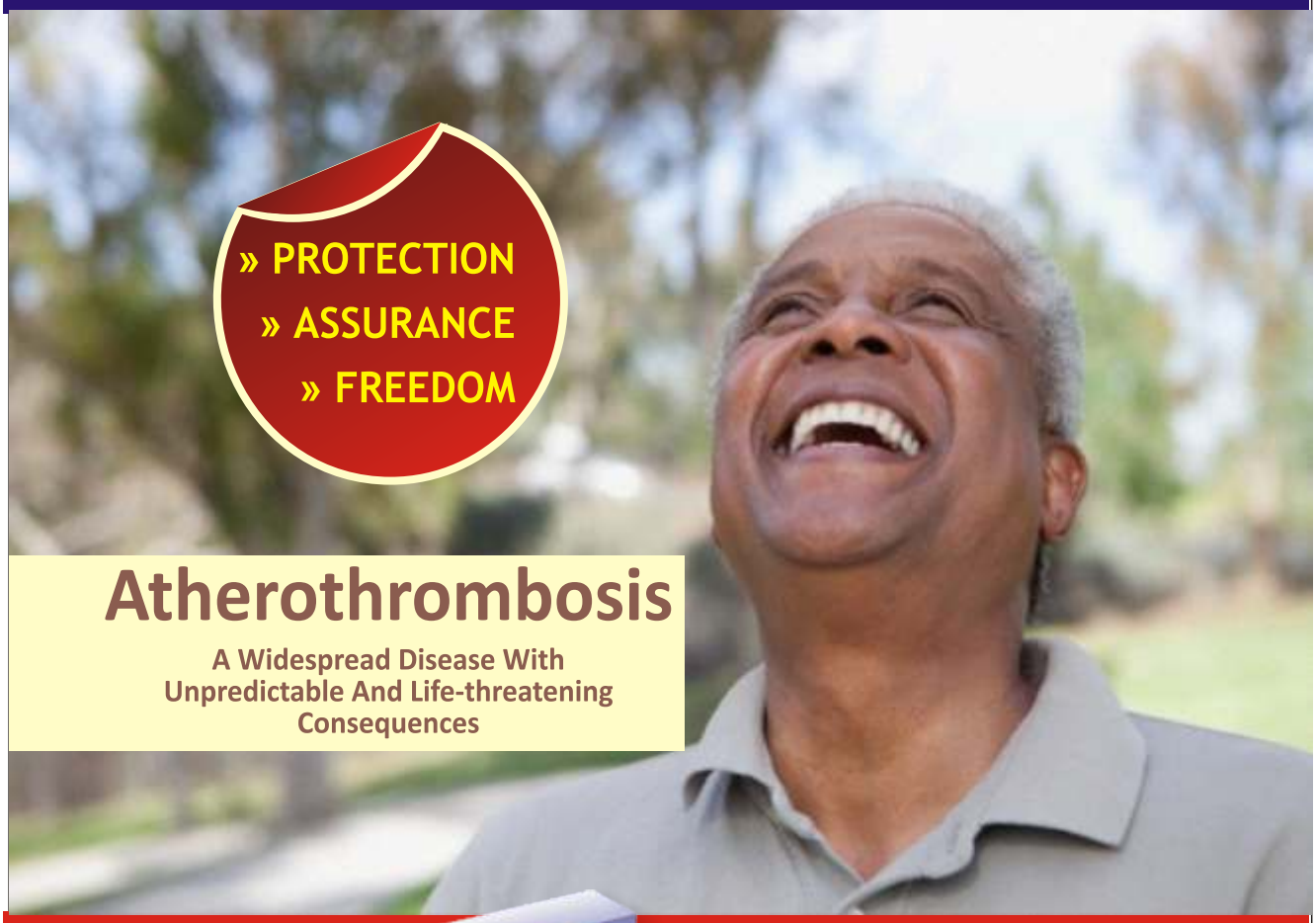

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