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ORIGINAL PAPER

JMS Journal of Medical Science

Isolation of Some Bioactive Compounds in the Methanol Extract of *Ficus exasperata* Leaves and the Effect of the Extract on Inflammatory Markers in 1,2 Dimethylhydrazine Induced Colorectal Cancer in Rats

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Keywords: colorectal cancer, 1,2-dimethylhydrazine, inflammation, bioactive compounds, antioxidants

ABSTRACT

Background. Colorectal cancer remains the third dominant cancer and is one of the leading cancer-related deaths in the world. The current investigation explores the chemoprotective roles of *Ficus exasperata* against inflammation and oxidative stress in 1,2-dimethylhydrazine (DMH)-induced colorectal cancer in rats. Some bioactive compounds were also isolated.

Material and methods. Forty-eight Wistar rats were grouped in 8 cages; group 1; control, group 2 was treated with 500mg/kg body weight of extract, group 3 received DMH twice a week, group 4 was treated with both the extract (500mg/kg b.w) and DMH, group 5 was treated with the extract (750mg/kg b.w) and DMH, group 6 was pretreated with the extract before DMH administration, group 7 was given DMH before the commencement of extract and group 8 was given the carcinogen and treated with 12.5mg/kg b.w of 5-fluorouracil simultaneously. Using high-performance liquid chromatography some bioactive compounds were isolated from the leaves extract of *Ficus exasperata*.

Results and conclusions. The bioactive compounds present in high quantity include; alpha-caryophyllene, isoquinoline, quercetin, kaempferol and rutin. After the 12th week, the animals were sacrificed. Total protein, catalase, superoxide dismutase and glutathione peroxidase activities were statistically significantly lower in group 3 ($p < 0.05$) compared with other groups. Gene expression of the Interleukins and cyclooxygenase 2 were statistically reduced and significant in all the groups except group 3. The extract suppressed the inflammatory cascade and also boosted antioxidant activities. This might be a result of some anticancer compounds that were discovered during the isolation of the compounds present in the plant.

Introduction

Colon cancer remains a challenge to human health. Cancer of the colon is common in both developed and developing countries. This cancer is also a major cause of death in both males and females which affects both the old and young [1]. Genetics, epigenetics and environmental factors are the causative agents of colorectal cancer. The cancer of the colon is accompanied by inflammation, a product of the immune response, proinflammatory and anti-inflammatory cytokines play different roles in tumor progression [2]. Tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-1β, are proinflammatory cytokines that accelerate tumor progression [3]. Inflammation activates the Janus kinase/signal transducers and activators of transcription (JAK-STAT) and nuclear factor (NF)–κB signaling pathways hereby promoting the proliferation of cells, migration, and invasion. inflammatory pathway [4,5]. Inhibition of the inflammatory cascade slows cancer cell growth and delays tumor progression [6]. Oxidative stress; an imbalance between reactive oxygen species and antioxidants is another factor implicated in the development of colorectal cancer [7].

Despite innovations and advancements in technology toward the production of a permanent cure for colon cancer, there remain setbacks and drawbacks as a result of the adverse effects of synthetic chemicals released by the drugs used in the treatment of this ailment. Natural products, especially those derived from plants, have been used to help mankind sustain its health since the dawn of medicine [8]. Herbal and natural medicine with lower side effects is crucial for lowering the mortality and morbidity rate related to colorectal cancer. Plants' active ingredients serve as a promising treatment with little or no adverse effects. Reports have shown that plants contain numerous active compounds ranging from antimicrobial, anthelmintic, antidiabetic, antihypertensive and anticancer compounds [9,10]. The presence of these active biological molecules is responsible for the medicinal and therapeutic contribution of plants in the treatment of various diseases [11,12].

The current investigation isolated some bioactive compounds present in *Ficus exasperata* and also explored the chemoprotective roles of *Ficus exasperata* against inflammation and oxidative stress in 1,2-dimethylhydrazine (DMH)-induced colorectal cancer in rats. 1,2- dimethylhydrazine (DMH) is a specific colon procarcinogen [13]. It has been shown in animal studies that experimental colonic tumors induced by DMH were similar in histology, morphology and anatomy to human colonic neoplasms [14].

Material and methods

Plant extract and preparation

The *Ficus exasperata* leaves was obtained from a local garden in Benin City and were authenticated by Dr. Akinnibosun of the Department of Plant Biology and Biotechnology of the University of Benin. The voucher number was UBH-F319. The plucked leaves were dried under shade for some weeks, after which it was ready for pulverization. Extraction was carried out by soaking the pulverized *Ficus exasperata* leaves in methanol for 72 hours. Extracts were concentrated over a rotary evaporator, freeze-dried, and stored in an airtight container in the freezer.

Experimental animals

A total of 48 male Wistar rats weighing above 150g were kept in different cages and given 14 days to acclimatize under typical laboratory circumstances. The 48 Wistar rats were grouped into 8 different cages of 6 rats each. Written approval for the study was obtained from the Research Ethics Committee Guideline Principles on Handling of Animals of the Faculty of Life Sciences, University of Benin, and was strictly adhered to.

- **Group 1:** Control group (feed only).
- **Group 2:** Leaf Extract only (500 mg/kg b.w) for 12 weeks.
- **Group 3:** DMH only (40 mg/kg b.w) for 12 weeks.
- **Group 4:** Leaf Extract (500 mg/kg b.w) + DMH (40 mg/kg b.w) for 12 weeks.
- **Group 5** Leaf Extract (750 mg/kg b.w) + DMH (40 mg/kg b.w) for 12 weeks.
- **Group 6:** Leaf extract for 4 weeks before DMH (500 mg/kg and 40 mg/kg respectively).
- **Group 7:** DMH for 8 weeks before leaf extract (40 mg/kg and 500 mg/kg respectively).
- **Group 8:** 5fluorouracil (12.5 mg/kg b.w) + DMH (40 mg/kg b.w).

The stock solution of the extract was prepared and kept refrigerated at 4°C. For the groups that received DMH, it was administered subcutaneous-

ly twice a week, while leaf extracts were administered orally. 5-fluorouracil was administered intraperitoneally. The experiment lasted for 12 weeks. The animals were fasted overnight and sacrificed. The colon was excised. A small portion was used for relative gene expression of cyclooxygenase-2 (COX-2) and tumor necrosis factor-alpha (TNF-α). The other portion was used for antioxidant assays. Blood samples were collected in plain bottles, allowed to stay for some hours at 4°C, and centrifuged at 3000 rpm for 5 minutes. The serum was collected, and stored at -80°C which was used for interleukin (IL) 6 and 10 asssays.

Biochemical assays: Relative gene expression of COX-2 and TNF-α was carried out according to the method described by Elekofehinti *et al*. [15]. Total RNA was extracted from colon samples using the Quick-RNA MiniPrep™ Kit (Zymo Research). DNA contamination was eliminated through DNAse I treatment (NEB, Cat: M0303S). RNA concentration was determined at 260 nm, and purity was assessed at 260 nm and 280 nm using an A&E Spectrophotometer (A&E Lab. UK). For cDNA conversion. One microgramme (1 µg) of DNA-free RNA underwent reverse transcriptase reaction with a cDNA synthesis kit based on ProtoScript II first-strand technology (New England BioLabs). The reaction occurred in three steps: 65 °C for 5 min, 42 °C for 1 h, and 80 °C for 5 min [15]. Polymerase chain reaction (PCR) for gene amplification utilized OneTaqR2X Master Mix (NEB) with specific primers (Inqaba Biotec, Hatfield, South Africa). The 25 µl reaction mixture contained cDNA, forward and reverse primers, and Ready Mix Taq PCR master mix. The conditions were as follows: Initial denaturation at 95 °C for 5 min, 30 cycles of amplification (95 °C for 30 s, annealing for 30 s, and extension at 72 °C for 60 s), and a final extension at 72 °C for 10 min. Amplicons were resolved on a 1.0% agarose gel. Normalization and quantification of gene expression were performed using the GAPDH gene and "ImageJ" software. The serum level of interleukin 6 and 10 were assayed using ELISA.

Total protein was determined using lowry's method [16] and catalase (CAT) was assayed according to the method of Cohen et al. [17]. Superoxide dismutase (SOD) was determined according to the method of Misra and Fridovich [18]. Glutathione Peroxidase (GPx) was determined according to the method of Paglia and Valentine [19]. Isolation of the bioactive compounds was done using high-performance liquid chromatography (HPLC).

Statistical analysis

Data obtained from the study were analyzed using one-way ANOVA and Graphpad prism 8.0.1 was used to compare the means and plot the graph. Data were presented as mean ± SEM. Values were considered statistically significant at $p < 0.05$.

Results

Identification of some bioactive compounds present in *F. exasperata*

The results obtained from the high-performance liquid chromatography (HPLC); isolation of the bioactive compounds present in *Ficus exasperata* are shown in **Figure 1**. The bioactive compounds present in high quantity include; alpha-caryophyllene, isoquinoline, quercetin, kaempferol and rutin. Garcin, caffeic acid, luteolin and linalool were moderately present but some bioactive compounds were present in minute quantity; catechin, epigallocatechin, stigmasterol, sitosterol, orientin, naringerin, hesperidin, isovitexin and isorhamnetin.

Effect of methanol extract of *Ficus exasperata* leaves (MEFE) on enzymatic antioxidants and total protein level

Colon tissues' total protein was significantly decreased in group 3 compared to other groups. Group 3 CAT activity was significantly reduced, the pretreated group also had a reduced level of CAT activities but not as low as group 3. This enzyme activity was significantly high in rats that were treated with a higher dose of the extract. SOD activity was significantly low in the pretreated group but higher in post-treated rats. GPx activity was significantly lower in groups 3 and 7 compared to groups 1 and 2. Groups 4, 5, 6 and 8 showed a significant increase in this enzyme activity as shown in **Figures 2–5**.

Effect of methanol extract of *Ficus exasperata* leaves (MEFE) on inflammatory markers

The relative gene expression of the inflammatory markers; IL-6, IL-10, COX-2 and TNF-α are shown in **Figures 6–9**. All the inflammatory markers were significantly high in group 3 compared to other groups.

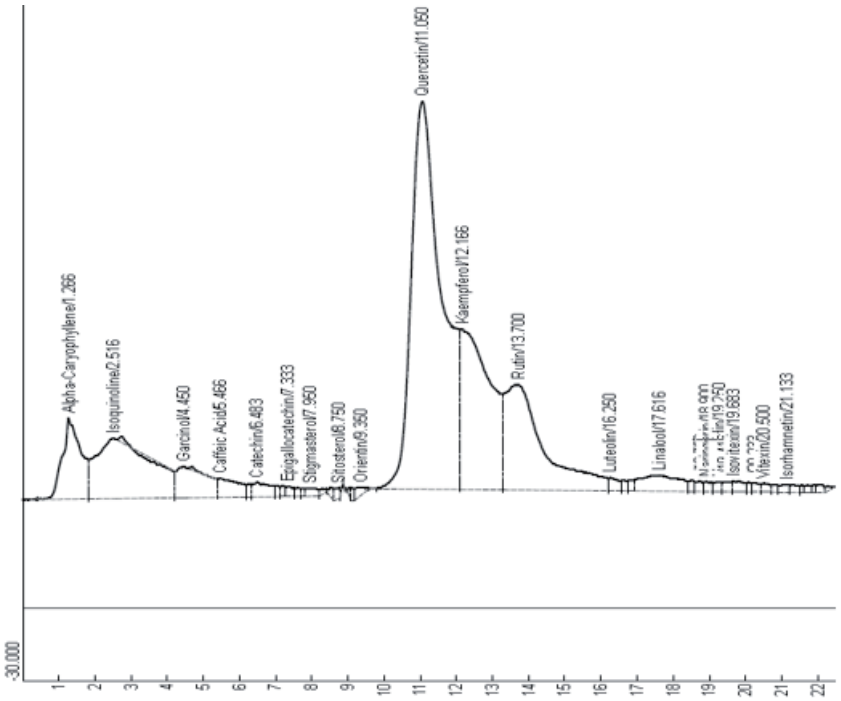

Figure 1. Phytochemical profile of the leaf extract using HPLC. Quercetin was the most numerous active compound present in this plant extract.

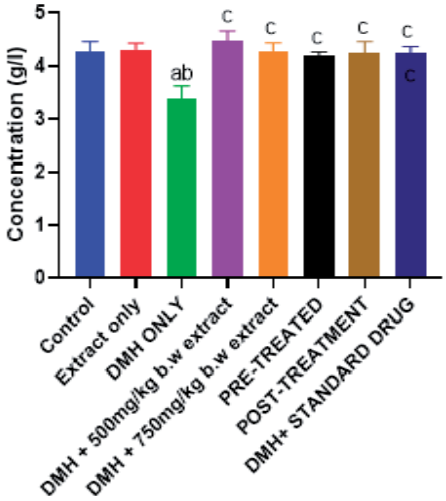

Figure 2. Colon total protein level. Values are expressed as mean ± SEM, n = 6/group. Lowercase letters represent a significant difference at P < 0.05. Values with a lowercase "a" denote a significant departure from standard control. It was statistically distinct from Extract only, as indicated by the local case letter "b". Values with the lowercase letter "c" denote significant differences from the DMH Only group. The total protein of the groups that received methanol extract of Ficus exasperata leaves (MEFE) was statistically different from untreated group.

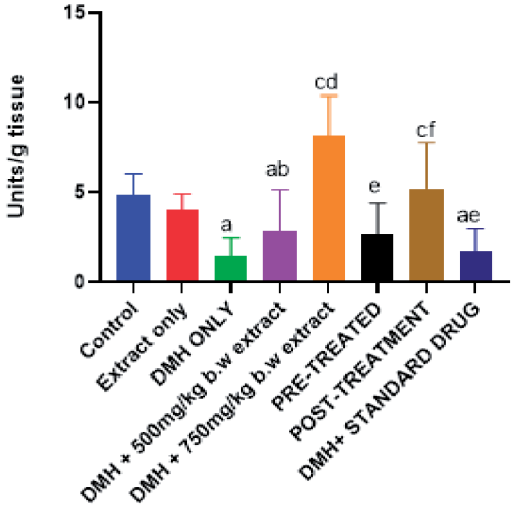

Figure 3. Colon catalase (CAT) activity. Values are expressed as mean ± SEM, n = 6/ group. Lowercase letters represent a significant difference at P < 0.05. Values with a lowercase "a" denote a significant departure from standard control. It was statistically distinct from Extract only, as indicated by the local case letter "b". Values with the lowercase letter "c" denote significant differences from the DMH Only group. The lowercase letter "d" stands for a significant departure from co-treatment (DMH+ 500mg/kg MEFE). The lowercase letter "e" stands for a significant departure from co-treatment (DMH+ 750mg/kg MEFE). A statistically significant divergence from pre-treated is denoted by the lowercase letter "f". Post treated group expressed a catalase activity close to the control. All the MEFE-treated groups showed an increase in this enzyme activity, it was statistically significant in group 5 and 7 compared to group 3.

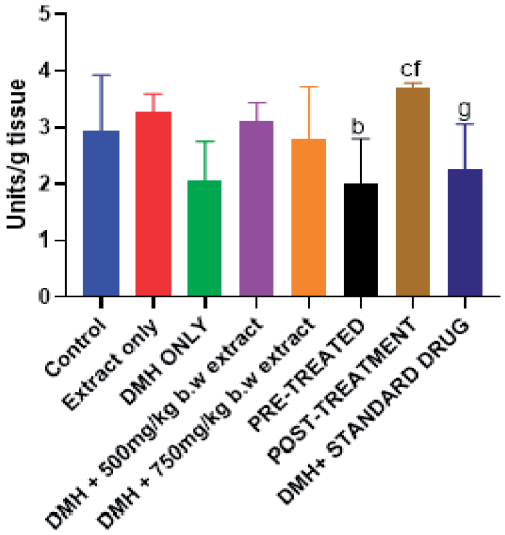

Figure 4. Colon superoxide dismutase (SOD) activity. Values are expressed as mean ± SEM, n = 6/ group. Lowercase letters represent a significant difference at P < 0.05. It was statistically distinct from Extract only, as indicated by the local case letter "b". Values with the lowercase letter "c" denote significant differences from the DMH Only group. A statistically significant divergence from pre-treated and post-treated is denoted by the lowercase letters "f" and "g" respectively. SOD activity in group 7 was statistically significant from group 6 and group 8 differs significantly from group 7. The enzyme activity didn't differ among the remaining groups.

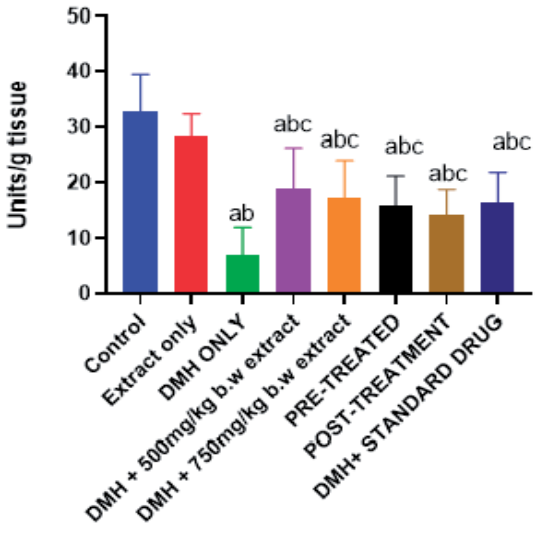

Figure 5. Colon glutathione peroxidase (GPx) activity. Values are expressed as mean ± SEM, n = 6/ group. Lowercase letters represent a significant difference at P < 0.05. Values with a lowercase "a" denote a significant departure from standard control. It was statistically distinct from Extract only, as indicated by the local case letter "b". Values with the lowercase letter "c" denote significant differences from the DMH Only group. Cotreated and pretreated groups' GPx activities didn't differ from the group treated with standard drugs (p > 0.05) but were higher compared to group 3.

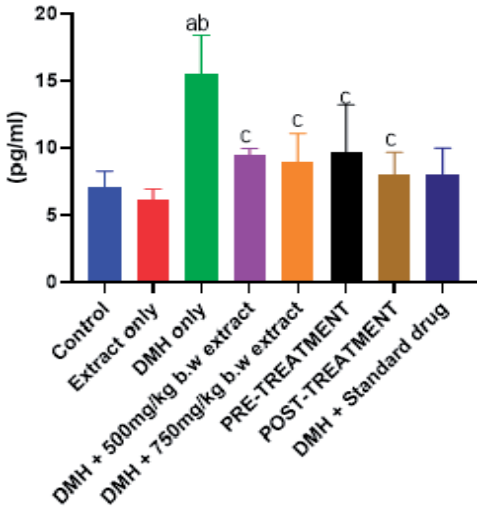

Figure 6. serum interleukin-6 (IL-6) level of rats in different groups. Values are expressed as mean ± SEM, n = 6/ group. Lowercase letters represent a significant difference at P < 0.05. Values with a lowercase "a" denote a significant departure from standard control. It was statistically distinct from Extract only, as indicated by the local case letter "b". Values with the lowercase letter "c" denote significant differences from the DMH Only group. Interleukin 6 is a pro-inflammatory marker. MEFE-treated groups expressed a reduced level of IL-6 compared to group 3. The post-treated group's mean value was the closest to the standard drug, control and extract-only groups.

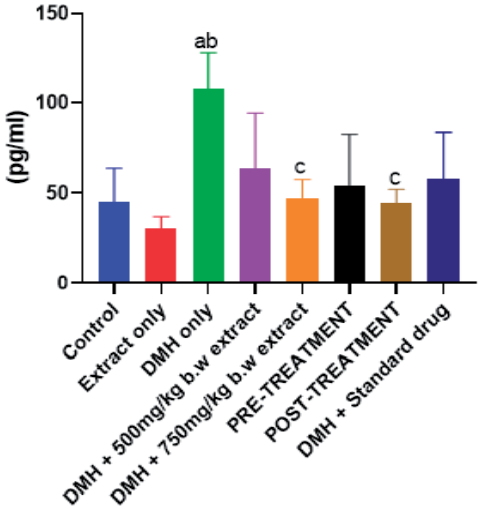

Figure 7. Interleukin-10 (IL-10) level of rats in different groups. Values are expressed as mean ± SEM, n = 6/ group. Lowercase letters represent a significant difference at P < 0.05. Values with a lowercase "a" denote a significant departure from standard control. It was statistically distinct from Extract only, as indicated by the local case letter "b". Values with the lowercase letter "c" denote significant differences from the DMH Only group. Interleukin 10 is an anti-inflammatory marker. The post-treated group's IL-10 level was lowered compared to other groups that also received MEFE and carcinogen.

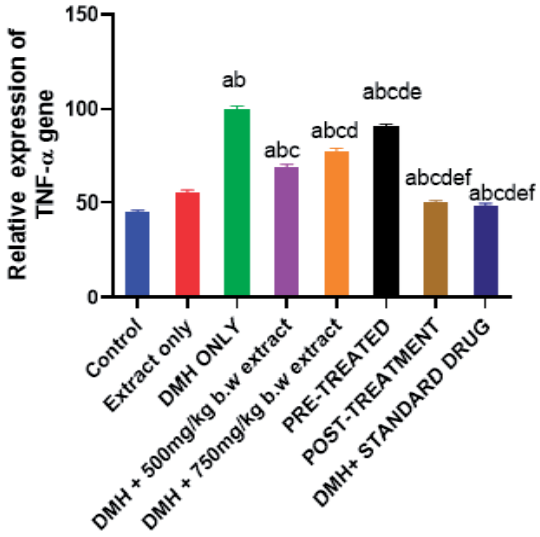

Figure 9. Relative expression of tumor necrosis factor-alpha (TNF-α) gene of rats in different groups. Values are expressed as mean ± SEM, n = 6/ group. Lowercase letters represent a significant difference at P < 0.05. Values with a lowercase "a" denote a significant departure from standard control. It was statistically distinct from Extract only, as indicated by the local case letter "b". Values with the lowercase letter "c" denote significant differences from the DMH Only group. The lowercase letter "d" stands for a significant departure from co-treatment (DMH+ 500mg/kg MEFE). The lowercase letter "e" stands for a significant departure from co-treatment (DMH+ 750mg/kg MEFE). A statistically significant divergence from pre-treated is denoted by the lowercase letters "f". The pre-treated and co-treated groups could not reduce inflammation, unlike the post-treated groups. Tumor necrosis factor-alpha levels in the post-treated group did not differ from the groups treated with standard drugs.

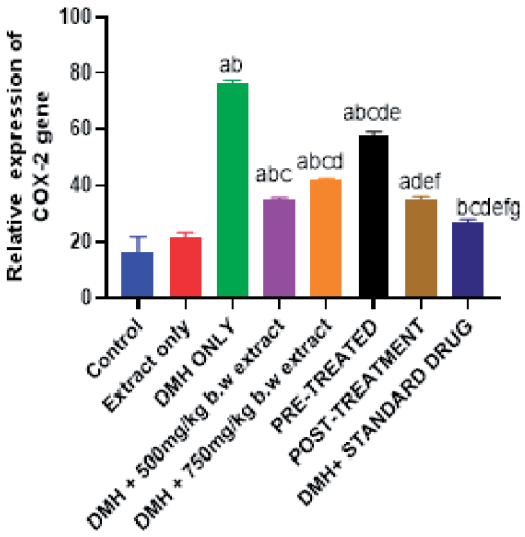

Figure 8. Cyclooxygenase-2 (Cox-2) gene expression of rats in different groups. Values are expressed as mean ± SEM, n = 6/ group. Lowercase letters represent a significant difference at P < 0.05. Values with a lowercase "a" denote a significant departure from standard control. It was statistically distinct from Extract only, as indicated by the local case letter "b". Values with the lowercase letter "c" denote significant differences from the DMH Only group. The lowercase letter "d" stands for a significant departure from co-treatment (DMH+ 500mg/kg MEFE). The lowercase letter "e" stands for a significant departure from co-treatment (DMH+ 750mg/kg MEFE). A statistically significant divergence from pre-treated and post-treated is denoted by the lowercase letters "f" and "g" respectively. Cyclooxygenase-2 level was greatly increased in the DMH-only and pre-treated groups compared to other groups.

Discussion

The high-performance liquid chromatography phytochemical profile of *Ficus exasperata* leaves revealed the abundance of bioactive compounds contributing to its potency against inflammation and cancer. Quercetin, a potent antioxidant, was found in high amounts, capable of scavenging free radicals and inhibiting lipid peroxidation [20]. Studies suggest that quercetin down-regulates mutant p53 protein expression in cancer cell lines and inhibits the JAK-STAT signaling pathway in inflammatory disorders. It also plays a crucial role in cancer prevention and tumor suppression through activation of caspase-3 and cas-9, and increased translocation of proapoptotic Bax to the mitochondria membrane [21–24]. Kaempferol, also abundant in *F. exasperata*, exhibits its antioxidant potency by reducing free radicals and reactive oxygen species production [25]. Kaempferol, found abundantly in *Ficus exasperata*, exhibits cytotoxic effects on various human colorectal cancer cell lines, including HCT116, HT-29, HCT-15, LS174-R colon, and SW480 cells. It induces apoptosis, causes cell cycle arrest at G2/M, and reduces cell migration and invasion [26–29]. These properties likely contribute to the pharmacological actions of MEFE. Rutin, another compound present in appreciable amounts, is known for its anticancer, chemopreventive, and chemosensitizing properties against various cancers [30–32]. Studies have demonstrated rutin's anticancer effects on human neuroblastoma LAN-5 cells and its cytotoxicity against human colon adenocarcinoma SW480 cells, with additional antitumor and anti-angiogenic effects in vivo [33–35]. The presence of rutin in MEFE adds to its beneficial effects. Isoquinoline, identified in *Ficus exasperata* extract (MEFE), possesses antiproliferative and anticancer properties, inducing cell death in various cancer cell lines through mechanisms like cell cycle arrest,

apoptosis, and autophagy [36–42]. Additionally, though present in small amounts in MEFE, caffeic acid exhibits diverse pharmacological properties such as immunomodulation, neuroprotection, anti-inflammatory, antioxidant, and anticancer activities [43]. Caffeic acid is known for reducing oxidative stress, inhibiting DNA damage by free radicals, and demonstrating potential antitumor effects in cell cultures and animal models, suggesting a protective role against colorectal cancer [44–46]. The presence of caffeic acid in MEFE confirms its anticarcinogenic capability.

DMH is a colon-specific carcinogen used to induce CRC in rodents. The methyldiazonium ion promotes oxidative stress through methylation of biomolecules in the epithelial cells of the colon. Most colon cancer is initiated by exposure to carcinogens, the cells may then progress through a series of precancerous lesions, premalignant, and malignant stages [14]. Superoxide dismutase (SOD), glutathione (GPx), and catalase (CAT) are more sensitive to oxidative damage induced by carcinogen treatment. These antioxidants play a crucial role in breaking down free radicals into less reactive molecules. During carcinogenesis, there is increased production of free radicals leading to more utilization of cells using antioxidants to break down this reactive species hence a reduction in antioxidant level. The reduced activities of these antioxidant enzymes contribute to an increase in the production of free radicals, surpassing the scavenging capacity of the antioxidant system in cancer. This imbalance can lead to a state of oxidative stress, which is known to play a pivotal role in the initiation and progression of cancer. The findings underscore the importance of maintaining a balanced antioxidant system to counteract the detrimental effects of oxidative stress associated with carcinogenesis. Thus, decreased activities of SOD and CAT observed in the DMH-treated rats in this study, may suggest their increased utilization to scavenge the dangerous increase in reactive oxygen species in the cancer tissues [47,48]. An increase in the activities of this enzyme in the administration of MEFE justifies that the plant is very potent in ameliorating colorectal cancer.

There was a decrease in total protein levels in colon tissues from animals in group 3 compared to animals that were treated with the methanol extract of *Ficus exasperata*. This might be a result of cancer cachexia [49]. The cachexia-inducing factors (CIFs) include tumor necrosis factor α (TNF-α), Interleukin 1 and 6 (IL-1, IL-6), Interferon γ [50]. These inflammatory markers were significantly high in group DMH-only group.

A high systemic level of pro-inflammatory cytokines (IL-6 and TNF-α) and an anti-inflammatory cytokine (IL-10) have been reported in colorectal cancer patients [51,52]. Data has suggested a potential role of the cytokine IL-6 in colon cancer. For instance, it has been shown that levels of IL-6 are increased in the serum of patients suffering from colon carcinoma, and IL-6 levels correlate with tumor size in colorectal cancer [53–55]. Gunasekaran *et al*. [56] also reported an increase in these inflammatory markers including COX-2 in Wistar rats treated with carcinogen. COX-2 is mainly expressed in cancerous conditions via the activation of inflammatory cytokines. It directs cancer cell proliferation by inhibiting apoptosis and enhancing cancer-induced angiogenesis. A similar trend was observed in this study, IL-6, IL-10, COX-2, and TNF-α were significantly increased in group 3 compared to groups 1 and 2. However, the levels of these markers were restored to normal on administration of the plant extract. The post-treatment appeared more effective in combating inflammation.

Conclusion

This study ascertained that *Ficus exasperata is* rich in anticancer compounds which contributed immensely to the potency of the plant in suppressing inflammation and oxidative stress induced by DMH colorectal cancer in rats.

Acknowledgements

Author contribution
Ngozi Paulinus OKOLIE and Olayemi Mujidat OLUDE prepared the materials used in this study. Olude OM. wrote the initial draft of the manuscript, while Okolie NP. designed the study and edited the written manuscript. The final paper was reviewed and approved by both authors.

Conflict of interest statement
The authors declare no conflict of interest.

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REVIEW PAPER

JMS Journal of Medical Science

Innovations in inductively coupled plasma-mass spectrometry: bridging scientific fields

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ABSTRACT

Inductively coupled plasma mass spectrometry ICP-MS is a versatile analytical tool with several research uses and regular applications in many domains, including biological materials, environmental analysis, and geochemistry. This technique detects trace components in water, soil and clay, blood, urine, pharmaceutical products, and medicinal cases. Although other methods, such as atomic absorption and atomic emission, are still used by researchers, there has been a noticeable shift toward ICP-MS, notably over the last decade. Developing accurate and precise methods for measuring components at low concentrations is crucial for detecting abnormalities in the human body and detecting trace amounts of metal in many other species. ICP-MS is a viable approach for the elemental determination of biological fluids, water, clay, and pharmaceuticals because it allows for reliable analysis at trace and ultra-trace levels while maintaining a wide dynamic range. Many breakthroughs have been made in ICP-MS analytical capabilities over the last few years. This review discusses the most recent works that use trace element analysis by ICP-MS in several fields.

Introduction

Measuring trace elements in biological and other samples has numerous clinical applications. The rise in xenobiotics in the environment directly results from technological advancement. Biomonitoring is an essential tool for estimating human exposure to pollutants, and their concentrations in the blood are commonly utilised as biomarkers [1]. This highlights the need for faster and more sensitive procedures requiring less sample handling. Thus, traditional techniques like atomic absorption or electrochemical approaches are gradually replaced by multi-element techniques with suitable sensitivity, such as ICP-MS [2]. These methods may be able to detect components at trace quantities with the necessary sensitivity, but they lack speed and usability. Because of the high sensitivity and low detection limits of ICP-MS, toxicologists can accurately assess ambient metal exposure and toxic levels, making it a valuable tool for various clinical applications [3]. Additionally, this approach opens up new opportunities in several disciplines, includ-

ing environmental exposure, workplace testing, clinical toxicology, and forensic toxicology. It is also suitable for epidemiological investigations and detecting many elements in urine, pharmaceuticals, water, and clay samples [4–8].

Mass spectrometry's sensitivity and selectivity make it ideal for monitoring tiny concentrations of components in biological materials [9]. These low-abundance substances often play essential roles in their corresponding chemical or biological systems [10]. This review aims to present the most recent research and some noteworthy older works, applying trace element analysis by ICP-MS in several fields to understand clinical and environmental conditions better.

ICP-MS Instrument

A single quadrupole ICP-MS comprises six basic compartments: the sample introduction system, inductively coupled plasma (ICP), interface, ion optics, mass analyser and detector [11]. Ions with varying mass-to-charge ratios are separated in MS using the basic characteristics of electric, magnetic, and radio frequency fields. In the case of ICP-MS, the inductively coupled high-frequency plasma serves as the ion source, where the ions to be separated are produced. A sample introduction system that transforms the sample into a physical condition most suited to the ion source's operation. Pneumatic nebulisation of a liquid sample is the most common method used for sample introduction in ICP-MS. The plasma ion source uses external energy from a high-frequency electromagnetic field coupled inductively to create ions from atoms. The aerosol is dried, broken down, dissociated, atomised, stimulated, and positively ionised in the plasma source. The interface system removes the ions from the plasma and comprises a vacuum fore pump, a sample cone, and a skimming cone. This apparatus is also required to lower the ion source's pressure to the necessary vacuum in the mass analyser area. The ions are focused into the mass analyser using a lens mechanism. The ions are separated based on their mass-to-charge ratio in the mass analyser (quadrupole [ICP-QMS], magnetic sector field [ICP-SF-MS], and time of flight analyser [ICP-TOFMS]).

A device known as a Faraday cup or secondary electron multiplier is also used to detect the ions. Lastly, the computer that manages every aspect of the mass spectrometer gathers information and outputs the mass spectrum, showing the ions' mass-to-charge ratios and their measured intensities. ICP-MS is a method commonly used for liquid analysis. To generate an aerosol from a solid or particle sample, it must be transformed into a solution using a pneumatic nebuliser. Aerosol is continually carried to the plasma ion source, which operates at atmospheric pressure, via a transport gas (often Argon) and tubing [12,13]. **Figure 1** is a schematic of an ICP-MS machine [14]. The advancements in ICP-MS technology have enabled the detection of challenging analytes at trace and ultra-trace levels in many samples. For example, comparing the technique with atomic absorption techniques shows that ICP-MS has incredible speed, precision, and sensitivity. The limitations of the technique include the equipment's relatively high cost and a group of elements that cannot be detected [15].

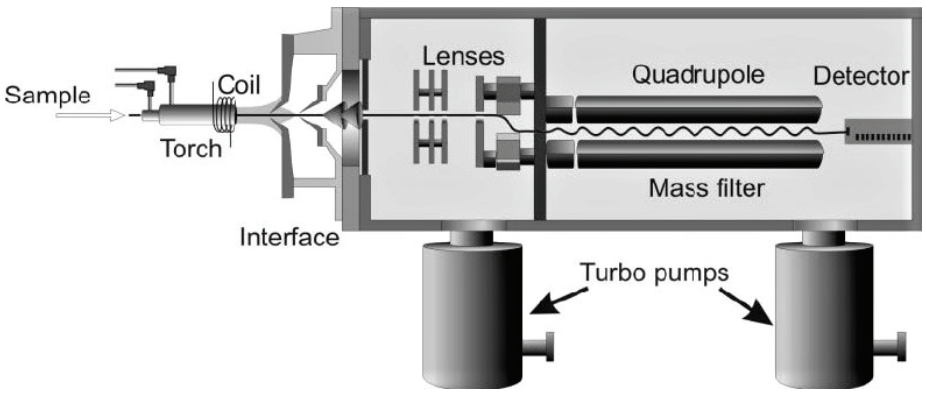

Figure 1. Cross-section schematic of an ICP-MS (ref. 14).

Determination of elements in blood samples

The focus on trace element content in biological fluids has led to the development of analytical techniques specifically for this purpose. The low quantities of specific components in human fluids, combined with the complexity of the matrix, challenge analytical methods [14].

The various biological functions that trace elements play in human health indicate their significance in clinical research, diagnosis of deficiency disorder, or prevention of unintended exposure to toxic metals. It is crucial to examine the trace element profile and functional elemental biomarkers in biological fluids to learn more about an individual's nutritional status, the diagnosis and treatment of diseases, and the relationship between these conditions and other contributing factors [16,17]. Based on their chemical characteristics and binding affinities, trace elements in blood are dispersed between the extracellular compartment (blood plasma) and the intracellular compartment (mostly in erythrocytes) [18]. ICP-MS was the most effective and frequently used analytical technique for measuring numerous trace elements in biological fluids such as blood and plasma [19].

ICP-MS was used in microsampling, which became more popular in recent decades, and it is used for standard analyses such as trace element quantification. Researchers compared dried blood spots and microtubes to assess their capacity for analysing 12 trace components in human whole blood [20]. The technique detected trace elements (K, Zn, Se, Cu, Mn, Fe, Mg) in serum and whole blood. The suggested methodology was validated by analysing certified human serum and whole blood with known amounts of all elements. The method is suitable for routine usage in biomonitoring investigations [21]. An enhanced micro-sampling ICP-MS technique was developed in another study to measure the concentrations of Ca, Mg, Cu, Zn, Fe, Mn, Se, and Pb in uremic patients receiving long-term hemodialysis [22].

On the other hand, the technique was used to investigate baseline blood levels of 12 toxic and/or essential metals and metalloids in Wuhan, central China, including As, Cd, Pb, Hg, Cr, Tl, Mn, Cu, Zn, Ca, Fe, and Mg [23]. Researchers developed a method to measure the content and size of silver nanoparticles in blood for use in in vivo toxicological assessments. The approach can be applied to characterise AgNPs in toxicity research [24]. Cu content in human red blood cells was studied using time-resolved ICP-MS. Human red blood cells (1.5 × 105 /mL) were transformed into fine aerosols using a modified nebuliser and spray chamber for efficient single-cell insertion into the ICP [25]. A new high-precision and high-throughput technique for directly identifying major and trace elements in whole blood samples was developed, and it was based on the laser ablation ICP-MS equipment. This technique significantly increased precision by using a specially made cryogenic ablation cell to prevent droplet splashes during the ablation procedure by solidifying liquid whole blood samples [26]. The effects of storage temperature and stability of various clinical trace elements in human blood and plasma were investigated over an extended storage period. It was found that human blood and plasma specimens could be stored for up to six months at low temperatures (4 °C and -20 °C) without experiencing significant changes in elemental content [27].

Urine elemental analysis

Urine is a mixture of waste metabolites that are soluble in water and is produced when the kidneys filter blood at a consistent rate. Urine analysis is well-established and has been used to analyse exposure to harmful elements or chemicals, find irregularities in absorption, or study diseases to determine causes and enhance prognosis [28,29]. Urine analyses are less complicated than blood or faeces because sample pre-treatment is made easier by the comparatively low concentrations of organic and inorganic solutes. Additionally, collecting urine samples is a simple and non-invasive procedure that may be completed without the help of qualified medical professionals [30].

Numerous studies have shown that ICP-MS is the most effective method for identifying elements in urine. An analysis method for the detection of six arsenic compounds, trivalent arsenic, pentavalent arsenic, methyl arsenic, dimethyl arsenic, arsenical choline and arsenical betaine

in urine was established by high-performance liquid chromatography combined with ICP-MS to provide a theoretical basis for health assessment of arsenic poisoning patients [31]. ICP-MS was used to assay elements in the urine alternation and correlation of Mg, Ca, Cu, Zn, Fe, Cr, and Se among diabetic peripheral neuropathy patients and healthy people using multivariate statistical analysis [32]. Chelation therapy was tried for a patient whose symptoms were thought to be consistent with Chronic Fatigue Syndrome, suggesting that the patient may have been intoxicated with metals. Simultaneously, the elemental excretion profile in urine was established. Most toxic elements showed an excretion peak in 12–24 hours after EDTA treatment [33]. An investigation was carried out to examine the impact of physical exercise on the concentrations of Cu in both intracellular (erythrocytes and platelets) and extracellular (serum, plasma, and urine) using ICP-MS [34]. A 14-day excretion study with 20 volunteers involved daily applications of 1 mg of CoCl2 or 1 mg of cyanocobalamin. The samples were obtained from 7 days before treatment to 7 days after. Total Co concentrations found by ICP-MS indicated considerably increased values exclusively after inorganic cobalt consumption [35]. Fifteen metals and metalloids (As, Be, Bi, Cd, Co, Cr, In, Mn, Mo, Ni, Pb, Sn, Tl, V, and Zn) were determined using ICP-MS spectrometry. All elements were detected in urine samples above the limit of quantification in ng/L ranges, except indium [36]. An experiment was done using ICP-MS to see if acid-washed containers were required for the 24-hour urine copper analysis. Assay diluent and unidentified urine samples were spiked with the copper calibrator to produce copper solutions at concentrations relevant to clinical decision limits. It was found that measuring the amount of copper in 24-hour urine does not require acid-washed containers [37].

Medicinal applications

In living organisms, numerous metal ions have structural and catalytic activities in proteins and enzymes, and they contribute to several physiological processes, such as antioxidation, metabolism, signalling, and gene expression [38]. Approximately ten elements are required for life: Na, K, Mg, Ca, Mn, Fe, Co, Zn, Ni, Cu, and Mo. Biologically necessary metals are classified into two types: non-transition elements (Na, K, Mg, and Ca) and transition elements (Fe, Co, and Cu) [39]. Ions like divalent Ca2+, which are found in relatively high concentrations, are among the necessary metal ions: Ca2+ is a required component of bones and teeth and accounts for 1% to 2% of the human body weight [40]. Mg2+ is also an essential element in rather substantial amounts in the human body and constitutes about 0.05% of body weight [41]. Metal ions frequently act as cofactors for enzymes and are required for their proper function, permitting catalytic activity. Metal ions are also responsible for the structural stability of proteins and for controlling various biological events. A metal binding site's shape may be distorted by the binding of a non-specific metal ion or an ion lacking a specific binding capacity, which could reduce the activity of the corresponding metalloprotein [42]. Because of their increased quantities as a direct result of human activity, the requirement to identify the species (oxidation state/chemical form) of elements present in the environment and biological matrices has excellent importance [43]. It was found that the search for a superior proteomics quantification method has essentially been resolved with the aid of ICP-MS [44].

ICP-MS analysis of a single cell has significant promise for evaluating components within cells [45]. In cells, trace elements are essential. It was found that ICP-MS is crucial for examining trace elements and their species in cells and that it can help with both clinical and biological research [46]. The technique was used to map elements in mouse brain tissue [47]. Results of a study on the impact of oral deferiprone treatment on Cd accumulation and the homeostasis of vital components in the brains of mice exposed to Cd were presented. The results showed that, in comparison to untreated controls, mice exposed to Cd for 14 days had considerably higher Cd concentrations and significantly lower brain levels of Mg, P, and Zn [48]. A sensitive and specific assay has been developed to detect platinum in biofluids. This technique allowed for the characterisation of patients' long-term platinum exposure after receiving oxaliplatin treatment [49]. Sector-field ICP-MS is a versatile tool for quantifying target elements, such as iron and sulfur, in bio-nano systems. When combined with ultra-

filtration, it creates an adaptable screening platform for assessing the pharmacological properties of engineered iron oxide nanoparticles [50].

Elemental analysis of pharmaceutical products

Substances in pharmaceuticals, excipients, and drug formulations are known as elemental impurities. They can originate from any raw components used in the drug product [51]. Controlling pharmaceutical products is vital to maintaining the high quality of pharmaceutical manufacturing. Official pharmacopoeias and authorised supervising bodies have called for more thorough and accurate quantitative screening of specific elemental impurities in medications since some may be seriously harmful to human health [52].

ICP-MS was utilised to identify plant-derived therapeutic compounds, with Cu being the most prevalent [53]. The technique was used to determine the factors influencing titanium dioxide nanoparticle size in cosmetic samples [54]. A study shows how effective high-resolution ICP-MS is for qualifying nanoparticles. It showed that crucial requirements for biomedical applications, like resistance to the action of the human serum milieu or reactivity toward serum biomolecules, can be accurately evaluated by recording the signals of gold or sulfur isotopes using novel gold nanoparticles stabilised by N-heterocyclic carbenes as test nanoparticles [55]. To investigate the toxicity levels of 22 nasal spray saline samples, Al, Sb, As, B, Cd, Cr, Co, Cu, Fe, Mn, Ni, Si, and Zn were analysed using ICP-MS [56]. It was also used to assess the concentration of metals (Ag, Ba, Bi, Cd, Pb, Sr, Tl) in 94 eye shadow samples from the Polish market [57]. It was also used to distinguish between ultra-trace quantities of transition metals (Co, Cr, Cu, Fe, and Ni) that interact with therapeutic proteins and free metal in solution in the drug formulation [58]. It was also used to determine Ag and Zn in microcapsules, as they are mighty antibacterial metals [59], and to assess 18 plasticiser residues (phthalates, adipates, sebacates, and others) in sixteen drugs that are sold in Tunisian pharmaceutical markets [60]. ICP-MS, with other techniques, was used to analyse a poison vial found in the remains of a soldier who died in 1944 in Normandy, France [61].

Assessing elements in water

Pure drinking water is crucial to survival and essential for optimal nutrition. Different natural and artificial processes pollute many water sources worldwide, causing various health issues for humans [62]. Water quality is deteriorating due to the ongoing addition of harmful chemicals and bacteria and the constant addition of domestic and industrial sewage sludge, garbage, and other hazardous waste that is damaging to humans and the environment [63]. Spectroscopic methods such as ICP-MS enabled the determination of the total metals and metalloid content in the water at low concentration levels [64].

Single particle ICP-MS was used to examine the presence of Ti- and Pb-based particle nanomaterials in the aquatic environment in 63 locations in the Melbourne area of Australia [65]. It was also used to evaluate a variety of elements in water, such as Ra-226 [66], Cr(III) [67], and F, by measuring BaF+ ions [68]. A technique for determining the total concentrations of the rare earth elements La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu in mineral water was developed and validated [69]. Another ICP-MS study examined the environmental geochemical properties of rare-earth elements in surface waters in the Anhui Province, China's Huainan mining area [70]. ICP-MS was used to compare water lead measurements made by two field analysers using anodic stripping voltammetry and fluorescence spectroscopy to reference laboratory measurements [70]. The technique was employed in a study on exposure to the high fluoride concentration in spring water in the Bazman volcanic Area in southeast Iran [72]. A cathodic stripping voltammetry electroanalytical technique on a miniature platinum working electrode was used to develop a new set of miniature sensors for Mn determination [73]. Combined with cobalt ions, the method enhanced photochemical vapour production and highly sensitive analysis of trace antimony in water samples [74].

Clays and soil analysis

Since human activity and industrial development are expanding at alarming rates, scientists are focusing on the issue of detecting these pollut-

ants in the environment. ICP-MS has proven sufficient for this area's analysis [75]. On the other hand, the technique is widely used for analysing several elements in geological samples for trace element levels [76]. The study of the provenance of archaeological pottery has extensively used elemental chemical analysis in archaeometry. The method involves determining the highest number of major, minor, and trace elements in ceramics and comparing them to known or assumed origins [77].

ICP-MS was used to determine sediment samples from the Itapicuru-Mirim River in Jacobina, Bahia, Brazil, for As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn concentrations [78]. Three Lemnian and three Silesian medicinal earths from the University of Basel's Pharmacy Museum were examined for antibacterial activity using [79]. The technique was used to study the *in vitro* release of aluminium from the geophagic clay Chacco in the Peruvian highlands [80]. In a study comparing the multi-element composition of forest trees to soil chemical and physical properties, 46 elements were measured [81]. A study examined the distribution of Li during the evaporation of brine ponds that produce halite and gathered the first data on the amount of Li in the salt plugs in southern Iran [82]. Another study examined metal(loid) presence and size-dependent variations in concentration in recent marine sediments from coastal and open-sea habitats in the eastern Adriatic [83]. Single particle-ICP-MS was used to analyse the size distribution of copper oxide nanoparticles in aqueous test soil extracts [84]. ICP-MS and other techniques were used to identify the mineral compositions of 28 soil samples collected from various places in the Disi area (South East Jordan) [85]. The technique was combined with Laser ablation to study the clay fraction of archaeological pottery [86]. The particle size distribution of colloids containing Cr(III) and Cr(III) species in mobile colloids was determined using the technique [87].

Conclusions

Even while researchers continue to use other methods, including atomic absorption and atomic emission, to detect metals in various species, there has been a discernible movement toward ICP-MS, particularly over the last decade. Because it enables dependable analysis at trace and ultra-trace levels while preserving a broad dynamic range, ICP-MS has proven to be a practical method for elemental measurement of biological fluids, water, soil, and clay, as well as pharmaceuticals. In this review, we demonstrated that the capabilities of the ICP-MS analytical technique have witnessed various developments.

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Conflict of interest statement
The authors declare no conflict of interest.

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REVIEW PAPER

JMS Journal of Medical Science

Factors determining the prognosis of people with type 1 diabetes – current perspective

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ABSTRACT

A multitude of factors strongly influences the prognosis of subjects with type 1 diabetes. In the literature, it's notable that only a limited number of studies simultaneously address multiple factors. Our objective is to identify and compile current papers that thoroughly examine these factors, offering a comprehensive overview of the various elements that can influence both life expectancy and the prevalence of complications among individuals with type 1 diabetes. In the overview, we included modifiable and non-modifiable factors. The paper covers technology development, comorbidities as well as acute and chronic complications as predictors. Greater focus on the significance of sex and age as a risk of macrovascular diabetes-related complications, age at onset of diabetes, and episodes of acute complications, can lead to more targeted management of type 1 diabetes and therefore, higher life expectancy. The article also discusses such environmental

factors as lifestyle, education, and access to the healthcare system affecting better handling of type 1 diabetes. This overview emphasizes the plurality of factors that are considered in type 1 diabetes, which might be crucial to prolonging life expectancy and reducing the prevalence of complications.

Introduction

Diabetes type 1 (T1D) remains one of the most commonly occurring chronic diseases with its incidence steadily increasing in recent years [1]. Despite advancements in various aspects, including diagnosis, treatment, and management, leading to more positive outlooks and reduced overall mortality, individuals with type 1 diabetes still experience a notably lower life expectancy compared to the general population [2,3]. Various factors can influence the lifespan of subjects with type 1 diabetes, ranging from modifiable lifestyle choices to non-modifiable demographic characteristics [4]. In the literature, it's notable that only a limited number of studies simultaneously address multiple factors. Our objective is to identify and compile current papers that thoroughly examine these factors, offering a comprehensive overview of the various elements that can influence both life expectancy and the prevalence of complications among individuals with type 1 diabetes.

The significance of technology in diabetes management

There is no doubt, that the greatest progress in recent years in the treatment of patients with type 1 diabetes has occurred in the field of new technologies, those supporting the measurement of glycemia, and those facilitating precise insulin administration.

Self-Monitoring of Blood Glucose (SMBG) and Continuous Glucose Monitoring (CGM) Systems

In recent years, tremendous technological advances have significantly improved the prognosis for patients with type 1 diabetes (T1D), enhancing their daily functioning and simplifying the monitoring and management of their condition. Glucometers have been the most fundamental and widely used devices for daily blood glucose monitoring for many decades by the vast majority of diabetes patients [5,6]. One of the recent advancements in diabetes management includes the development of smartphone applications (apps) specifically designed for diabetes management. These apps offer various features to help individuals with diabetes monitor their blood glucose levels, track insulin dosages, record food intake, and log physical activity. Despite the glucometer's relatively high reliability in blood glucose measurements [7,8], it has drawbacks, notably the need for daily finger pricking, and above all, only current, glycemia measurement without insight into the past or future. Consequently, more technologically advanced methods of blood glucose measurement have been developed, namely the CGM systems.

CGM systems consist of sensors that continuously monitor interstitial glucose levels, and transmitters that send wirelessly data to a receiver or smartphone app, providing real-time glucose readings and trend information [9]. This significantly enhances the ability to manage glycemia fluctuations in response to meals or exercise. By providing alerts CGM systems increase the safety of treatment and reduce the risk of hypoglycemia, especially severe and nocturnal ones. In the DIAMOND trial, the mean glycated hemoglobin level (A1c) after twelve weeks of CGM use decreased by 1.1% in the type 1 diabetes group and only by 0.5% in the control group. Additionally, the mean time below range (TBR) <70 mg/dl in the two groups was 43 min/day and 80 min/day respectively [10]. CGM systems are an excellent educational tool and can motivate patients to improve self-care. They have also demonstrated long-term positive effects on increased physical activity, weight loss, reduced calorie intake, and greater treatment satisfaction, indirectly improving the prognosis of patients with T1D [11].

Insulin administration

The most crucial aspect of insulin therapy is selecting the appropriate type of insulin and its

proper administration. The first commercially available insulin was of animal origin. Only in the 1980s did human insulin preparations obtained through genetic engineering appear. The next generation of preparations are insulin analogues. Compared to human insulin, insulin analogues have an action profile closer to physiology. Undoubtedly insulin analogues offer advantages in daily life, among others preventing nocturnal hypoglycemia and improving postprandial glycemia [12]. Thanks to advancements in genetic engineering, insulins are now being developed to reduce the frequency of daily injections or, in case of basal insulin, are planning to be administered weekly [13]. Regarding insulin delivery, there are various methods available. Insulin syringes were the first to appear on the market, now being replaced in many countries by pen injectors [14,15]. Additionally, so-called 'smart pens' are being further developed to transmit information on insulin administration times and doses via Bluetooth to a dedicated smartphone application, which also provides dose reminders and monitors insulin levels [16]. Moreover, unconventional methods of insulin administration, such as oral, or inhalable delivery, have gained interest among researchers and may potentially replace subcutaneous administration in the future [17].

Insulin pumps. closed-loop systems

Closed-loop insulin delivery systems, also known as artificial pancreas systems, represent the most advanced technology mimicking the function of the natural pancreas. These systems integrate CGM with an insulin pump and algorithm to automatically adjust insulin delivery based on real-time glucose readings. Initially introduced in Europe in 2015, they have rapidly evolved, with increasingly sophisticated solutions available today [18].

While most closed-loop systems primarily administer insulin, some incorporate glucagon or a combination of both hormones [19]. They demonstrate high efficiency in automatic glucose regulation, notably reducing the risk of hypoglycemia. Despite potential inaccuracies in carbohydrate estimations, adaptive algorithms effectively compensate meal estimation, enhancing overall system performance. This represents an amelioration in patients' quality of life and a reduction in associated stress, ultimately improving their prognosis [20].

Patients also have the option of choosing standalone insulin pumps, which provide them with simplified daily routines and enhanced treatment satisfaction. However, their effectiveness outside the closed-loop setting is notably diminished. Research suggests that two years after training in flexible insulin therapy, the reduction in A1c levels was considerably better on a pump compared to MDI, with pumps resulting in a decrease of 0.85% compared to 0.42% for MDI [21].

The role of immuno-based therapy and transplantations

Currently, the sole known cure for T1D is pancreas transplantation, with over 900 procedures performed annually in the United States alone. These surgeries offer the opportunity to maintain euglycemia permanently, prevent hypoglycemia, and sometimes mitigate or eliminate the effects of the disease [22,23]. Due to co-existing renal-related complications, patients often undergo kidney transplantation simultaneously. Despite potential complications, the majority of patients achieve therapeutic success, with estimated survival rates of 87% at five years post-surgery and 70% at ten years for simultaneous pancreas-kidney (SPK) transplantation [24]. Patients experience increased empowerment leading to improvements in health, mental well-being and social interactions. Systemic changes are observed in glomerular structure and cardiovascular function [23].

An alternative to whole pancreas transplantation is islet cell transplantation, which is a simpler and lower-risk procedure. Although it does not guarantee total insulin independence, achieved by 52% of patients, it reduces the frequency and severity of hypoglycemic episodes. A1c level is reduced to < 7.0% in most patients, reaching the median value of 5.6% at 1-year post-transplant [25]. However, graft survival is influenced by continued immunosuppression, which poses challenges for recipients. As a result, ongoing research aims to investigate alternative cell

sources capable of producing functional pancreatic beta cells or to develop transplantation techniques that minimize immune system activation. Promising studies focus on macro-encapsulated human islets and pluripotent stem cells (iPSCs) obtained from patient tissues and reprogrammed in culture into stem cell-derived islets (SC-islets). These can be used not only for autologous transplantation but also for studying the pathogenesis of T1D [26,27].

Recently, immune-based therapies have gained significant interest in treating T1D due to their potential to modify the underlying autoimmune response responsible for destroying insulin-producing beta cells in the pancreas. These therapies aim to halt or slow down the progression of the disease, preserving beta cell function and improving glycemic control. Teplizumab, a humanized monoclonal antibody targeting CD3, has emerged as the first FDA (Food and Drug Administration) approved therapy for modifying the course of preclinical stage 2 diabetes [28].

The significance of late diabetic complications and selected comorbidities in diabetes prognosis

Chronic kidney disease (CKD), Diabetic peripheral neuropathy (DPN) and Cardiac autonomic neuropathy (CAN)

Stages of CKD were found to be one of the most significant factors predicting all-cause mortality in type 1 diabetes. Advanced eGFR stages G4-G5 were found to have some of the highest mortality rates per 100 person-years, at 9.11 and 11.42 respectively [29,30]. Patients with end-stage renal disease have more than three times increased risk of all-cause death compared to patients without such ailments [29,31]. Individuals on renal replacement therapy have a median survival time of 3.84 years since the beginning of the mentioned procedure [32]. Diabetic retinopathy is a highly specific neurovascular complication with prevalence strongly related to both the duration of diabetes and the level of glycemic management. Diabetic retinopathy is the most frequent cause of new cases of blindness among adults aged 20–74 years in developed countries [33]. Distal symmetrical polyneuropathy is the cause of severe discomfort, significantly deteriorates the quality of life of patients, and is a recognized risk factor for the development of diabetic foot syndrome in the form of ulcers and Charcot's neuroarthropathy. Neuropathy increases the risk of amputations, fractures, and falls, as well as the costs of treatment, and is a predictor of increased mortality risk. Cardiovascular autonomic neuropathy is an independent risk factor for increased mortality in diabetes [34].

Cardiovascular disease (CVD)

Patients with T1D with a history of cardiovascular disease were found to have almost twice the risk of all-cause death than individuals without it. History of heart failure worsens the prognoses even more [29]. The co-existence of CVD and CKD further increases the risk of death [29]. In the past years, deaths and hospitalizations from CVD declined substantially in people with type 1 diabetes. In Sweden from 1998 through 2014 patients with type 1 diabetes had roughly even 40% greater reduction in cardiovascular outcomes than controls. Unfortunately, the risk of CVD events still remains clearly higher in the population with T1D [30,35].

Overweight and obesity

Normal body mass index (BMI) isn't indicative of the longest lifespan in individuals with T1D. Research across various BMI groups reveals that those with a BMI of 20 kg/m² tend to have the shortest predicted lifespans, while those with a BMI of 25 kg/m² demonstrate the best outcomes [4]. This seems consistent with the findings of other studies which suggest the lowest adjusted mortality rates within the 25–29.9 kg/m^2 BMI range [29]. However, the prognosis worsens with further increases in BMI. Nonetheless, differences in predicted lifespans between class I obesity and class II obesity are inconsistent. Some cases suggest that more obese patients might have slightly longer life expectancy than less obese ones, even though the exact opposite is often true [4]. This contrasts with the general population, where increasing BMI typically correlates with an increased risk of death [36]. The association between BMI and mortality in subjects with T1D may be influenced by confounders such as differences in age, duration of diabetes,

glycemic control, and the presence of comorbidities like cardiovascular disease or nephropathy. Lower BMI in T1D individuals might be indicative of poor glycemic control, catabolic states, or the presence of diabetes-related complications that adversely impact longevity.

COVID-19

Patients with diabetes diagnosed with COVID-19 had a 2.26 times increased risk of experiencing diabetic ketoacidosis (DKA) incidents than those without infection [37]. People hospitalized with a COVID-19 infection and type 1 diabetes have a 2.86 times increased risk of death than those without diabetes [38]. This trend was more visible in the younger population with 6.39 times odds of death increase [38].

Depression

Depressive scores are higher in people with T1D overall and increased 7-fold in men as compared to the general population. Higher levels of depressive symptoms are associated with both lower engagement in self-management behaviors and physical activity [39]. Depressed patients with type 1 diabetes have more than 3 times increased risk of DKA event and more than 2 times increased risk of severe hypoglycemia [40]. A positive connection between a patient's history of depression diagnosis and the progression of diabetic nephropathy has been found. A study shows a 1.5 increased chance of renal damage progression in affected patients [41]. These data demonstrate that there is an urgent need to screen adults with T1D for depressive symptoms as part of routine medical care and to test interventions to minimize their impact on physical health outcomes.

Impact of age at diagnosis of type 1 diabetes on mortality, life expectancy and acute complications

Type 1 diabetes ranks as the second most prevalent chronic disease affecting children. Notably, the severity of its complications varies significantly based on the age at diagnosis. Individuals with type 1 diabetes face a considerable reduction in life expectancy, evident in the two- to eightfold increase in mortality rates. According to Rawshani et al. in the Swedish National Diabetes Register the onset of type 1 diabetes before the age of 10 is associated with a loss of 16 life-years, estimated at 17.7 life-years lost in females and 14.2 life-years lost in males. Conversely, diagnosis after the age of 20 results in a loss of 10 life-years. This highlights an inverse relationship between age at diagnosis and the risk of mortality, with cardiovascular complications being the primary cause. Moreover, in a Finnish population-based cohort of T1D subjects, the mortality risk from ischemic heart disease is exceptionally high in women with early-onset T1D compared with women in the background population. These observations underscore the importance of identifying risk factors early in women and delivering more aggressive treatment after diagnosis. [42,43] Cardiovascular-related deaths constitute a significant proportion, accounting for 70% and 61% of all major contributors to mortality in individuals diagnosed before the age of 10 and between 26–30 years of age, respectively. Interestingly, the data of Vuralli D et al in Turkey girls were 1.9 times more likely than boys to have two or more risk factors for CVD. Factors associated with risk for CVD in multiple logistic regression analyses were being a girl, followed by higher daily insulin dose, higher hemoglobin A1c, and longer duration of diabetes [44].

Diabetic ketoacidosis and severe hypoglycemia as acute complications of T1D

Diabetic ketoacidosis (DKA) and severe hypoglycemia (SH) are still life-threatening acute complications of type 1 diabetes. Poor glycemic control, considered as higher levels of A1c, remains one of the major factors contributing to the increased risk of DKA, SH, microvascular and macrovascular complications of T1D [45]. The highest frequency of DKA events concerns the suboptimal glycemic control with a rate of A1c > 9.5% in patients aged 13 to < 18 years [46,47]. The incidence of SH by its highest rate relates to 2–6 years old patients in the T1D Exchange Clinic Registry [47]. The frequency of this acute complication is widely influenced by

the level of A1c. Patients with lower A1c (<6%) as well as with higher A1c (>13%) have a greater risk of SH (respectively 6.9% and 13.5%) than those patients with A1c > 6.5% to 7% (3.3% of risk) [45]. Priorly highlighted the importance of firm glycemic control is crucial to preventing significant fluctuations of glycemia and further occurrences of hypoglycemia [45,47]. However, glycemic control and therefore prevalence of DKA and SH closely correlate with other relevant factors such as BMI. Patients with BMI < 30kg/m^2 (normal weight and overweight) have better glycemic control than obese patients and reduced risk of T1D-related complications [45]. It is remarkable that female sex and ethnic minority status correlate with a 23% and 27% increase in the likelihood of experiencing DKA, respectively [46,47]. Coexistent lower household income and lack of private health insurance are associated with elevated incidence of DKA and SH [45,47]. Younger patients are substantially exposed to a higher risk of DKA and SH resulting in morbidity and mortality. Having regard to age and reasonable management of T1D, including monitoring glucose levels and performing blood or urine tests to detect possible considerably increased ketone bodies, could distinctly reduce the occurrence of acute complications in type 1 diabetes patients [45]. Moreover, the wide use of closed-loop systems might significantly minimize the risk of severe hypoglycemia. Fortunately, data from multiple countries, T1D Exchange Quality Improvement Collaborative, and the SWEET initiative gathered between the years 2013–2022 show a decrease in the incidence of DKA from 3.1 events per 100 person-years to 2.2 events per 100 person-years in 2022. This progress is associated with a simultaneous increase in the frequency of insulin pump and CGM systems' usage [48].

Gender as a risk factor for CVD in patients with T1D

Certainly, when examining sex as a risk factor for cardiovascular disease, noticeable differences exist between the general population and individuals with T1D. Statistics report conducted by the European Society of Cardiology shows that males have both higher incidence and prevalence of CVD per 100.000 people compared to females after age standardization [49].

However, in a population of patients with T1D, an alarming statistic emerges. A study combining registries from multiple countries showed, that in patients with T1D, after age standardization females have a greater incidence of CVD such as stroke (1.37 female: male ratio) and have more than twice greater incidence for coronary heart disease (2.54 female: male ratio) with a mortality from CVD being almost twice as much in females than in males (1.86 female: male ratio) [50]. That statistic confirms previous studies in which a relation was found between females with T1D and more rapid arterial endothelial dysfunction such as artery calcification than in males [51,52]. However, more recent studies need to be made to find the underlying causes for the previously shown statistics.

Age as a factor for CVD in patients with T1D

Cardiovascular diseases (CVD) are the leading causes of death in modern society. In the overall American population, in 2020 about 25% of all deaths were contributed to CVD [53]. Moreover, the percentage of CVD and associated deaths are directly proportional to advancing age. In the age group of 25–44 years old, CVD were responsible for 10.6% of all deaths and in the age group of 65 and older, amount of deaths associated with CVD increased to 27.7% [53].

Among patients with T1D age is still considered the strongest risk factor for CVD (including stroke and acute/silent myocardial infarction), followed by mean A1c levels [54]. Subsequently, atherosclerosis as a consequence of endothelial dysfunction plays a crucial role in the pathogenesis of CVD [55]. Studies show, that patients with T1D (mean age of ~46 years) had decreased reactive hyperemia index (RHI, a measure of endothelial function) when compared to healthy control group [56]. The difference in RHI was however not visible in younger patients with T1D (mean age of ~21 years) when compared to the healthy control group of similar age [57]. These results reveal that age as a risk factor for CVD in patients with T1D is significantly more important as a risk factor when compared to a healthy population.

The significance of health care access and education

Complications of T1D can be reduced or delayed by efficient control of A1c, blood pressure, and cholesterol levels (ABCs of diabetes). The strict relation between access to health care and managing disease among people is visible. The importance of access to healthcare in managing the disease is evident, with current health insurance coverage and frequency of medical visits serving as key indicators of access. Individuals with chronic illnesses, such as T1D, require regular communication with medical professionals for effective glycemic control [58,59]. Those without insurance tend to visit medical doctors less frequently compared to their insured counterparts (12.2% vs 2.1%; at least one visit during the last 12 months respectively), leading to poorer diabetes management outcomes [58]. Therefore, people with poor access to medical care (state insurance instead of private or lack thereof) showed a higher probability of having an A1c level > 9% and blood pressure > 140/90 mmHg [58,59]. Moreover, the frequency of medical visits correlates with better diabetes control, with individuals reporting over four visits in the previous year demonstrating significantly improved A1c levels compared to those who did not disclose any medical visits (mean A1c 9.1 vs. 7.4% respectively) [58]. There is a strong and clinically relevant correlation between inadequate glycemic control, lack or poor level of insurance, and frequency of visits to the physician [59]. Enhancing the availability of healthcare services for individuals with T1D is crucial, as it plays a vital role in controlling risk factors and decreasing diabetes-related complications [58].

Adolescents diagnosed with T1D encounter difficulties adjusting to a way of life that necessitates self-management of food habits, exercise routines, and insulin dosage adjustments. The purpose of diabetes education is to assist patients in gaining the skills, information, self-care routines, coping mechanisms, and attitudes necessary for efficient diabetic self-control [60,61]. Patients who had obtained diabetes education (59%) were more inclined to conduct self-management than their counterparts who had not. It is associated with A1c level: 42% of educated patients had a level of A1c > 8% vs 52% in a group of non-educated. These findings unequivocally demonstrate the advantageous effects of diabetes self-management on glycemic target achievement and the favorable correlation between diabetes education and self-management [62]. There are a dozen of established diabetes education programs. For individuals with T1D, PRIMAS is one of self-management-oriented education programme. Research proves that PRIMAS is effective in lowering A1c, as evidenced by a 0.4 percentage point decrease in A1c compared to the control group [61]. Presented data shows that to improve the effectiveness of patient treatment and avoid acute complications all diabetic centers should incorporate health education throughout their diabetes care programs [60]. Adolescents with T1D may benefit from education intervention as a preventative measure against potential declines in their quality of life. Moreover, it has been proven that deterioration of glycemic control can be prevented through educational intervention, which translates into more effective treatment and better patient outcomes [60,62]. In conclusion, diabetes education fosters a favorable attitude toward patients' active involvement in the control and treatment of their condition in addition to imparting the knowledge and skills necessary to maximize self-management [62].

Lifestyle features are important for people with type 1 diabetes

Self-Management

It is known that self-management programs can significantly improve the quality of life for people with T1D. These programs encourage independence and empower individuals to take control of their health, potentially leading to improved emotional well-being. However, the situation is not without its challenges. Depression is a possible comorbidity in T1D and can significantly hinder a teenager's ability to stay motivated and adhere to the demanding regimen. Furthermore, research indicates that while self-management programs may improve the quality of life, their impact on metabolic control, as measured by A1c, might be less pronounced. This highlights the need for a multi-directional approach that addresses not just the physical aspects of diabetes manage-

ment but also the emotional well-being of the adolescent [34].

Physical activity

A sedentary lifestyle often correlates with a diminished quality of life for individuals with T1D. Physical inactivity can lead to fatigue, reduced motivation, and a decline in overall well-being. However, there is promising evidence: incorporating regular physical activity into one's daily routine can bring about transformative changes. Studies demonstrate that exercise improves sleep quality, enhances overall enjoyment of life, and increases motivation for further physical activity. Systematic physical exercise increases insulin sensitivity and allows people with type 1 diabetes to optimize glycemia with lower insulin demand [34]. This positive cycle not only improves quality of life but also offers significant health benefits.

One major concern for people with T1D is the risk of hypoglycemia during exercise. However, this fear can be addressed by implementing a well-designed training plan. High-Intensity Interval Training (HIIT) has emerged as a promising approach. By incorporating short bursts of intense activity followed by recovery periods, HIIT allows for effective exercise while minimizing the risk of hypoglycemia. Additionally, regular exercise offers a plethora of benefits beyond blood sugar control. It improves fasting glucose levels and reduces cardiovascular risk, potentially leading to a longer and healthier life. However, research suggests that achieving a significant reduction in A1c levels through exercise is more likely when adhering to a structured training plan like HIIT. This underscores the importance of tailoring exercise regimens to individual needs and preferences to maximize their effectiveness [64–66].

Diet

Diet plays a crucial role in managing blood sugar levels in T1D. Studies suggest that there is a need for a personalized approach to dietary planning, taking into account factors such as age, gender, and physical activity level. The standards of care of Diabetes Poland state that individuals with type 1 diabetes should avoid easily digestible carbohydrates and follow the general principles of a properly balanced diet. There is insufficient scientific evidence to determine one optimal amount of carbohydrates for individuals with diabetes, so carbohydrates should make up about 45% of total energy [34]. There is a growing body of research on dietary management strategies for individuals with type 1 diabetes. Turton et al. conducted a single-arm non-randomized clinical trial to investigate the effects of a low-carbohydrate diet in adults with type 1 diabetes management. The preliminary findings suggest that a professionally supported low-carbohydrate diet may lead to improvements in markers of blood glucose control and quality of life with reduced exogenous insulin requirements and no evidence of increased hypoglycemia or ketoacidosis risk in adults with T1D. Muntis et al. indicate a possible link between high protein intake and improved glycemic control after exercise. This suggests that incorporating lean proteins into the diet can further optimize blood sugar management during physical activity. Gluten-free diets, however, have not been shown to improve quality of life or glycemic control in individuals with T1D who are not diagnosed with celiac disease. Interestingly, research on adolescent dietary habits reveals a concerning trend. A study suggests that the average diet of teenagers with T1D is often lacking in essential nutrients. Addressing these challenges through education and personalized dietary counseling can significantly improve the quality of life for adolescents with T1D [67–70].

Conclusions

Prognosis and management of people with type 1 diabetes involve many crucial determinants. These factors are strongly connected with morbidity, mortality, and life expectancy of patients. This review highlights two main groups of features influencing the prognosis of patients with T1D. The first group includes key-role factors such as sex, age at onset of T1D, presence of comorbidities, and cardiovascular and acute complications of T1D. The data emphasize the importance of considering these factors, especially in patients with early onset T1D, and implementing more targeted guidelines, particularly in cardiovascular prevention, which could significantly lower the mortality of people diagnosed with type 1 diabetes at a young age. Greater focus on these characteristics during early diag-

nosis and treatment is crucial for mitigating the risk of lower life expectancy and achieving better control of the disease in individuals with T1D. The second group comprises environmental aspects, including consistent self-management, access to diabetes educational programs, enhanced healthcare system access, and recent technological advancements. These factors are closely associated with improved prognosis in patients with T1D. Strengthening these areas could significantly simplify T1D management, and improve quality of life and daily functioning while reducing the occurrence of severe diabetes-related complications. The review discusses the current perspective on the prognosis of patients with T1D, which is markedly dependent on a wide range of factors. Incorporating their relevance into guidelines could significantly prolong life expectancy, reduce morbidity, and enhance daily well-being for individuals living with T1D.

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REVIEW PAPER

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The microbiome-mind connection: exploring gut health's impact on depression

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ABSTRACT

Introduction. The gut-brain axis is considered to be a crucial component of mental health, significantly influenced by the gut microbiota. This axis operates through neural (vagal nerve), hormonal (Hypothalamic-Pituitary-Adrenal axis), and immune pathways. Key mechanisms include microbial production of neurotransmitters like serotonin and gamma-aminobutyric acid, modulation of inflammatory responses and metabolic pathways involving short-chain fatty acids. Dysbiosis - a microbial imbalance - is associated with increased inflammation and neurotransmitter disruptions, both contributing to depressive symptoms.
Material and methods. The search strategy was centered on gathering high-quality articles focusing on the gut-brain axis and its implications for mental health, particularly depression. Databases including PubMed,

Scopus, and Google Scholar were searched using keywords such as "gut-brain axis," "microbiota and mental health," "depression and gut microbiome," "gut neurotransmitters," "probiotics," and "inflammation and mood disorders." Studies were selected with a focus on research published mainly within the last two years.
Results. Potential interventions, such as administration of probiotics, prebiotics, dietary modifications, and innovative therapies like fecal microbiota transplantation and vagus nerve stimulation intend to restore the gut microbiota equilibrium.
Conclusions. Despite the limitations of current research, such as reliance on animal models, small human sample sizes, and methodological inconsistencies, expanding these studies remains highly valuable. Conducting large-scale human trials with standardized protocols and deeper exploring the interactions of specific microbial species could create a foundation for new approaches to supporting the treatment of depression effectively.

Introduction to human microbiota

The human gastrointestinal (GI) tract, particularly the intestines, hosts a vast array of microorganisms, predominantly bacteria, alongside smaller populations of viruses, fungi, and archaea. The largest concentration of these microorganisms reside in the colon, where they form a complex microbial community that interacts with the host, influencing a range of physiological and pathological processes.

The bacterial composition of the gut microbiota is dominated by *Firmicutes* and *Bacteroidetes phyla*, although *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia* are also present in smaller proportions, contributing to its diversity and functionality [1].

Development of gut microbiota

The development of the gut microbiota begins at birth and undergoes significant changes during infancy and early childhood [2]. Factors shaping this dynamic process include:

- Mode of Delivery: Vaginally delivered infants acquire a microbiota resembling the maternal vaginal flora, whereas those born by cesarean section show microbiota patterns more similar to the maternal skin microbiome [3].
- Diet: Breastfeeding supports a distinct microbiota profile, with bacteria like *Bifidobacterium*. Formula feeding, however, leads to a more diverse microbiota composition, resembling that of adults [4]. While such diversity is generally considered beneficial in older children and adults, in infants, it may not offer the same immune-protective benefits as a bifidobacteria-dominant profile.
- Environmental Exposures: Both prenatal and postnatal antibiotic exposure can disrupt microbial colonization, increasing the risk of metabolic and allergic diseases later in life [5]. Antibiotics interfere with the passing of beneficial bacteria from mother to child, causing reduced microbial diversity and imbalance in species.

Geographic and dietary influences on gut microbiota composition

Geographic factors, especially regional dietary habits, significantly impact the diversity and structure of the gut microbiota. Western diet pattern, rich in fats and proteins, is associated with an increase in the *Bacteroides* enterotype and generally contributes to lower microbial diversity. In contrast, fiber-rich diets common in non-Western countries are typically associated with higher levels of *Prevotella*, a genus linked to carbohydrate-rich, plant-based diets [6].

Polyphenols are found abundantly in plant-based foods such as fruits, vegetables, tea, wine, and cocoa. Due to their prebiotic-like properties, polyphenols bypass digestion in the small intestine and reach the colon, where they serve as a nutrients for beneficial microbes, thereby enhancing microbiota composition [7].

Within individual countries, urbanization also affects gut microbiota diversity. Rural populations, who often maintain traditional diets rich in fiber and low in processed foods, display higher microbial diversity than urban populations, whose diets tend to be abundant in processed and fat-dense foods. Another impacting element is seasonal dietary variations and lifestyle. For example, rural populations in Mongolia dem-

onstrate seasonal shifts in microbiota composition due to changes in available food sources, supporting a microbiota that is both diverse and adaptable [6].

Functional roles of gut microbiota

The gut microbiota plays various essential roles in maintaining host health, acting as a metabolic, immunologic, and protective barrier. Key functions include:

- Metabolic Functions: The gut microbiota ferments dietary fibers to produce short-chain fatty acids (SCFAs) like butyrate, acetate, and propionate. These SCFAs serve as an energy source for colon cells and help regulate glucose and lipid metabolism [8]. Unlike other cells that primarily use glucose, colonocytes rely on SCFAs to produce ATP through the citric acid cycle [9].
- Immune Modulation: The microbiota influences both the innate and adaptive immune responses [10]. Microbial products and metabolites stimulate immune cells - including macrophages, dendritic cells, and neutrophils - enhancing their ability to recognize pathogens and modulate inflammatory responses. Certain bacterial species also contribute to the development of T helper 17 (Th17) cells and the differentiation of T regulatory cells (Tregs). One pathway through which these bacteria exert their influence is by conjugating bile acids. Preclinical experimental studies in mice have demonstrated that this conjugation significantly impacts the intestinal microbiota, thereby promoting the differentiation of Th17 and Treg cells [9].
- Barrier Integrity and Defense: The gut microbiota competes with pathogenic organisms for resources and attachment sites, thereby preventing colonization by pathogens. It strengthens gut epithelial integrity by enhancing tight junctions and occupies binding sites along the intestinal lining, blocking pathogens from attaching [11,12].

Dysbiosis and disease associations

Disruptions in the composition of microbiota - known as dysbiosis - are increasingly associated with various disease states:

- Metabolic Disorders: Alterations in gut microbiota composition are associated with metabolic diseases, including obesity, type 2 diabetes, and non-alcoholic fatty liver disease [13].
- Gastrointestinal Diseases: The intestinal microbiota supports nutrient absorption, maintains gut barrier integrity, and contributes to peristaltic movement, facilitating efficient digestion [14]. Dysbiosis has been associated with inflammatory bowel diseases, irritable bowel syndrome (IBS), and other gastrointestinal disorders [15].
- Immune-Related Diseases: Altered microbiome has been linked with immune dysregulation, which can predispose to conditions such as allergies, asthma, and autoimmune diseases [16].
- Neurological and neuropsychiatric Disorders: Dysbiosis has been connected to mood disorders such as anxiety and depression, as well as to attention deficit hyperactivity disorder [17], Parkinson's disease, and Alzheimer's disease [18].

The following section will explore the mechanisms underlying these interactions, highlighting how gut health influences neurological and psychological outcomes.

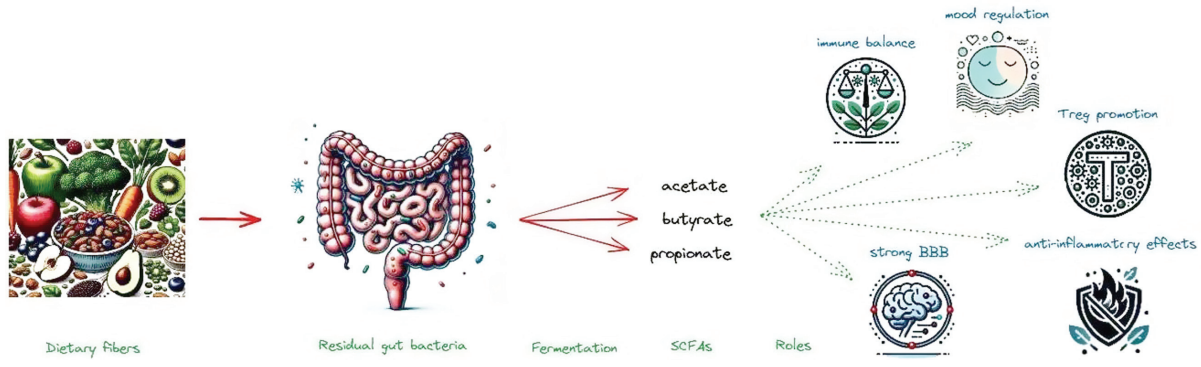

Figure 1. The pathway of SCFAs formation and their role in maintaining health.

Gut-brain axis: pathways of interaction

The gut-brain axis is a complex, bidirectional communication system between the central nervous system (CNS) and the gastrointestinal tract. The key pathways are mediated by neural (particularly the vagus nerve), hormonal, and immunological routes. Each one contributes uniquely to the intricate dialogue between the gut and the brain, helping regulate functions from digestion and mood to immune responses and cognition.

Vagus nerve pathway

The vagus nerve transmits critical information from the GI tract to the brain via neurotransmitters such as serotonin, dopamine, gamma-aminobutyric acid (GABA), and acetylcholine, each of which influences mood, stress responses, and overall mental health [19].

Approximately 90% of the body's serotonin is synthesized in the gut by enterochromaffin cells, a process profoundly influenced by microbial metabolites [20]. While gut-derived serotonin does not cross the blood-brain barrier, it modulates mood and emotional states indirectly through vagal signaling, ultimately affecting central serotonergic neurons [21]. Gut bacteria also play a role in serotonin production by synthesizing its precursor - tryptophan, with species like *Bacteroides* regulating its availability and conversion [22]. Short-chain fatty acids contribute to the promotion of serotonin release [23].

Certain gut bacteria, particularly strains of *Lactobacillus* and *Bifidobacterium*, convert glutamate to gamma-aminobutyric acid, an inhibitory neurotransmitter essential for reducing neuronal excitability and regulating anxiety [24]. When gut-derived GABA binds to vagal receptors, it triggers excitatory pathways that signal to the brainstem, specifically affecting areas such as the locus coeruleus and hypothalamus, demontrating promising anxiolytic effects [23,25]. For example, *Lacticaseibacillus rhamnosus* has been shown to alter GABA receptor expression in the prefrontal cortex, impacting mood and behavior [26]. In a study by Strandwitz, GABA- producing bacteria were linked to reduced anxiety-like behaviors in mice. Introducing these bacteria into germ-free mice increased brain GABA levels and reduced stress-induced hyperactivity [27].

Short-chain fatty acids synthesized by bacteria like *Clostridium butyricum* and *Bacteroides thetaiotaomicron* can influence dopaminergic pathways via vagal signaling, potentially impacting reward-related brain regions. In a study by Dalile et al., healthy participants received a mixture of SCFAs (acetate, propionate, and butyrate), which modulated activity in brain regions associated with reward and motivation, particularly the nucleus accumbens [28]. SCFAs interact with G-protein-coupled receptors (GPCRs) such as GPR41 and GPR43, which are expressed on enteroendocrine cells and vagal afferent neurons. Activation of these receptors by SCFAs can modulate the release of gut hormones like peptide YY and glucagon-like peptide-1, which in turn influence brain function [29].

Although most dopamine in the CNS is synthesized locally, gut-derived dopamine affects local GI functions and systemic signaling rather than directly modulating CNS dopamine levels. It interacts with D1-D5 dopamine receptors, where signaling through D2, D3 and D4 receptors influences the release of acetylocholine and vasoactive intestinal peptide [30]. While these effects have been observed primarily in animal models, further research is necessary to confirm these mechanisms in human studies [31]. Some bacterial strains, including *Lacticaseibacillus rhamnosus* and *Limosilactobacillus reuteri*, influence acetylcholine release, stimulating the vagus nerve to activate the cholinergic anti-inflammatory pathway. Acetylcholine binds to nicotinic acetylcholine receptors located on immune cells like macrophages [31]. This pathway reduces pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), contributing to systemic inflammation control [1,32]. The vagus nerve pathway illustrates how gut-derived neurotransmitters, microbial metabolites, and specific bacterial strains influence CNS function and mental health. While serotonin and dopamine from the gut influence brain function indirectly, others, such as GABA and SCFAs, have direct impacts on neural signaling and neuroinflammation.

Hormonal pathway (hypothalamic-pituitary-adrenal axis)

The Hypothalamic-Pituitary-Adrenal (HPA) axis is a key hormonal pathway in the gut-brain axis, par-

ticularly relevant to stress responses. When physical or psychological stressors activate the HPA axis, it initiates a cascade that begins with the release of corticotropin-releasing hormone (CRH) from the hypothalamus, stimulating the pituitary gland to secrete adrenocorticotropic hormone (ACTH). ACTH then acts on the adrenal glands, prompting the release of cortisol, a glucocorticoid that modulates immune function, mobilizes energy, and helps maintain homeostasis [33].

SCFAs can influence HPA axis activity indirectly by modulating cortisol production, enhancing immune responses, and supporting the integrity of the blood-brain barrier - all factors that contribute to stress resilience [29,34]. Additionally, SCFAs promote the production of regulatory T-cells, which help stabilize immune responses [28].

Beyond SCFAs, gut bacteria also affect HPA axis through tryptophan metabolism. Tryptophan, an essential amino acid from dietary sources, can follow one of two primary metabolic pathways: conversion to serotonin or to kynurenine, with the direction depending on immune conditions. In states of chronic inflammation, tryptophan is more likely to be metabolized into kynurenine, which activates the HPA axis and promotes cortisol release. Elevated kynurenine levels have been associated with mood and stress-related disorders [19].

Certain probiotic strains, such as *Bifidobacterium longum* and *Lacticaseibacillus rhamnosus*, have shown potential in modulating HPA axis activity. Studies on *Lacticaseibacillus rhamnosus* indicate its ability to lower corticosterone levels and alleviate anxiety-like behaviors. These findings align with research showing that *Lacticaseibacillus rhamnosus* affects GABA signaling through the vagus nerve, highlighting its role in regulating both neurotransmitters and hormones [26,35].

The HPA axis indicates that through the modulation of cortisol production and tryptophan metabolism, gut microbiota may significantly impact mental health and stress resilience [18].

Immunological pathway

The immune system plays a significant role in gut-brain communication, as changes in the microbiota can modify immune responses, potentially triggering inflammation and neurobiological alternations [36].

Dysbiosis can increase intestinal permeability, allowing endotoxins such as lipopolysaccharides (LPS) from Gram-negative bacteria to enter the bloodstream. LPS can trigger systemic inflammation and, once crossing the blood-brain barrier (BBB), may lead to neuroinflammation and contribute to neurological and neuropsychiatric disorders [36,37].

An imbalanced microbiota can overstimulate immune cells, resulting in the release of pro-inflammatory cytokines, including IL-6, TNF-α, and interleukin-1 beta (IL-1β), which reach brain through vagus nerve or by crossing BBB [38].

Microglia, the resident immune cells of the central nervous system, play a critical role in responding to injury and maintaining brain health. However, when overactivated by signals from peripheral inflammation, often due to gut dysbiosis, microglia can become neurotoxic, disrupting synaptic function [39].

The immunological pathway shows how gut dysbiosis and immune dysregulation drive neuroinflammation, emphasizing the role of beneficial bacteria in maintaining balance.

Gut microbiota influence on depression

While the gut-brain axis has broad implications for neurological and mental health, depression repre-

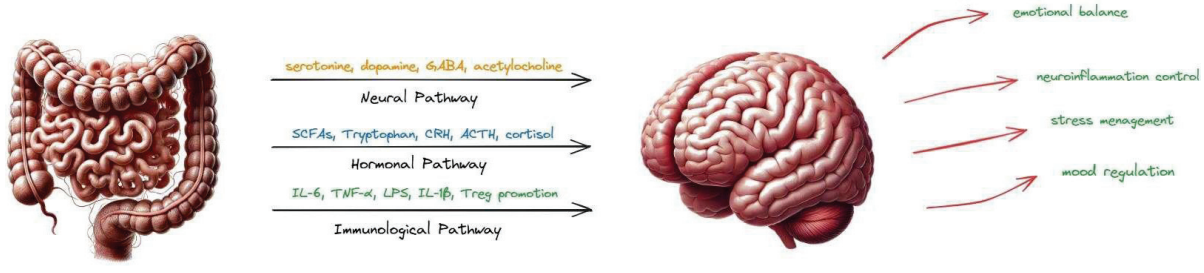

Figure 2. Key pathways in gut-brain axis and the main components contributing in each.

sents an area where microbial interventions hold particular promise. By examining neurotransmitter synthesis, immune modulation, and metabolic pathways, we can gain a clearer understanding of how gut microbiota might interact with and potentially reduce depressive symptoms.

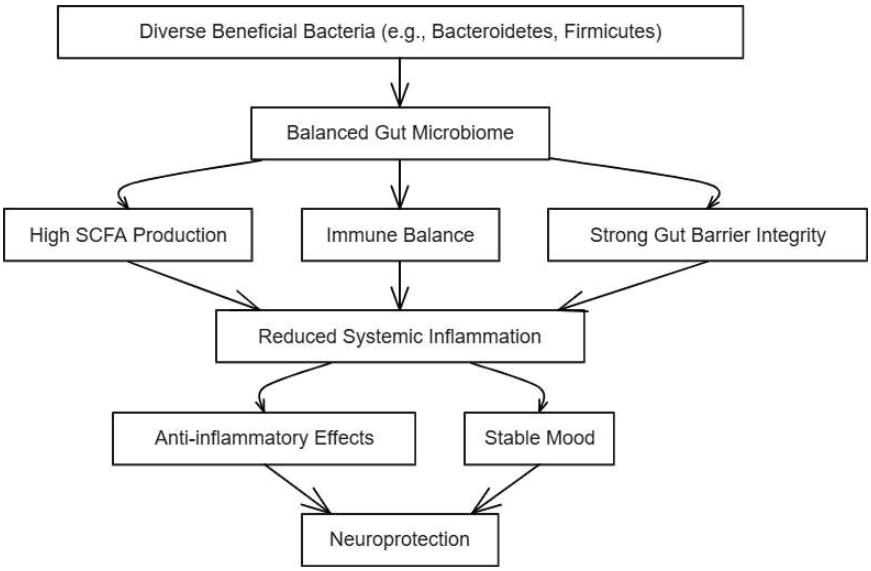

Figure 3. Influence of the balanced gut microbiota on the nervous system.

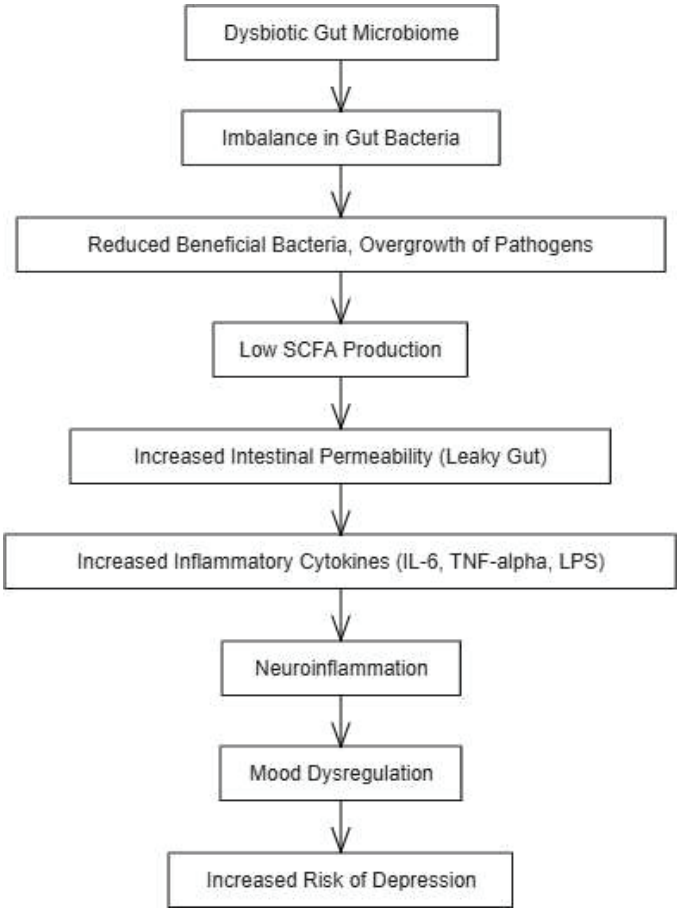

Figure 4. Influence of the imbalanced microbiota on the nervous system.

Neurotransmitter production and depression

As previously discussed, neurotransmitter production in the gut is crucial for maintaining CNS function. In the context of depression, neurotransmitters like serotonin, dopamine, and gamma-aminobutyric acid are essential for mood regulation. The shikimate pathway is worth mentioning. It is a crucial metabolic route found in bacteria, fungi, algae, and plants. It begins with simple carbohydrate precursors and proceeds through seven enzymatic steps, ultimately leading to the formation of chorismate. This compound is further converted into aromatic amino acids, which are vital precursors for the production of the mentioned neurotransmitters [40].

Research has demonstrated that certain bacterial strains, such as *Lacticaseibacillus paracasei*, enhance serotonin production, alleviating depressive symptoms related to gastrointestinal distress [41]. This effect is facilitated by increased tryptophan uptake in the gut. In a randomized clinical trial Peijun Tian demonstrated that *Bifidobacterium* breve CCFM1025 decreased major depressive disorder (MDD) by modulating gut microbiome composition and tryptophan metabolism [42]. In people with MDD, tryptophan is often shifted more toward the kynurenine pathway, leading to metabolites that can contribute to neuroinflammation and neurotoxicity.

Bifidobacterium breve CCFM1025 might reduce this switch, promoting a healthier balance by favoring serotonin production over kynurenine pathway metabolites [42].

Studies on germ-free animal models show that the absence of *Lactobacillus* and *Bifidobacterium* species can lead to lower levels of serotonin and dopamine, which may be partially restored through microbial reconstitution [43,44]. Another compelling piece of evidence linking neurotransmitter levels and depression is the study conducted by Wu, which used chronic restraint stress (CRS) in mice as a depression model. These mice exhibited classical depressive-like behaviors. Compared to the control group, the depressed mice demonstrated significantly decreased levels of norepinephrine, 5-hydroxyindoleacetic acid (5-HIAA), and serotonin (5-HT) in the hypothalamus, underscoring the critical role of neurotransmitter dysregulation in the pathophysiology of depression [45].

Immune system modulation in depressive pathways

As noted earlier, lipopolysaccharides can enter the bloodstream, triggering systemic inflammation. LPS binds to Toll-like receptor 4 which activates a signaling cascade, ultimately stimulating the production of pro-inflammatory cytokines and inflammatory mediators [46].

Elevated levels of interleukin-6 and tumor necrosis factor-alpha have been associated with depressive symptoms due to their neuroinflammatory effects upon crossing the blood-brain barrier [47,48].

Chronic inflammation is a hallmark of MDD and studies show that dysbiosis-driven inflammation stimulates microglial activation in the brain [43]. Overactive microglia release further pro-inflammatory mediators, disrupting neuronal function and synaptic plasticity, contributing to neurodegenerative and neuropsychiatric disorders [49]. Certain probiotics, such as *Lacticaseibacillus rhamnosus* and *Bifidobacterium longum*, have demonstrated efficacy in reducing inflammation by restoring gut barrier function and decreasing the levels of pro-inflammatory cytokines [41,50].

Metabolic pathways and short-chain fatty acids in depression

The metabolic impact of gut microbiota on depression is largely mediated through SCFAs. Butyrate, in particular, is recognized for its neuroprotective and anti-inflammatory properties, supporting mood stabilization by reducing neuroinflammation and strengthening the blood-brain barrier [47]. SCFAs bind to G-protein-coupled receptors (GPCRs) like GPR41 and GPR43 on immune cells, leading to decreased production of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β, while enhancing the secretion of anti-inflammatory cytokines like IL-10. Qi Xu in his research linked SCFAs downregulating function to the NOD-like receptor protein 3 inflammasome, a critical driver of neuroinflammation in the hippocampus, to the alleviation of depression-like behaviors in mouse models [51].

Zeng and Tang also investigated the impact of SCFAs on psychiatric symptoms in individuals during the COVID-19 pandemic. Their findings indicated a reduction in depression and anxiety

levels, which correlated with an increased presence of SCFA-producing bacteria [52].

Clinical implications and practical therapeutic approaches for depression

Given the growing understanding of the gut-brain axis, several therapeutic approaches target gut microbiota to manage depression. These strategies include probiotics, prebiotics, dietary interventions, and emerging techniques such as vagus nerve stimulation and fecal microbiota transplantation.

Probiotics and prebiotics in depression management

As previously discussed, probiotics and prebiotics show promise in modulating the gut-brain axis [53]. Specific strains, including *Lacticaseibacillus rhamnosus* and *Bifidobacterium longum*, have been associated mood improvement, likely due to their roles in neurotransmitter synthesis and immune modulation [41,50]. Prebiotics, that stimulate SCFA production, further enhance this process by stabilizing immune responses and supporting overall mental health.

These probiotics have shown significant benefits in reducing both gastrointestinal symptoms and depressive symptoms in IBS patients [54]. In Sanjay Noonan's review, probiotic supplementation significantly reduced depressive symptoms, as measured by scales like Hamilton Depression Rating Scale (HAM-D) and Beck Depression Inventory (BDI) [55]. Furthermore, probiotics were noted to enhance the effects of conventional antidepressants, suggesting potential as an adjunctive therapy. This additive effect may result from probiotics' ability to modulate systemic inflammation and improve the overall gut environment, which positively affects brain function.

However, the review also points out that the beneficial effects were often temporal, with symptoms reappearing after cessation of probiotic use.

In a randomized clinical trial 110 patients with MDD were assigned to one of three groups: probiotic supplementation (*Lactobacillus helveticus* and *Bifidobacterium longum*), prebiotic supplementation (galactooligosaccharide), or placebo [56]. After 8 weeks of intervention the results in BDI scores and changes in kynurenine/tryptophan and tryptophan/branched-chain amino acids (BCAAs) ratios were taken. The probiotic group exhibited a significant reduction in BDI scores (from 18.25 to 9.0), compared to the placebo group (18.74 to 15.55) and the prebiotic group (19.43 to 14.14). While no significant differences were observed between groups in kynurenine/tryptophan and tryptophan/BCAA ratios, the probiotic group showed a significant decrease in the kynurenine/tryptophan ratio after adjusting for serum isoleucine levels.

The arising field of personalized medicine uses microbiome profiling to tailor treatments based on an individual's unique microbial composition. Studies explored the use of personalized probiotics based on individual microbiome profiles to treat depressive symptoms [57].

Researchers conducted a clinical trial involving patients with MDD, who received a customized probiotic regimen tailored to their specific microbiome composition. By customizing therapies to the individual microbiome profiles, personalized medicine can optimize microbial balance, reduce inflammation, and enhance neurotransmitter synthesis, thereby alleviating depressive symptoms. However, the limited effects observed with prebiotics and mixed results regarding metabolic markers underscore the complex relationship between diet, microbiota, and mental health. In the future, with standardized treatment protocols in place, routine gut microbiota diagnostics for patients with depression undergoing psychiatric treatment may become a standard practice, allowing for the selection of appropriate probiotics to support the primary treatment of the disease.

Dietary interventions and depression

Dietary modifications are a non-invasive approach to enhancing gut microbiota balance and improving mental health. The Mediterranean diet, known for its anti-inflammatory effects, and the low-FODMAP diet, which reduces fermentable carbohydrates, have both shown potential in alleviating depressive symptoms by promoting SCFA production and reducing gut inflammation [50,58]. The Mediterranean diet is rich in fruits, vegetables, whole grains, nuts, seeds, olive oil, and moderate amounts of fish and poultry - foods high in fiber and polyphenols. While the low-FOD-

MAP diet is mainly used to manage symptoms of irritable bowel syndrome, emerging research suggests its potential to alleviate depression-related symptoms. Although low-FODMAP diet tends to reduce overall microbial diversity, it also selectively decreases the abundance of certain gas-producing bacteria, potentially easing GI symptoms and inflammation.

In a randomized clinical trial, Stilling demonstrated that increasing dietary fiber intake correlates with improved markers of neuroinflammation and microglial function in older adults [9].

Participants who consumed more fiber had higher levels of SCFAs, which were linked to reduced expression of inflammatory cytokines and microglial overactivity in brain imaging studies. These dietary interventions offer accessible strategies to improve microbiota composition and, in turn, support mood regulation [59]. Additionally, a study by Tončić observed that diet approaches promoting SCFA production were associated with sustained improvements in mood over time [47].

In clinical studies, such as the "SMILES" trial, participants with major depressive disorder who followed a modified Mediterranean diet (ModiMedDiet) experienced significant reductions in depressive symptoms, along with lower cortisol levels and improved stress resilience. After 12 weeks, participants in the dietary group showed a notable decrease in their Montgomery–Åsberg Depression Rating Scale (MADRS) scores, from 26.1 at baseline to 14.8, highlighting the potential of the Mediterranean diet as an adjunctive therapy for depression [60].

Diets high in polyunsaturated fatty acids (PUFA), particularly omega-3 fatty acids from fish, have been associated with lower depressive symptom scores. Participants following a Mediterranean diet supplemented with omega-3 PUFAs reported a 45% reduction in depression scores, compared to a 26.8% reduction in the control group [61].

Similar findings were observed in the comparison of 17 randomized controlled trials on the role of dietary interventions, particularly in relation to depression and anxiety, as presented in the work of Rachelle S. Opie [62]. In this study, outcomes were measured using the Beck Depression Inventory and the Hospital Anxiety and Depression Scale (HADS), revealing that approximately 47% of the studies reported a significant reduction in depressive symptoms in the intervention groups. Notably, interventions focusing on Mediterranean diets or increasing polyunsaturated fatty acids were more likely to yield positive results. These findings underscore the interplay between diet, microbiota, and hormonal balance, suggesting new avenues for managing depression [63]. Given these studies, it would be beneficial for clinicians to implement the Mediterranean diet for patients with depression. Such a diet should include a high intake of fruits, vegetables, whole grains, nuts, seeds, olive oil, and moderate amounts of fish and poultry. Mentioned foods are rich in fiber, polyphenols, and omega-3 fatty acids, which can help improve gut microbiota balance and support mental health. Clinicians may consider collaborating with dietitians to create personalized dietary plans for patients, ensuring optimal nutritional support for the treatment of depression [64].

Vagus nerve stimulation and depression

Vagus nerve stimulation (VNS) has emerged as a promising intervention for treating both psychiatric and gastrointestinal conditions, reflecting its role in the gut-brain axis. Initially approved for epilepsy, VNS has shown effectiveness in treating treatment-resistant depression. This neuromodulatory therapy works by modulating the autonomic nervous system, reducing sympathetic activity, and enhancing parasympathetic tone. Applied modulation results in decreased inflammation and improved autonomic control of the GI tract [65]. This dual efficacy highlights the interconnected nature of psychiatric and GI symptoms mediated through the vagus nerve [66]. A systematic review by Guerriero evaluated the efficacy of transcutaneous vagus nerve stimulation (tVNS) for treating depression, revealing significant improvements in mood and anxiety levels [67]. Similarly, a clinical trial by Shi demonstrated that transcutaneous auricular vagus nerve stimulation reduced symptoms of functional dyspepsia while also easing depression-related effects [68]. Clinical applications of VNS also have shown marked improvements in patients suffering from IBS experiencing concurrent mood disorders – patients undergoing VNS reported both a decrease in GI discomfort and an enhanced mood stability.

Fecal microbiota transplantation and depression

Fecal microbiota transplantation (FMT), based on the transfer of a healthy donor's gut microbiota to a recipient, aims to alter the recipient's microbiome to confer health benefits. The procedure's goal is to optimize the complex bidirectional communication between the gut microbiota and the central nervous system [58].

In a clinical trial conducted by Zhang, 18 patients with irritable bowel syndrome and mild to moderate symptoms of depression and anxiety were divided into two groups: one receiving fecal microbiota transplantation and a control group [69]. The FMT group demonstrated marked improvements in both gastrointestinal symptoms and mental health parameters (Quality of Life measures and Gastrointestinal Symptom Rating Scale). Post-treatment analysis revealed significant reductions in levels of isovaleric and valeric acids, as well as notable changes in gut bacterial profiles. Similar conclusions have been reached by Kurkowa in her study, where 17 patients with Functional Gastrointestinal Disorders (FGIDs) were observed after undergoing FMT therapy [70]. At baseline, 12 out of 17 patients had a HAM-D score of 8 or higher, indicating notable depressive symptoms. After treatment, patients experienced significant improvements in scores for depression (HAM-D), anxiety (HAM-A), and quality of life – all with statistically meaningful results.

Acupuncture in depression

Acupuncture is increasingly recognized as a complementary therapy for depression, valued for its therapeutic effects and minimal side effects, which have garnered global research interest.

Acupuncture has been shown to decrease levels of pro-inflammatory cytokines in the gastrointestinal tract, potentially reducing systemic inflammation linked to depressive symptoms. Additionally, this procedure may shift gut microbiota composition by promoting populations of beneficial bacteria [71]. Hiang-Yun Yan study aimed to evaluate the effect of acupuncture on gut microbiota in 80 patients with functional constipation and 28 healthy controls [72]. The composition and predictive metabolic function of the gut microbiota from fecal samples were analyzed using 16S rRNA gene sequencing, while fecal SCFAs were identified via gas chromatography-mass spectrometry (GC-MS). Results showed that acupuncture restored the composition of gut microbiota. Specifically, the abundance of beneficial bacteria such as *Lactobacillus* increased, while that of pathogenic bacteria like *Pseudomonas* decreased. These changes were significantly correlated with alleviated constipation symptoms. Additionally, ten microbes, including *Lactobacillus* and *Eubacterium coprostanoligenes* group, were identified as acupuncture-specific microbes and formed a stable interaction network. Research on animal models of depression has shown that acupuncture, by interacting with the brain-gut axis, can improve this communication and help balance neurotransmitter levels, such as serotonin and dopamine [73]. Acupuncture supports the regulation of gut microbial homeostasis, reduced intestinal inflammation by lowering pro- inflammatory cytokines, and enhanced intestinal barrier function by increasing the expression of tight junction proteins. These combined effects contribute to enhanced communication between the gut and brain, mediated through the vagus nerve [74]. Although acupuncture has demonstrated favorable results, limitations include the need for consistent methodology in terms of dosage and points of stimulation across studies.

Limitations of current research

While existing studies provide valuable insights, there are several limitations. Key areas of concern include the reliance on animal models, methodological variability, and individual differences in microbiome composition. It highlight the need for further standardization and more extensive human trials to confirm findings and improve therapeutic potential.

First, most research relies on animal models, limiting the direct applicability of findings to human physiology. For instance, studies examining the anti-inflammatory effects of short-chain fatty acids on the brain often use animal models and translating these findings to human applications remains uncertain due to physiological variances in SCFA pathways. Further, while germ-free mice models have shown a correlation between certain microbiota and behav-

ioral changes, these models do not fully replicate human complexity, limiting the robustness of the findings.

Moreover, studies that include human participants often suffer from small sample sizes and lack longitudinal data, which weakens the ability to understand long-term effects and causal relationships. For example, while FMT and VNS have demonstrated short-term improvements in clinical symptoms, the lack of long-term follow-up studies means the durability of these benefits remains unclear. Individual variability in gut microbiota composition significantly impacts therapeutic outcomes, underscoring the need for precision medicine approaches tailored to individual microbiome profiles. For example FMT outcomes depend heavily on donor microbiome quality. Diversity in microbiome responses to interventions such as probiotics and dietary changes has also been observed, suggesting that personalized treatments may be essential for effective long-term management.

Additionally, there is significant variability in methodologies, such as differences in dietary interventions, strains of probiotics used, and biomarkers measured, which complicates comparison across studies. For instance, studies on probiotics often use different bacterial strains, such as *Lacticaseibacillus rhamnosus* in some trials and *Bifidobacterium longum* in others. The field would benefit from standardizing protocols for interventions like FMT, VNS, and probiotic administration. Establishing standardized protocols could enhance the comparability and reproducibility of studies, allowing researchers to better evaluate the efficacy and safety of these treatments. To mitigate current limitations, future research should incorporate multi-omics approaches, which integrate wide data to provide a comprehensive understanding of microbiome functions and interactions [75]. Furthermore, larger sample sizes and longitudinal studies seem to be essential to capture the variability and long-term effects of microbiome interventions [76].

Collaborative efforts across research institutions could help standardize methodologies and protocols, ensuring consistency and reliability in findings. By addressing these limitations, the field can advance towards more effective and personalized microbiome-based therapies.

Conclusions

The reviewed literature on the gut-brain axis clarifies its complex, bidirectional communication pathways involving neural, hormonal, and immunological mechanisms. This axis, significantly impacted by gut microbiota, mediates critical physiological processes, that influence both gastrointestinal and central nervous system health. It should be kept in mind that the gut microbiota represents a promising therapeutic target for addressing both the physiological and psychological dimensions of depression. Interventions such as probiotics, prebiotics, dietary modifications and emerging therapies like fecal microbiota transplantation and vagus nerve stimulation offer innovative avenues for treatment. Future research should focus on standardizing methodologies, personalizing interventions based on individual microbiome profiles, and conducting long-term studies to fully explore the therapeutic potential of these approaches.

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Ethical consideration
This review is based exclusively on previously published data, all of which are publicly accessible through academic databases and journal publications. As no new patient data was collected or analyzed, ethical approval was not required.

Conflict of interest statement
The authors declare no conflict of interest.

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REVIEW PAPER

JMS Journal of Medical Science

Neurological and renal complications in obese children with cancer: a systematic review of cardiovascular risk factors

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ABSTRACT

Obesity in children, especially those with cancer, is a growing concern due to its impact on health outcomes. These children are at increased risk for neurological, renal, and cardiovascular complications, which can worsen their prognosis. This systematic review aims to examine the role of obesity in the development of these complications in children with cancer, highlighting the associated cardiovascular risk factors. A comprehensive literature search was conducted across databases such as PubMed, Scopus, Web of Science, Embase, and Google Scholar for studies published between 2014 and 2025. Eligible studies included interventional, cohort, case-control, and observational studies that examined the impact of cancer treatments on neurological and renal outcomes in obese pediatric patients. The review followed PRISMA guidelines to ensure methodological rigor, with quality assessment using validated tools such as the Newcastle-Ottawa Scale and STROBE checklist. Thirteen studies involving 14,723 participants met the inclusion criteria. Obesity was associated with poorer survival outcomes, particularly in children with ALL and CNS tumors, showing lower EFS and OS rates. Obese children undergoing chemotherapy had higher incidences of treatment-related toxicities, including hepatotoxicity, nephrotoxicity, and thrombotic events. Renal complications, including acute kidney injury and electrolyte imbalances, were more prevalent in obese patients. Obesity also increased cardiovascular risk, with higher rates of hypertension and insulin resistance. Additionally, it contributed to neurocognitive impairments and poor psychosocial outcomes. Lastly, obesity affected growth trajectories, with many survivors remaining obese long-term. Early weight management and personalized treatment strategies are crucial to mitigate these risks. Addressing obesity in pediatric cancer care is essential to improve treatment outcomes and long-term survivorship, with further research needed to develop effective interventions.

Introduction

Childhood obesity is a growing global health concern, with its prevalence increasing at an alarming rate over the past few decades [29]. According to the World Health Organization (WHO), the number of overweight and obese children under the age of five has risen to over 37 million worldwide, with higher prevalence rates in developed and developing nations alike [25]. Obesity is associated with numerous metabolic, cardiovascular, renal, and neurological complications, many of which persist into adulthood, leading to increased morbidity and mortality [2]. Among children diagnosed with cancer, obesity further exacerbates disease progression, treatment complications, and overall prognosis [24].

The prevalence of obesity in pediatric cancer patients varies across different regions and cancer types [34]. Studies have reported that 15–40% of children undergoing chemotherapy develop obesity, with the highest rates observed in survivors of acute lymphoblastic leukemia (ALL) and brain tumors [13, 33]. The pathophysiology behind this increased susceptibility includes hormonal imbalances, reduced physical activity, steroid treatments, and genetic predisposition [8]. Furthermore, obesity in childhood cancer survivors has been linked to a higher risk of cardiovascular diseases, renal dysfunction, and neurocognitive impairment [9, 21, 9]. Childhood cancer survivors are at an increased risk for cardiovascular diseases (CVD) due to both the effects of cancer treatments and the development of obesity [19]. Treatments such as chemotherapy, particularly anthracyclines, and radiation therapy can directly damage the heart and vascular tissues, leading to long-term issues like left ventricular dysfunction, arrhythmias, and coronary artery disease [3]. Additionally, obesity, which is common among childhood cancer survivors, exacerbates the risk by promoting atherosclerosis, hypertension, and insulin resistance, all of which are well-established cardiovascular risk factors [4]. Studies have shown that survivors with obesity have a significantly higher likelihood of developing heart disease, even years after treatment, underlining the need for ongoing cardiovascular monitoring in this population [4, 32].

Neurological complications in obese pediatric cancer patients can arise due to chronic inflammation, metabolic dysregulation, and treatment-induced neurotoxicity [16]. Cognitive impairment, memory deficits, and executive dysfunction are frequently reported in obese survivors of childhood leukemia and brain tumors [15]. Obesity exacerbates the neurotoxic effects of cancer treatments, as chemotherapy and radiation can directly impact brain structures and cognitive functions, while the added burden of obesity further complicates recovery [30]. Moreover, obesity-induced alterations in systemic metabolism and neuroinflammation may heighten susceptibility to long-term neurological sequelae in pediatric cancer survivors. Adipose tissue dysfunction and elevated pro-inflammatory cytokines, such as TNF-α and IL-6, contribute to blood-brain barrier disruption and neuronal damage, potentially exacerbating treatment-related cognitive decline [10].

Renal dysfunction is another critical consequence of obesity and cancer treatment [18]. Obesity-related glomerulopathy, hyperfiltration, and increased proteinuria are well-documented in pediatric populations [20]. Studies have shown that chemotherapy-induced nephrotoxicity is exacerbated in obese children due to altered drug metabolism and increased systemic inflammation [7, 28]. A study by Aldrink et al. concluded that obese pediatric cancer patients exhibited a higher incidence of renal toxicity compared to their nonobese counterparts [1], highlighting the need for individualized treatment strategies.

Despite the well-established risks of obesity in children with cancer, limited systematic reviews have comprehensively examined the neurological and renal complications in this population. Understanding these associations is crucial for early intervention strategies, targeted therapies, and improved long-term outcomes. This systematic review aimed to evaluate the prevalence, pathophysiology, and clinical implications of neurological and renal complications in obese children with cancer, with a specific focus on cardiovascular risk factors.

Method

Study Design

This study is a systematic review aimed at evaluating the neurological and renal complications

in obese children with cancer, with a particular focus on cardiovascular risk factors. The review follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure transparency and methodological rigor.

Search strategy

A comprehensive literature search was conducted using electronic databases, including PubMed, Scopus, Web of Science, Embase, and Google Scholar, to identify relevant studies published between 2014 and 2025. Articles were searched using Medical Subject Headings (MeSH) terms and Boolean operators (AND, OR) to refine the search strategy. The search was limited to English-language studies, and additional articles were identified through manual searches of reference lists from relevant studies. Two independent researchers determined the keywords and search terms, and the snowball method was applied to ensure inclusivity of pertinent studies (**Table 1**).

Inclusion criteria

The inclusion criteria for this systematic review were established based on the PICO framework. Studies were eligible if they focused on obese pediatric cancer patients (aged 0–18 years) and examined the impact of cancer treatments on neurological and renal complications. Interventional studies that assessed chemotherapy, radiation therapy, or targeted therapy in relation to these complications were considered. Comparative studies evaluating differences between obese and nonobese pediatric cancer patients were included when available. Additionally, eligible studies reported on primary outcomes such as cognitive impairment, neurotoxicity, neuropathy, nephrotoxicity, glomerulopathy, renal dysfunction, and cardiovascular risk factors associated with these complications. Only observational studies, cohort studies, case-control studies, and clinical trials published in English were included.

Exclusion criteria

Studies that focused exclusively on adult populations, investigated complications unrelated to obesity, or examined general pediatric cancer treatment without specific reference to neurological or renal outcomes were excluded. Furthermore, grey literature, including conference proceedings, dissertations, and unpublished studies, was not considered. Review articles, letters, editorials, case reports, and commentaries were also excluded. Additionally, studies not available in English or without a reliable translation were not included. Research that failed to assess obesity as a contributing factor to neurological or renal complications in pediatric cancer patients was similarly excluded.

Study selection

Two independent researchers screened the titles and abstracts of retrieved studies. Full texts of eligible articles were reviewed, and disagreements were resolved through discussion with a third reviewer.

Quality assessment

The quality of included studies was assessed using validated checklists: the Newcastle-Otta-

Table 1. Search Strategies for Systematic Review

Concept	Search Terms
Obesity and Cancer in Children	"Obesity" [MeSH] OR "Pediatric Obesity" [MeSH] OR "Childhood Obesity" [MeSH] OR "Overweight" [MeSH] OR "Body Mass Index" [MeSH] OR "BMI" OR "Adiposity" [MeSH] AND "Neoplasms" [MeSH] OR "Cancer" [MeSH] OR "Malignancy" OR "Pediatric Cancer" OR "Childhood Cancer" OR "Leukemia" [MeSH] OR "Lymphoma" [MeSH] OR "CNS Tumors"
Neurological and Renal Complications	"Neurological Manifestations" [MeSH] OR "Cognitive Dysfunction" [MeSH] OR "Neurotoxicity" [MeSH] OR "Cognitive Impairment" OR "Brain Injury" [MeSH] OR "Neurocognitive Function" [MeSH] OR "Kidney Diseases" [MeSH] OR "Renal Insufficiency" [MeSH] OR "Nephrotoxicity" OR "Chronic Kidney Disease" [MeSH] OR "Renal Dysfunction" [MeSH] OR "Acute Kidney Injury" [MeSH]
Cardiovascular Risk Factors	"Cardiovascular Diseases" [MeSH] OR "Hypertension" [MeSH] OR "Dyslipidemia" [MeSH] OR "Hyperlipidemia" [MeSH] OR "Atherosclerosis" [MeSH] OR "Cardiovascular Risk" OR "Metabolic Syndrome" [MeSH] OR "Insulin Resistance"
Final Search Strategy	#1 AND #2 AND #3

wa Scale (NOS) for cohort and case-control studies, the Joanna Briggs Institute (JBI) checklist for qualitative studies, and the STROBE checklist for observational studies. Studies were not excluded based solely on quality assessment scores, but low-quality studies were considered with caution in the final analysis.

Data extraction

Two authors independently extracted data, including study characteristics (author, year, location, study type), sample size, interventions, assessment tools, and key findings. Any discrepancies were resolved through discussion with a third researcher. Extracted data were summarized in **Table 2**.

Data synthesis

A qualitative narrative synthesis was employed to integrate findings from different studies. Neurological and renal complications were categorized thematically based on their reported incidence and severity. Where possible, quantitative data were pooled for descriptive statistical analysis. The synthesis considered variations in study designs, populations, and treatment regimens to provide a comprehensive overview of obesity-related complications in pediatric oncology patients.

Results

Eventually, 13 studies were compatible with inclusion criteria. The total number of participants was 14,723. The procedure of study selection based on PRISMA guidelines in shown in **Figure 1**.

Impact of obesity on survival outcomes in childhood cancer

Obesity at the time of cancer diagnosis has been consistently associated with poorer survival outcomes in pediatric patients. Multiple studies have demonstrated that obese children with acute lymphoblastic leukemia (ALL) and central nervous system (CNS) tumors experience significantly lower event-free survival (EFS) and overall survival (OS) rates compared to their non-obese counterparts. Hazard ratio (HR) analyses indicate that obesity independently predicts worse survival, with a particularly pronounced effect in ALL and CNS malignancies. These findings underscore the critical need for early weight management interventions in this vulnerable population.

Obesity and treatment-related toxicities (TRT) in pediatric cancer patients

Obesity has been linked to an increased risk of severe treatment-related toxicities (TRT) in children undergoing chemotherapy. Studies report

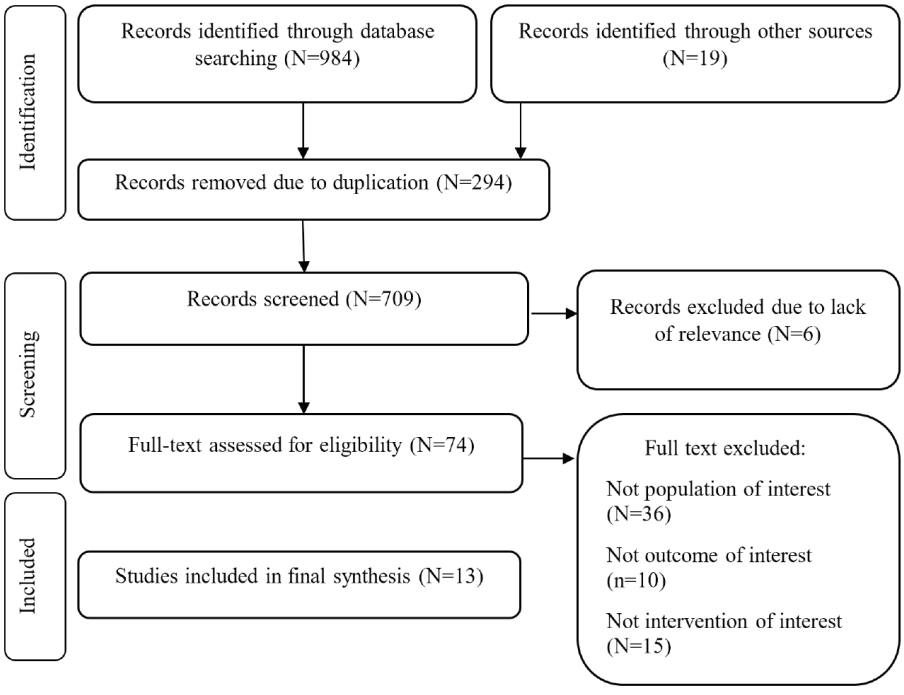

Figure 1. PRISMA flowchart of selected studies.

Table 2. Overview of included studies

Author, Year	Study Design	Sample Size	Population	Intervention	Neurological Outcomes	Renal Outcomes	Cardiovascular Risk Factors	Key Findings & Conclusion
Sassine et al., 2025	Retrospective cohort study	11,291 children (2–18 years)	Age: 2–18 years; Obesity: BMI ≥ 95th percentile; Cancer types: Acute lymphoblastic leukaemia (ALL), central nervous system (CNS) tumors, others	Various cancer treatments (chemo, radiation, etc.)	-	-	Obesity at diagnosis was associated with inferior event-free survival (EFS) and overall survival (OS)	Obesity at diagnosis was independently associated with inferior EFS (aHR 1.16) and OS (aHR 1.29) for the entire cohort. Specifically, for ALL patients (n = 3458), obesity was associated with worse EFS (aHR 1.55) and OS (aHR 1.75). For CNS tumor patients (n = 2458), obesity was also linked to worse EFS (aHR 1.38) and OS (aHR 1.47). The study suggests that obesity at diagnosis negatively impacts survival outcomes, particularly for ALL and CNS tumors.
Sassine et al., 2024	Retrospective cohort study	11,291	Children with newly diagnosed cancer (2001–2020, Canada), aged 2–18 years. Cancer types: Leukemias (37.1%), Lymphomas (14.5%), CNS tumors (21.8%), Non-CNS solid tumors (26.6%)	-	-	-	Obesity is a known cardiovascular risk factor	In ALL patients, obesity remained significantly associated with worse EFS (aHR 1.55) and OS (aHR 1.75). In CNS tumors, obesity was linked to worse EFS (aHR 1.38) and OS (aHR 1.47). No adverse survival impact was seen in other cancer types.
Ehrhardt et al., 2023	Retrospective cohort study	38 children (18 girls, 20 boys)	Median age at diagnosis: 9.75 years (Range: 0.92–17.7 years); Median age at evaluation: 13.7 years (Range: 2.1–22 years); All had CNS tumors (medulloblastoma, high-grade glioma, PNET, anaplastic ependymoma, germ cell tumor)	Chemotherapy (vincristine, etoposide, carboplatin, cisplatin, cyclophosphamide, ifosfamide, lomustine) and radiotherapy (protocol-based treatment)	patients were treated with chemotherapy and radiation therapy which may lead to potential cognitive impairment or neurotoxicity	58% of patients developed subclinical chronic kidney disease (eGFR 90–60 ml/min/1.73 m²); 16% had renal insufficiency (eGFR 30–60 ml/min/1.73 m²); 34% developed drug-induced tubulopathy (decreased tubular reabsorption of phosphate and renal tubular threshold dysfunction); No significant correlation with NGAL levels	-	Statistically significant negative correlation between eGFR and cystatin C concentration ($p < 0.0001$); negative correlation between eGFR and beta-2 microglobulin concentration ($p < 0.02$); no correlation between eGFR and NGAL levels. Drug-induced nephrotoxicity (including glomerular and tubular damage) is common in these children. Cystatin C and beta-2 microglobulin are useful markers for detecting chronic kidney damage, while NGAL is not.
Egnell et al., 2022	Cohort study	1,443	Children aged 2–17.9 years with acute lymphoblastic leukaemia (ALL)	Chemotherapy (asparaginase-based regimen)	-	Liver and kidney failure, abdominal complications, bleeding, and hyperlipidemia were more frequent in obese children	Obesity is a known cardiovascular risk factor	Obese children had a higher incidence of severe treatment-related toxicities, including liver and kidney failure, bleeding, abdominal complications, and hyperlipidemia (IRR 1.55). In children aged ≥ 10 years, obesity was associated with an increased risk of asparaginase-related toxicities, including thrombosis (IRR 2.87), anaphylaxis (IRR 7.95), and a higher risk of asparaginase treatment truncation (IRR 3.54). These toxicities may contribute to the poor prognosis in obese children aged ≥ 10 years with ALL.
Iijima et al., 2021	Retrospective Cohort Study	Survivors of childhood ALL treated on St. Jude Total XV protocol	ALL survivors, ≥8 years old, ≥5 years post-diagnosis, no HCT, relapse, secondary cancer, or neurodevelopmental disorders	Total XV therapy: Induction, consolidation, continuation (chemo with prednisone, dexamethasone, MTX, and intrathecal therapy)	Neurocognitive assessment showed deficits in executive function, attention, and processing speed	-	BMI tracked from diagnosis to follow-up; obesity prevalence assessed	Obesity prevalence in ALL survivors and its correlation with long-term neurocognitive outcomes; ongoing BMI monitoring recommended.
Bhandari et al., 2020	Retrospective Study	221	Pediatric patients with solid tumors; 22% malnourished (10% underweight, 12% obese); ≥15 years classified as adolescent/young adult	Chemotherapy (Cisplatin-containing regimens)	-	Acute or chronic kidney injury (significantly higher in obese patients, p = 0.014)	-	Obesity at diagnosis increased risk of severe TRT (>3x, p = 0.037); Obesity & age ≥ 15 years linked to worse event-free survival (HR 2.32, p = 0.024) and overall survival (HR 3.69, p = 0.006); Older and obese patients at higher risk for poor outcomes.
Karimi et al., 2020	Cross-sectional, biopsychosocial model	N = 144	Children treated for oncology conditions, various cancer types	Chemotherapy and other cancer treatments	Depression and low mobility are significant factors affecting fatigue and quality of life	-	-	Fatigue in childhood cancer survivors improves over time but is influenced by depression and low mobility. Additionally, older survivors and those not receiving chemotherapy tend to have higher BMI. Findings highlight the importance of addressing psychosocial factors in this population.
Gance-Cleveland et al., 2020	Retrospective chart	321	Childhood cancer survivors (CCS)	-	-	-	Long-term cardiovascular health concerns	Findings from this study indicate that childhood cancer survivors who are overweight or obese are at an increased risk of long-term cardiovascular complications
Moke et al., 2019	Case–Control Study	A total of 59 cases and 130 controls	Pediatric patients (<21 years) with invasive cancer at CHLA (1988–2014), including obese, overweight, and normal-weight patients	Chemotherapy (alkylating agents, anthracyclines, epipodophyllotoxins, platinum-based chemo) and radiation	-	Kidney injury (acute/chronic)	-	Cases with obesity had higher risk for severe treatment-related toxicities (TRT); Matching criteria ensured comparable treatment exposures between cases and controls; Genetic predisposition variables considered (e.g., BRCA, Li-Fraumeni, etc.).
Meenan et al., 2019	Retrospective cohort study	155 pediatric ALL patients	Age at diagnosis: Not specified; Obesity: BMI ≥ 95th percentile; Diagnosis: Acute lymphoblastic leukaemia (ALL)	Pre-maintenance chemotherapy for ALL	-	-	Obesity was associated with increased incidence of hypertension, insulin-requiring hyperglycemia, and febrile neutropenia (FN) admissions	Obese patients had a significantly higher incidence of treatment-requiring hypertension (17.5% vs 6.1%), insulin-requiring hyperglycemia (25.0% vs 11.3%), recurrent infections (IRR 1.64), and recurrent FN admissions (IRR 1.53). Obesity was a significant risk factor for these AEs ($p < 0.05$).
Browne et al., 2018	Prospective Cohort Study	372 children with ALL	Children and adolescents (2-18 years) with ALL, both sexes, diverse racial background (Black, White, Native American, other)	Total XV protocol treatment including chemotherapy and reinduction therapy (induction, consolidation, continuation)	Monitoring of neurotoxicity through CNS disease status and intrathecal treatments, no cranial irradiation	Monitoring of renal function for nephrotoxicity during therapy	Monitoring for cardiovascular risk due to steroid use and chemotherapy	The study observed growth and BMI changes over time, with a focus on final height and permanent height loss post-treatment. It also assessed the impact of chemotherapy on BMI and growth velocity, identifying permanent short stature in some patients. Treatment details highlighted variations between male and female therapy durations, and follow-up was comprehensive, including yearly visits for up to five years after therapy.
Touyz et al., 2017	Retrospective Cohort Study	184	Children with standard- and medium-risk ALL, treated without cranial radiation or glucocorticoids	Chemotherapy-based protocol omitting prophylactic cranial radiation and glucocorticoids in maintenance	-	-	Increased BMI z-score associated with cardiovascular risk	BMI z-score increased significantly during treatment and persisted up to 7 years post-diagnosis. Height z-scores declined, and weight z-scores fluctuated. Early interventions are needed to mitigate long-term obesity-related risks.
Aldrink et al., 2014	Retrospective Analysis	365 (63 obese, 302 nonobese	Pediatric, Obese vs. Nonobese, Leukemia/Lymphoma & Solid Tumors	Chemotherapy	-	Higher renal toxicity in obese patients (38.1% vs. 26.2%, p = 0.06	-	Increased wound complications in obese leukemia/lymphoma patients (13.2% vs. 1.6%, p = 0.0075

a higher incidence of hepatotoxicity, nephrotoxicity, hyperlipidemia, and thrombotic events among obese pediatric cancer patients. In particular, older obese children (≥10 years) receiving asparaginase-based chemotherapy are at significantly higher risk for thrombosis, anaphylaxis, and premature treatment discontinuation. These toxicities not only compromise treatment efficacy but also contribute to long-term morbidity, emphasizing the need for personalized therapeutic strategies for obese patients.

Renal outcomes and drug-induced nephrotoxicity

Childhood cancer survivors, particularly those treated with nephrotoxic agents such as platinum-based chemotherapy, are at heightened risk for chronic kidney disease (CKD) and renal dysfunction. Evidence suggests that obesity exacerbates renal complications, with obese patients exhibiting higher rates of acute and chronic kidney injury, renal tubular dysfunction, and electrolyte imbalances. Biomarker analyses indicate that cystatin C and beta-2 microglobulin are reliable indicators of nephrotoxicity, while neutrophil gelatinase-associated lipocalin (NGAL) does not significantly correlate with glomerular filtration rate (GFR) decline. These findings highlight the importance of early nephroprotective strategies in pediatric oncology.

Cardiovascular risk factors in childhood cancer survivors

Obesity in pediatric cancer patients has been identified as a significant risk factor for cardiovascular complications both during and after treatment. Studies have documented an increased prevalence of hypertension, insulin resistance, and hyperglycemia in obese children undergoing chemotherapy, particularly in those treated with steroids and alkylating agents. Moreover, long-term follow-up data indicate that childhood cancer survivors with obesity are at greater risk for developing metabolic syndrome and cardiovascular disease in adulthood. These findings underscore the necessity of routine cardiovascular monitoring and lifestyle interventions to mitigate long-term health risks in this population.

Neurocognitive and psychosocial outcomes

Emerging evidence suggests that obesity may contribute to neurocognitive impairments in

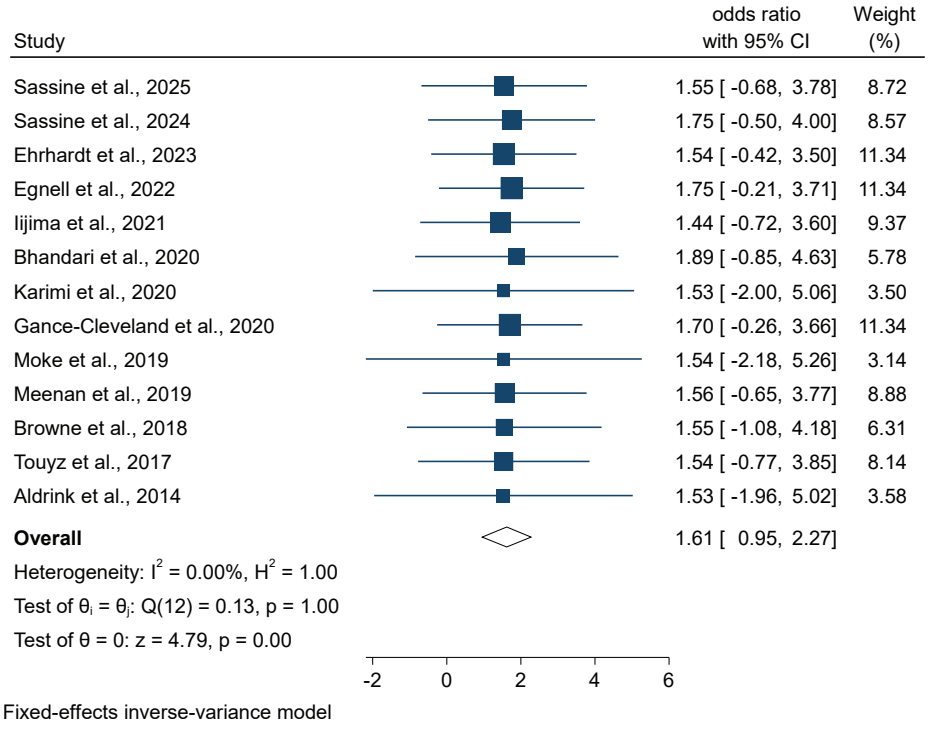

Figure 2. Forset plot showed Odds ratio of obesity prevalence in children and adolescents undergoing cancer treatment, compared to potentially healthy children.

childhood cancer survivors. Studies on ALL survivors reveal persistent deficits in executive function, attention, and processing speed, which may be exacerbated by obesity and metabolic dysregulation. Additionally, obesity has been associated with increased fatigue and poorer quality of life, with psychosocial factors such as depression and reduced mobility playing a critical role. These findings highlight the need for comprehensive survivorship care that addresses both cognitive and psychological well-being in pediatric cancer survivors.

Growth and BMI trajectories in childhood cancer survivors

Pediatric cancer treatment significantly impacts growth patterns, with obesity being a persistent issue among survivors. Longitudinal studies show that BMI z-scores tend to increase during and after treatment, with many children remaining obese up to seven years post-diagnosis. Additionally, chemotherapy regimens, particularly those involving corticosteroids, have been linked to permanent height reduction and altered growth velocity. Given these long-term consequences, early nutritional and physical activity interventions are crucial to promoting healthier weight trajectories and mitigating the risks of obesity-related complications in survivors.

The odds ratio of prevalence of obesity in children and adolescents undergoing cancer treatment occurs more frequently than in the potentially healthy pediatric population was 1.61 (OR: 1.61 95% CI; 0.59–2.27) (**Figure 2**).

Identifying which types of cancer are most commonly associated with obesity

The odds ratio of ALL tumors compared other types of cancer are most commonly associated with obesity 1.34 (OR: 1.34 95% CI; 0.68–2.00) (**Figure 3**).

The relationship between age groups in children with cancer and obesity

The age group of children aged 2–18 years with cancer is at higher risk of obesity (**Table 1**).

Percentage of children who become obese during cancer treatment and percentage who remain obese after treatment

33% of children develop obesity during cancer treatment and 23% of survivors remain obese (**Figure 4**).

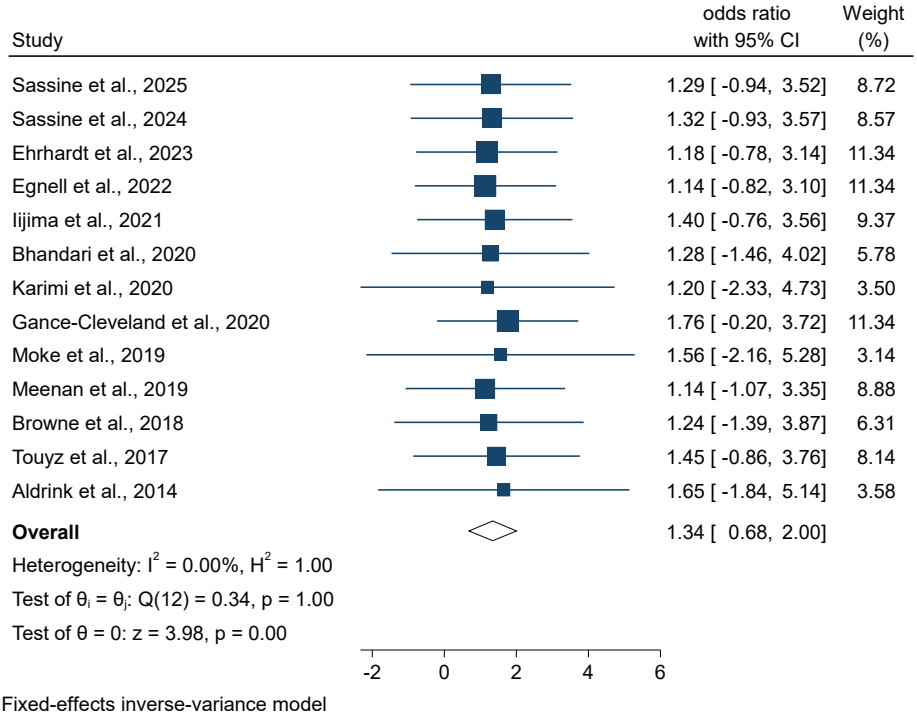

Figure 3. Forset plot showed Identifying which types of cancer are most commonly associated with obesity.

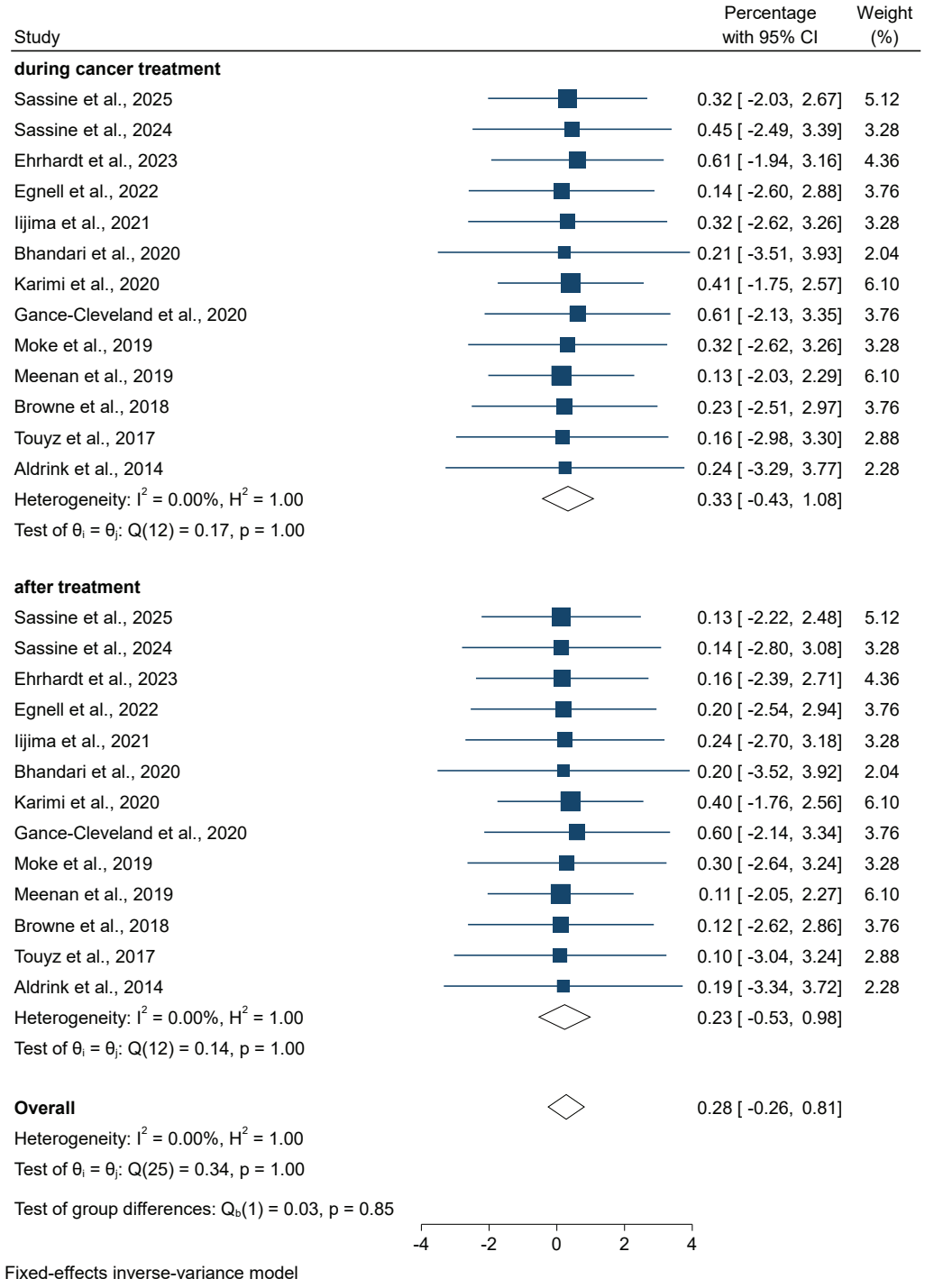

Figure 4. Percentage of children who become obese during cancer treatment and percentage who remain obese after treatment.

Most common complications associated with obesity in children undergoing cancer treatment

According to the study results in **Table 1**, the most common complications associated with obesity in children undergoing cancer treatment were diabetes, kidney failure, liver dysfunction, and stroke, respectively.

Discussion

This systematic review highlights the complex interplay between obesity and its neurological, renal, and cardiovascular consequences in pediatric cancer patients. The findings underscore that obesity at the time of cancer diagnosis significantly influences treatment outcomes and long-term health risks, particularly in children with ALL and CNS tumors. The studies analyzed provide compelling evidence that obesity is a significant prognostic factor, EFS, OS, renal function, and cardiovascular health in this vulnerable population.

Several studies, including those by Sassine et al. [26, 27], demonstrate a clear association between obesity and poorer survival outcomes in pediatric cancer patients. These studies indicate that obesity at diagnosis is independently linked to inferior EFS and OS, particularly in ALL and CNS tumor patients. Sassine et al. [27] reported adjusted hazard ratios (aHR) of 1.55 for EFS and 1.75 for OS in ALL patients, while CNS tumor patients showed an aHR of 1.38 for EFS and 1.47 for OS. These findings align with those of Bhandari et al. [5] and Meenan et al. [22], who identified obesity as a risk factor for increased treatment-related toxicity and adverse clinical outcomes.

Neurological impairments in obese pediatric cancer patients have been a growing concern. Iijima et al. [15] assessed the long-term neurocognitive impact of obesity in ALL survivors, finding deficits in executive function, attention, and processing speed. These cognitive impairments may be linked to steroid-based chemotherapy regimens, as well as systemic inflammation and metabolic dysfunction associated with obesity. Similarly, Ehrhardt et al. [12] documented cognitive impairments in CNS tumor patients receiving chemotherapy and radiation, highlighting the role of neurotoxicity in long-term morbidity. The findings suggest that BMI monitoring should be integrated into survivorship care plans to address obesity-related cognitive deficits.

The relationship between obesity and renal dysfunction in pediatric cancer patients is well-documented. Ehrhardt et al. [12] reported a high prevalence of subclinical chronic kidney disease (58%) and drug-induced tubulopathy (34%) in CNS tumor patients undergoing chemotherapy. Additionally, Bhandari et al. [5] and Aldrink et al. [1] identified obesity as a predictor of acute and chronic kidney injury, with significantly higher nephrotoxicity rates in obese children receiving cisplatin-based regimens. The negative correlation between estimated glomerular filtration rate (eGFR) and markers such as cystatin C and beta-2 microglobulin further emphasizes the need for early detection and intervention strategies to mitigate renal complications in obese pediatric oncology patients.

Obesity is a well-established cardiovascular risk factor in both healthy and oncologic pediatric populations. Multiple studies, including those by Egnell et al. [11], Meenan et al. [22], and Gance-Cleveland et al. [14], confirm that obesity exacerbates treatment-related cardiovascular complications. Egnell et al. [11] found that obese children with ALL had a higher incidence of asparaginase-related toxicities, including thrombosis (IRR 2.87) and anaphylaxis (IRR 7.95), which can contribute to treatment delays and inferior outcomes. Similarly, Meenan et al. [22] reported that obese ALL patients had significantly higher rates of treatment-requiring hypertension (17.5% vs. 6.1%) and insulin-requiring hyperglycemia (25.0% vs. 11.3%). These findings highlight the need for cardiovascular risk assessment and early intervention to improve long-term health outcomes.

The cumulative evidence presented in this review highlights the necessity of integrating obesity management into pediatric oncology care. Given the significant impact of obesity on survival outcomes, neurocognitive function, renal health, and cardiovascular risk, a multidisciplinary approach involving oncologists, endocrinologists, nephrologists, and nutritionists is essential. Future research should focus on targeted interventions to mitigate obesity-related complications, including personalized weight management programs, pharmacologic strate-

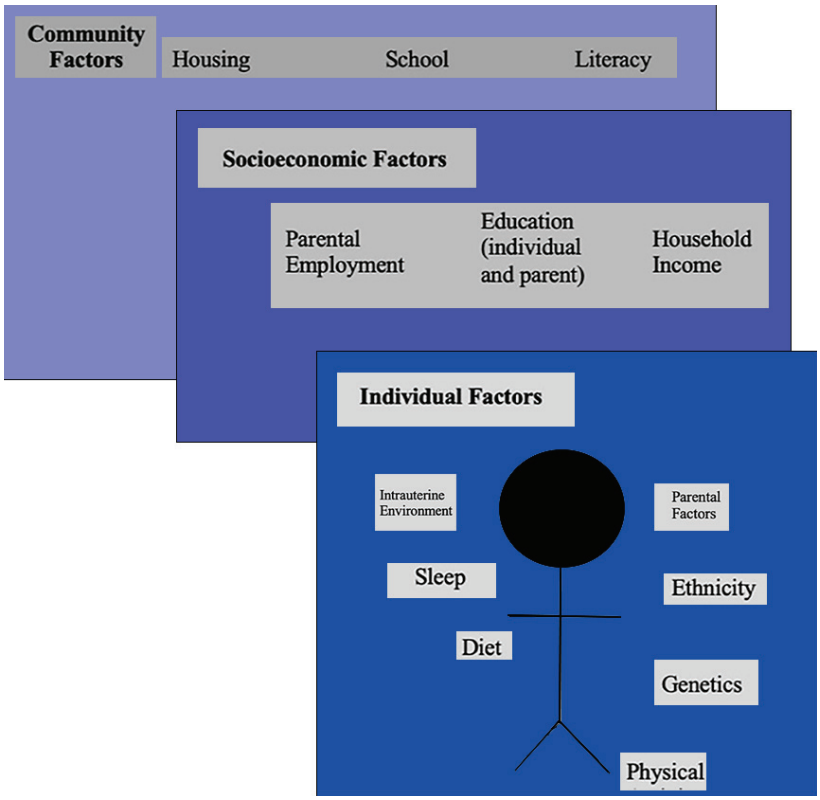

Figure 5. Strategies for combating obesity in children and adolescents undergoing cancer treatment.

gies, and lifestyle modifications tailored to pediatric cancer survivors.

Preventing obesity in early childhood and adolescence requires awareness and action. Early AR has long been known to increase the risk of adult obesity. As a result, healthcare professionals who treat children should concentrate on metrics like body mass index (BMI) while also offering proactive advice on nutritional counseling without stigmatizing or condemning parents for their children's diabetes. Anticipatory recommendations include teaching the families about bad and good eating habits, promoting more physical activity, and restricting screen time and other sedentary activities. Several societal sectors, including the family, impact the lifestyle choices of children and adolescents (**Figure 5**).

Conclusion

Obesity remains a critical determinant of morbidity and mortality in pediatric cancer patients, influencing survival, neurocognitive function, renal outcomes, and cardiovascular health. The findings from this review highlight the need for comprehensive weight management strategies and close monitoring of obesity-related complications throughout cancer treatment and survivorship. Addressing these factors through early interventions may significantly improve long-term outcomes in this high-risk population.

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Conflict of interest statement
The authors declare no conflict of interest.

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Acute heart failure with improved ejection fraction in a middle-aged patient with myocarditis and COVID-19 infection

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ABSTRACT

This clinical image describes the management of a 48-year-old male with a history of asthma, anxiety-depressive disorder, and prior COVID-19 infection, who was diagnosed with NYHA Class IV heart failure with reduced ejection fraction due to myocarditis.

The patient received an intensive heart failure treatment regimen, including ramipril, eplerenone, bisoprolol, dapagliflozin, ivabradine, and loop diuretics, resulting in a notable clinical improvement and a subsequent reduction in NT-proBNP levels. Upon follow-up, the initiation of sacubitril/valsartan over four months further enhanced functional capacity, leading to an increase in left ventricular ejection fraction to 41% and a decrease in NT-proBNP to 315 pg/ml. Due to these substantial improvements, the need for implantable cardioverter-defibrillator implantation was deemed unnecessary. This case underscores the necessity for early and intensive management of acute heart failure in patients with reduced ejection fraction to facilitate significant recovery. It highlights the rising incidence of myocarditis-related heart failure and emphasises the need for further investigations to optimise therapeutic strategies for patients with restored left ventricular function.

Literature review

The overall global incidence of myocarditis was estimated at approximately 1.5 million cases in 2021, with about 0.5% to 4% of these cases progressing to heart failure (HF). According to the latest ESC guidelines for managing HF, and in the absence of sufficient evidence, a standard HF with reduced ejection fraction (HFrEF) treatment approach is recommended for patients with left ventricular dysfunction. This includes the use of angiotensin-converting enzyme inhibitors (ACE-I) or an angiotensin receptor-neprilysin inhibitor (ARNI), beta-blockers, mineralocorticoid receptor antagonists (MRA), and sodium-glucose cotransporter 2 (SGLT2) inhibitors. Immunosuppression is considered only in select cases of chronic inflammation with inactive viral infection. Once the indicators of HF have resolved, therapy should be continued for at least six months [1,2].

Since the emergence of COVID-19, numerous studies have investigated its cardiovascular complications, including myocarditis and subsequent heart failure (HF). However, the literature addressing post-COVID-19 myocarditis leading to HF remains limited and heterogeneous. Current research focuses predominantly on epidemiological trends and recovery statistics rather than optimal treatment strategies, and guidelines have not been established.

Sawalha et al. conducted one of the first systematic reviews of published case reports on COVID-19-associated myocarditis, highlighting the wide variability in clinical presentations and outcomes, with reported recovery rates ranging from approximately 60% to as high as 91% based on small patient cohorts with short-term follow-up. The review considered therapeutic interventions for severe cases, suggesting a potential benefit from glucocorticoid therapy, IL-6 inhibitors, intravenous immunoglobulins, and colchicine. However, these were primarily applied to critically ill patients, many of whom required intubation, indicating that these recommendations might be more applicable to individuals with concomitant acute respiratory distress syndrome [4]. Also, Mrudula et al. assessed the recovery rate from post-COVID myocarditis as 92.1% (35/38 cases) [5].

Castiello et al. concluded that acute myocarditis related to COVID-19 may present even after the resolution of an upper respiratory tract infection and that, if promptly treated, it is associated with a moderate prognosis. Furthermore, his comprehensive review of the challenges in diagnosing and managing COVID-19-related myocarditis emphasised that current evidence cannot offer definitive treatment guidance. Castiello underscored the urgent need for further research to elucidate optimal therapeutic approaches for patients who develop HF secondary to post-COVID-19 myocarditis, highlighting the necessity for prospective, large-scale studies to identify prognostic markers and refine management strategies [6].

In 2024, Semenzato et al., in a nationwide population-based study, examined the long-term prognosis of patients with myocarditis attributed to various etiologies, including post-COVID-19. In the context of treatment for post-COVID-19 myocarditis (298 individuals), their findings indicate myocarditis secondary to SARS-CoV-2 infection displays a more heterogeneous prognosis, with a higher incidence of persistent cardiac dysfunction than myocarditis following COVID-19 mRNA vaccination. HF occurred in 7/298 (2.3%), compared to 3/558 (0.5%) patients for postvaccine myocarditis and 45/3779 (1.2%) for conventional myocarditis. These data suggest that patients with post-COVID-19 myocarditis may benefit from more aggressive therapeutic strategies, such as prolonged pharmacotherapy and tailored device therapy. Moreover, Semenzato et al. emphasise the importance of individualised management and close follow-up to optimise outcomes for this patient population [7].

Given the high potential for myocardial recovery in myocarditis-related HF, current guidelines for non-ischemic HFrEF offer a Class IIa recommendation for implantable cardioverter-defibrillator (ICD) implantation in selected patients. Additionally, the role of non-invasive alternatives, such as wearable cardioverter defibrillators (WCD), is discussed, particularly in patients with reversible HF etiologies [1]. Despite these insights, the optimal management strategy, including the timing and escalation of pharmacotherapy and the use of device therapy, remains to be clearly defined.

In summary, while preliminary data suggest that patients with post-COVID-19 myocarditis HF may experience significant recovery, the current literature is limited. This underscores an urgent

need for further research to establish optimal treatment protocols, identify reliable prognostic markers, and refine management strategies for patients with HF secondary to post-COVID-19 myocarditis.

Case report

A 48-year-old male with asthma and anxiety-depressive disorder, with no history of cardiovascular diseases, experiencing his first COVID-19 infection, confirmed by PCR testing fourteen days before hospitalisation, was referred to cardiology for reduced exercise tolerance and severe dyspnoea (NYHA IV), which began three weeks prior. Hospital imaging and tests led to a diagnosis of HF due to myocarditis. His NT-proBNP level was 10,846 pg/ml (normal: 0–125 pg/ml). Echocardiography showed severe left ventricular dysfunction with 10% left ventricular ejection fraction (LVEF), and cardiac magnetic resonance (CMR) confirmed myocarditis [**Figure 1A-C**]. Coronary angiography was negative for significant stenosis, and toxic and other viral etiologies were excluded. Given the patient's favourable clinical status and refusal to undergo the procedure, a myocardial biopsy was not performed.

The patient received intensive HF therapy, including ramipril, eplerenone, bisoprolol, dapagliflozin, ivabradine, and loop diuretics, resulting in NT-proBNP reduction to 7,255 pg/ml. After eight days, he was discharged with symptoms improvement to NYHA II. Sacubitril/valsartan was introduced and titrated at a follow-up over four months. This led to improved functional capacity, LVEF increase to 41%, and NT-proBNP reduction to 315 pg/ml after nine months [1–3]. Due to clinical improvement, CMR-confirmed LVEF recovery [**Figure 1D-F**] and absence of ventricular arrhythmia, using WCD or ICD implantation was deemed unnecessary.

This case highlights the rising incidence of post-COVID-19 HF and underscores the need for early, intensive HF treatment in patients with reduced LVEF, which can lead to significant recovery. Guidelines recommend sustained pharmacotherapy to maintain clinical stability and functional improvements in such patients [1–3,8]. Currently, there are no established guidelines for the follow-up of patients with myocarditis; however, frequent monitoring in the early phase after diagnosis is essential, combined with continuous escalation of medication dosages. In the initial period, follow-up visits are recommended every 2 to 4 weeks, followed by evaluations every 3 months, and subsequently annually. It is suggested that serial echocardiographic assessments, such as improvements in LVEF and reductions in left ventricular dimensions combined with serial laboratory assessments indicating declining levels of natriuretic peptides, may collectively serve as favourable prognostic indicators. These observations require further validation in more extensive, prospective studies to establish their predictive value in this patient population. Additional studies are needed to refine management strategies for restoring left ventricular function.

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Conflict of interest statement
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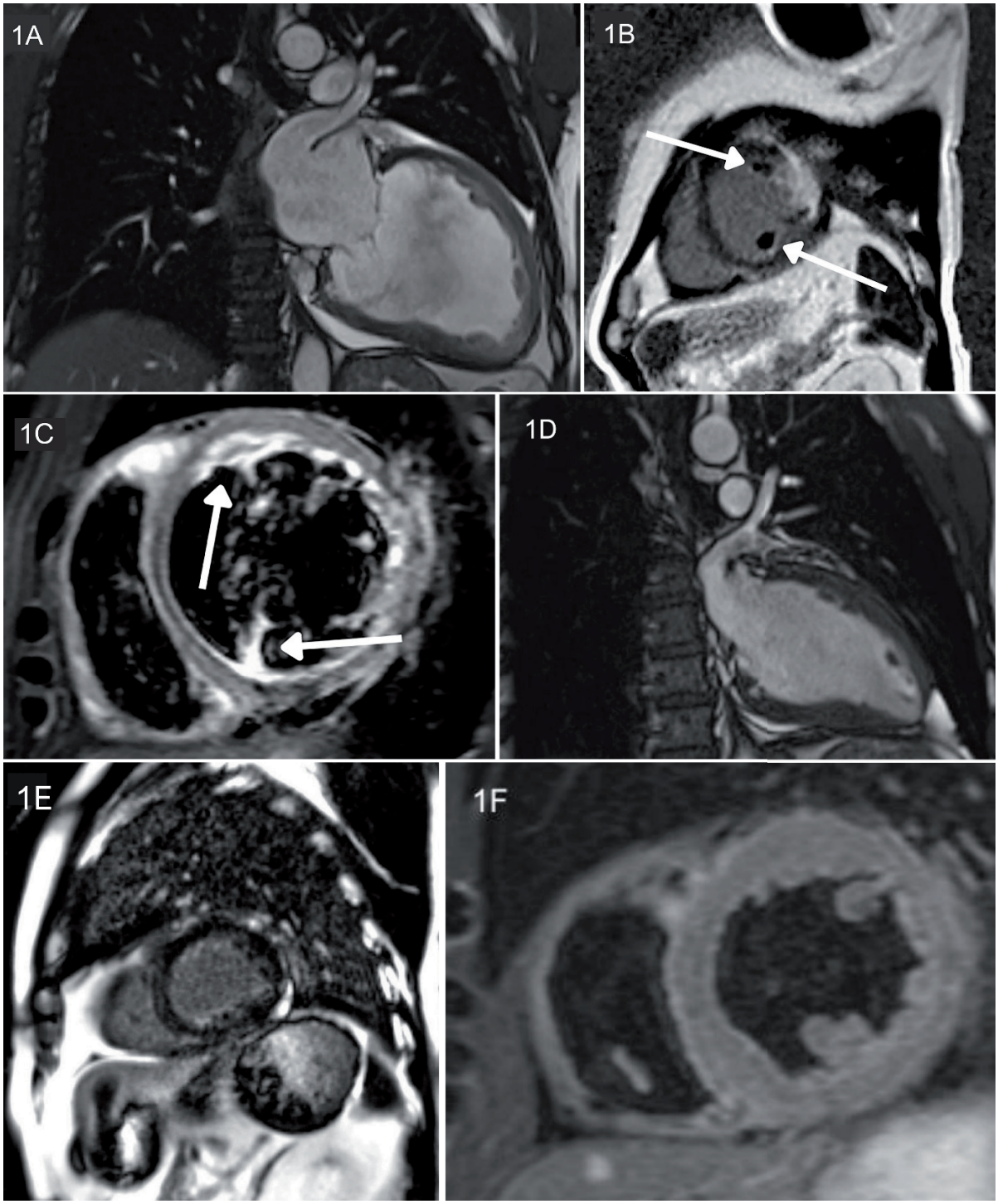

Figure 1. A – Cine-Magnetic Resonance Imaging 2-chamber view in end-diastole demonstrates left ventricular dilatation on admission. **B** – Magnetic Resonance Imaging 2-chamber short-axis, delayed enhancement images show intramural hyperenhancement (arrowheads) in the apical lateral wall and the adjacent mass (arrow) with no enhancement typical of thrombus formation in the left ventricle. **C** – Short-axis T2-weighted Magnetic Resonance Imaging shows myocardial oedema associated with acute myocarditis (arrows) predominantly involving the epicardial or transmural myocardium in the lateral wall on admission. **D –** Cine-Magnetic Resonance Imaging 2-chamber view in end-diastole demonstrates left ventricular reverse remodelling after 6 months of follow-up. **E –** Magnetic Resonance Imaging 2-chamber short-axis delayed enhancement images show a reduction of the lesions shown in Figure 1B after 6 months. **F –** Short-axis T2-weighted magnetic resonance imaging shows the resolution of oedema, as shown in Figure 1C, after 6 months. Abbreviations: MRI – magnetic resonance imaging.

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Long-term impact of physical activity on the prevention of cognitive function decline: study protocol for an extended randomized controlled trial (PA Protect study)

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ABSTRACT

The PA Protect study investigated the impact of increased daily physical activity on preventing cognitive decline, and maintaining healthy biomarker levels in individuals with mild cognitive impairment. Participants

aged 50–70 years, selected based on their performance in the Montreal Cognitive Assessment test were enrolled in a double-blind randomized controlled trial and randomly assigned to either an active or passive group. The active group was instructed to achieve over 10,000 daily steps, while the passive group maintained usual activity levels. Preliminary findings revealed a positive influence of increased physical activity on cognitive function, with significant differences observed between the two groups in selected cognitive tests. Building on these results, this study extends the PA Protect trial for an additional three years. All participants will undergo cognitive assessments and biomarker analyses at the beginning and after the extended intervention period, providing further insights into long-term intervention effects.

Research project objectives

Previously, 198 subjects aged 50–70 years with mild cognitive impairment (MCI), assessed using the Montreal Cognitive Assessment (MoCA) test, were recruited to a double-blind randomized controlled trial (PA Protect). Participants were randomly allocated into two groups: active and passive. The active group was instructed, encouraged, and motivated to increase their physical activity (>10,000 steps/day), while the passive group was advised to maintain their usual activity levels. All subjects underwent cognitive assessments, neuroimaging, and biomarker tests before and after the one-year intervention. The previous study has created a unique opportunity to extend the period of the observation for additional three years. This study aims to assess the effect of prolonged, 4-year increases in daily physical activity on cognitive decline prevention in subjects with MCI. We will also evaluate the effect of physical activity on neurodegenerative parameters, anthropometric, and densitometric parameters, body composition, blood pressure, glucose and insulin homeostasis, lipid metabolism and inflammatory markers. Moreover, we will investigate the usefulness of mobile applications to improve compliance with the recommended physical activity.

The research hypotheses are as follows:

1. Increased physical activity does not affect cognitive function in subjects with MCI.
2. Increased physical activity does not affect neurodegenerative parameters in subjects with MCI.
3. Increased physical activity does not affect anthropometric parameters in subjects with MCI.
4. Increased physical activity does not affect body composition in subjects with MCI.
5. Increased physical activity does not affect densitometric parameters in subjects with MCI.
6. Increased physical activity does not affect glucose and insulin homeostasis in subjects with MCI.
7. Increased physical activity does not affect lipid metabolism in subjects with MCI.
8. Increased physical activity does not affect inflammatory markers in subjects with MCI.
9. Increased physical activity does not affect blood pressure in subjects with MCI.

Basic concept and research plan

Concept overview

As stated by the World Health Organization [1], the global population of elderly individuals is steadily growing. This trend is associated with an increasing occurrence of various conditions, among which MCI is frequently mentioned. MCI is a transitional stage between normal cognitive function and dementia, described as a subtle cognitive decline, while maintaining the ability to perform everyday tasks [2,3]. The worldwide prevalence of MCI among individuals aged 50 and older is estimated to be 19.7%, which makes it rapidly becoming one of the most common clinical manifestations affecting the elderly [4]. The likelihood of developing MCI rises with age, is lower among individuals with higher education levels, and occurs more frequently in men [5]. MCI increases the likelihood of progressing to dementia and Alzheimer's disease (AD) [6]. About 54% of MCI patients eventually develop AD [7]. The underlying pathological and molecular mechanisms in individuals with MCI are not yet clearly defined. Besides age, sex, and education level, numerous other risk factors can impact

the development of cognitive disorders. Physical activity level is among these factors. Physical exercise has been proven to effectively enhance cognitive performance in older adults, regardless of their initial cognitive abilities [8]. Multiple studies, including meta-analyses [9,10], have demonstrated that regular physical activity provides protective effects against cognitive decline by promoting mechanisms such as neurogenesis, angiogenesis, synaptic plasticity, increasing brain volume, and enhancing cognitive function. Zhao et al. [11] highlighted in their meta-analysis a significant positive impact of physical activity on cognition among sedentary elderly individuals, particularly those with cognitive impairment. The effect was more pronounced in studies with intervention periods exceeding 12 weeks and involving aerobic training. Conversely, Li et al. [12] examined the impact of various training types on cognitive function in older adults with MCI and observed that strength training improved executive function and attention, whereas endurance training showed no significant effect. Huang et al. [13] reported that resistance training is most likely the optimal exercise type for mitigating cognitive decline in individuals with cognitive dysfunction. For patients with MCI, multicomponent exercise appears to be the most effective in preserving overall cognitive abilities and improving executive function. A comprehensive meta-analysis performed by Smith et al. [14], encompassing 29 studies, revealed that slow walking and jogging led to modest yet significant improvements in attention, executive functions, and memory processes. Alosco et al. [15] demonstrated that among older adults with heart failure, a higher daily step count was associated with improved attention, executive functions, memory, and language skills. Oliveira et al. [16] observed that accelerometer-measured physical activity has a positive impact on cognition. However, a recent umbrella review of randomized controlled trials revealed only minor exercise-related benefits, which were significantly reduced when accounting for various moderators and became negligible after adjusting for publication bias [17]. Despite this, Dougherty et al. [18] found that older adults at high risk for AD who adhered to physical activity guidelines (150 minutes of moderate-to-vigorous activity per week) exhibited larger temporal lobe volumes compared to those who did not. Regrettably, the current recommendations for physical activity levels are difficult for older adults to meet [19]. Moreover, current standards do not provide specific quantitative guidelines for physical activity levels that could help prevent cognitive impairment [20]. The aim of our study is to determine whether a prolonged 4-year intervention, focused on increasing physical activity levels using physical activity trackers integrated with a mobile application, significantly improves cognitive function and slows the progression of cognitive decline in 50–70 years old adults at risk, compared to a group with no intervention implemented. We hypothesize that a sufficiently prolonged intervention focused on increasing physical activity levels will demonstrate significant cognitive benefits, contributing to a slowdown in the progression of cognitive impairments. To support this process, we will incorporate the use of physical activity trackers integrated with a mobile application, enabling continuous activity monitoring. Ultimately, our intervention leverages advanced tracking tools to generate novel and objective insights into the relationship between physical activity and cognitive function over an extended duration. Extending the intervention period beyond typical short-term studies is crucial, as existing research suggests that cognitive benefits from physical activity become more pronounced with prolonged engagement. Many previous studies have been limited by short observation windows, potentially underestimating the long-term protective effects of sustained physical activity. By implementing a comprehensive 4-year-long intervention with continuous monitoring, we aim to capture more nuanced and meaningful changes in cognitive function that may not be detectable in shorter studies. We anticipate that this approach will not only validate the protective role of physical activity but also highlight effective strategies for implementing long-term interventions aimed at mitigating cognitive decline.

Study design

The study was designed as a prospective randomized controlled trial with parallel groups. Initially, the study was planned to last for one year; however, with the possibility of additional funding, a decision was made to extend the intervention period by an additional three years, enabling an

assessment of the intervention over a four-year timeframe. The first year of intervention was described in the previously published study protocol [21]. The study protocol was registered in the German Clinical Trials Register database (registration no. DRKS00020943, date of last update 23.10.2023) and was updated due to the prolongation of the study for the next three years. The study protocol has been prepared following the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines [22,23].

Ethical issues

The present study is conducted in accordance with the guidelines outlined in the Declaration of Helsinki. The study protocol was approved by the Bioethics Committee of Poznan University of Medical Sciences (refs. no. 813/23, date: 12.10.2023). Informed, written consent for further participation in the study and for data processing was obtained from all study participants, who agreed to continue the study. The previously published study protocol comprehensively described all aspects of participant data management, including data collection methods, personal data protection, participant rights, data storage duration, and potential protocol modifications [21]. At this stage, data will be collected online in the REDCap (Leavenworth, Omaha, NE) database. All questionnaires will be completed and entered into the online database during visits.

Study population

Previously, 198 subjects with MCI, aged 50–70 years old, were recruited to the study PA Protect. Inclusion and exclusion criteria were described in detail in the previous protocol from the first stage of the study [21].

At the beginning of the study, the participants were randomly assigned (1:1 allocation ratio) into two groups: active (Group A) and passive (Group P). The A group contained n = 98 participants and the P group contained n = 100 participants. Participants were evenly assigned to one of two groups, passive or active, as determined by the randomization code. The recruitment and randomization procedures were described in the original protocol from the first phase of the study [21].

The first phase of the study was successfully completed by 187 participants – 93 from the A group and 95 from the P group. All individuals who completed the study will be invited by the research team to take part in its continuation. It was anticipated that approximately 130 participants (69.5%) would consent to continue their involvement in the extended phase of the study; however, ultimately only 115 individuals agreed to continue the study.

Intervention

Individuals who decided to continue their participation in the study will remain in the group to which they were originally assigned and will be asked to follow the recommendations provided at the beginning of the study. From group A, 53 participants decided to continue participation in the study, and from group P, 54 participants agreed to continue their involvement.

Consistent with the approach taken during the first phase of the study, throughout the further intervention, participants will be asked to keep their current diet and medication regimen unchanged. Any changes in medication or health status should be reported to the research team via phone or email. The main measurements (cognitive functions assessed by MoCA test, neurodegenerative markers, glucose and insulin metabolism, lipid metabolism, inflammatory markers, anthropometric parameters, body composition, densitometric parameters, and blood pressure) will be performed before the second year of intervention and after the intervention period, while physical activity via Garmin Vívosmart 5 tracker (Garmin Inc., Olathe, KS, USA) will be monitored continuously throughout the study. Participants will also complete an IPAQ, health status, medications, smoking habits, alcohol use, profession and education questionnaires at the beginning and at the end of the intervention period. At the end of the intervention, cognitive functions measured by the CANTAB test will be assessed as well. The scheduled survey plan (Gantt's chart), is presented in **Figure 1**.

Adherence to intervention

Adherence to the intervention will be monitored by the dietitians using daily step count data collected from Garmin devices. Vívosmart 5 trackers will be connected with the Garmin Connect app, dedicated to tracking physical activity with more advanced data (e.g. Body Battery, stress level, etc.) for review by participants, which include

Tasks	2023	2024				2025				2026				2027
	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1
Submission of an application to the Bioethics Committee														
Recruitment of study participants for prolonged duration of the study														
Intervention period														
Outcomes measurements														
Data analysis														

Figure 1. Gantt's chart.

metrics such as daily step count, distance traveled, estimated energy expenditure, sedentary time, and minutes spent in low, moderate, and high-intensity activities, as well as sleep patterns. Daily step count data will be automatically synced to the participant's account and accessed by the research team through an application programming interface (API). The information collected from the Garmin device will provide an objective measure of adherence throughout the intervention period. Group P will have all notifications from the Garmin Connect app regarding increased physical activity turned off to avoid disrupting the representation of their current activity level. In Group A, a reminder will be enabled in the app settings to help participants meet the goal established at the beginning of the study. If the step requirements are still not met in this group, a phone call will be made to remind them about physical activity. To further enhance adherence, scheduled phone calls will be made to review compliance with physical activity recommendations. Participants will also have the option to request additional support calls if needed. If participants experience a medical problem or a situation that affects adherence to step count guidelines, the research team is informed and records all events. If a participant withdraws from the study, no further data will be collected from that individual.

Research methodology

Primary outcomes

Changes (Δ before – after) in cognitive function parameters and neurodegenerative markers will serve as primary outcomes. Data collection will take place at the Department of Pediatric Gastroenterology and Metabolic Diseases, Poznan University of Medical Sciences.

Cognitive assessments: Cognitive function will be assessed using the MoCA test and the Cambridge Neuropsychological Test Automated Battery (CANTAB). Following tests: Motor Screening Task (MOT), Reaction Time (RTI), Paired Associates Learning (PAL), Spatial Working Memory (SWM), Pattern Recognition Memory (PRM), Delayed Matching to Sample (DMS), Rapid Visual Information Processing (RVP) will be used.

Blood collection: Blood samples (approximately 15 ml) will be obtained through standard venopuncture from the antecubital vein by licensed staff nurses or phlebologists. The blood collection will occur after a 12-hour fasting period. Blood will be collected by a commercial laboratory (Diagnostyka, Poznań, 77a Dąbrowskiego Street), which performs part of the lab tests.

Neurodegenerative markers: The neurodegenerative parameters to be evaluated are beta-amyloid 1–40 and 1–42, total tau protein, and brain-derived neurotrophic factor (BDNF). Blood samples will be collected by a commercial laboratory, while biochemical analyses will be carried out using ELISA kits in the Laboratory of the Department of Pediatric Gastroenterology and Metabolic Diseases at Poznan University of Medical Sciences: Amyloid-beta (1–40) ELISA, IBL; Amyloid-beta (1–42) ELISA, IBL, Human Tau proteins ELISA kit, Cusabio; Human Free BDNF Quantikine ELISA Kit, R&D Systems.

MoCA test and neurodegenerative markers will be evaluated after the first year of the intervention

and after the intervention period. CANTAB test will be assessed after the intervention period.

Secondary outcomes

Secondary outcomes will include changes in biochemical parameters (glucose, insulin and lipid metabolism, inflammatory markers), anthropometric measurements, body composition and densitometric parameters, blood pressure assessment.

Biochemical analysis: The study will evaluate a range of other biochemical parameters, including fasting glucose, insulin, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) and inflammatory markers – high-sensitivity C-reactive protein (hs-CRP). All these parameters will be analyzed in a commercial laboratory.

Anthropometric measurements: Key anthropometric parameters, including height, weight, hip and waist circumferences, will be measured at the beginning and at the end of the extended intervention period. Body mass index (BMI) will be determined using the recorded height and weight data and waist-to-hip ratio (WHR) will be calculated using the measured hip and waist circumferences.

Body composition and densitometric parameters: Body composition (fat and free fat mass) and densitometric parameters (bone mineral density and content at the lumbar spine (L1-L4)) will be evaluated through dual-energy X-ray absorptiometry methods, utilizing the Hologic Discovery analyser (Bedford, Massachusetts, USA) before and after the extended intervention period.

Blood pressure: Blood pressure will be assessed before blood sample collection, adhering to the protocols established by the European Society of Hypertension (2021) [24]. Blood pressure will be measured on the arm at heart level and recorded as the average of three measurements for both systolic and diastolic pressure.

Assessment of dietary habits: Dietary habits and food group intake will be assessed during and after the extended intervention, using the Dietary Habits and Nutrition Beliefs Questionnaire (KomPAN) [25], created by the Behavioural Conditions of Nutrition Team from the Committee of Human Nutrition Science of the Polish Academy of Sciences. The main objective of assessing participants' dietary behaviors is to verify whether changes in dietary patterns are a variable affecting the obtained results in given individuals.

Physical activity: Physical activity will be measured objectively using the Garmin Vívosmart 5 tracker (Garmin Inc., Olathe, KS, USA) and subjectively through the Polish version of International Physical Activity Questionnaire (IPAQ) [26]. Previously, a Fitbit Inspire HR device (Fitbit Inc., San Francisco, CA, USA) was used; however, due to changes in Fitbit's privacy policy and the merger of Fitbit accounts with Google accounts, patient data management became challenging. Therefore, it was decided to choose another wristband model. Garmin Vívosmart 5 tracker is a wrist-mounted wireless device equipped with an accelerometer that monitors physical activity continuously throughout the day and can seamlessly synchronize with both smartphone applications and computers. Participants will be instructed to wear the Garmin device all day long, except showering, bathing or swimming, on their non-dominant wrist for the duration of three years. The Garmin Connect app will be installed on their smartphones, and individual anonymous Garmin Connect accounts will be created to facilitate data download. Participants will be reminded to regularly synchronize and charge their trackers. If a Garmin remains unsynchronized for a week, study staff will contact the participant to assist with synchronizing or provide technical support. Data on sedentary behavior, light, moderate, and vigorous activity, as well as steps taken, distance covered, and calories burned, will be collected.

Sociodemographic questionnaire: A sociodemographic questionnaire will be used to collect details about participants' backgrounds, including their place of residence, education level, family status, and financial situation. Furthermore, the questionnaire will inquire about lifestyle factors such as smoking habits and alcohol use.

Minimum sample size calculation: The minimum required sample size was determined using preliminary study (n = 152) findings and calculated with G*Power 3.1 software (University of Kiel, Kiel, Germany). Results were obtained from the MoCA and SWM tests. Preliminary outcomes are presented in **Table 1**. Based on the current trend, the study needed to include at least 94 participants to achieve statistical significance ($\alpha = 0.05$, $\beta = 0.2$).

Table 1. Preliminary (n = 152) outcomes of changes in cognitive test results (Δ) after one year of intervention.

	Active group (n = 76)	Passive group (n = 76)	p
	Median (Q1 – Q3)		
Δ MOCA [points]	1 (0 – 3)	0 (-2 – 2)	0.0197
Δ SWMBE468	-3 (-8 – 1)	0 (-5 – 6)	0.0312
Δ SWMBE6	-1 (-4 – 0)	0 (-2 – 2)	0.0061

Statistical analysis: The detailed statistical analysis methodology was comprehensively described in the previous project documentation [21]. Key analytical approaches will include descriptive statistics, normality testing, appropriate parametric or non-parametric tests based on data distribution, regression models, and multiple imputation methods for handling missing data. A two-tailed p-value of < 0.05 will be considered statistically significant.

Risk analysis: The following challenges were identified as potential factors influencing the study's feasibility:

Recruitment challenges (low risk): Participants from the PA Protect study will be invited to extend their involvement for an additional three years. Approximately 107 (54.04%) are willing to continue their participation.

Adherence to the intervention (moderate risk): Initial observations during the first year showed strong adherence among most participants. However, individuals with lower adherence levels have expressed limited interest in continuing the intervention for another year.

Drop-out rate (moderate risk): The longer duration of the intervention may lead to a slightly higher drop-out rate compared to studies with shorter time frames.

Collaboration with external partners (low risk): Biochemical analyses will be outsourced to a large, reliable commercial laboratory, reducing the risk of delays or complications. Alternative laboratories are available to provide similar services at comparable costs if needed.

Rising costs (moderate risk): Unstable market conditions and inflation could drive up the costs of materials and services, potentially affecting the project's financial feasibility.

Equipment availability (low risk): The Department of Pediatric Gastroenterology and Metabolic Diseases is fully equipped to perform all planned assessments, ensuring no equipment-related delays.

Data loss (low risk): Study data will be directly entered into the RedCap tool (Seattle, WA, USA), which provides secure and reliable data storage. The likelihood of data loss is minimal.

Measurable effects

Preliminary results from this study showed a promising effect of increasing physical activity on improving cognitive functions. Its prolongation may reveal differences between groups observed after the completion of the first phase of the study and offer valuable insights for optimizing and tailoring daily physical activity recommendations for individuals with MCI. We anticipate that the research will yield precise data on the intensity of physical activity needed to mitigate cognitive decline. The findings could also contribute to define desirable activity recommendations specifically designed to address this population's needs.

Expected results

This randomized controlled trial will involve an additional 3-year physical activity intervention targeting 115 individuals with MCI. Drawing on existing research and new data, the study aims to determine the optimal intensity and frequency of physical activity necessary to develop innovative guidelines for preventing cognitive decline in high-risk adults. Additionally, the study will explore the relationships between long-term physical activity, cognitive performance, and blood biomarkers. We hypothesize that specific thresholds of physical activity frequency and intensity can enhance overall cognitive function in at-risk individuals, and maintain biomarkers within normal ranges. The anticipated results will inform the creation of the first tailored physical activity guidelines focused on cognitive impair-

ment, designed to be both effective and practical for older adults. Walking, as an accessible and affordable form of exercise, offers the added benefit of reducing the risk of other chronic conditions such as diabetes, cardiovascular disease, obesity, and depression.

Study strengths and limitations

Strengths

The main strength of this study is its extended four-year intervention period, which represents one of the longest randomized controlled trials investigating the impact of increased physical activity on cognitive function in subjects with MCI. The study's design allows for comprehensive assessment of both cognitive and physiological outcomes through standardized tools and objective measurements. The use of wearable devices provides accurate, continuous monitoring of physical activity levels, while the mobile application offers a practical method for improving intervention adherence. The study benefits from the continuation of the previously established phase, allowing for longitudinal assessment of intervention effects. Additionally, the broad range of measured parameters, including neurodegenerative markers, metabolic parameters, and body composition, enables a thorough understanding of the multisystemic effects of increased physical activity in 50–70-years old subjects with MCI.

Limitations

Several limitations should be considered when interpreting the study results. The study population consists of individuals with MCI, which may limit the generalizability of the findings to populations with more severe cognitive impairments, such as dementia or Alzheimer's disease. Technical difficulties with smartphones or tracking devices may impact participants' ability to consistently follow physical activity recommendations and affect intervention adherence. The change in activity tracking devices from Fitbit to Garmin between study phases may affect data consistency, although both devices provide comparable basic metrics. Participants' awareness of their group allocation could potentially influence their behavior and the study outcomes.

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Contributors

Conceptualization, M.J., K.H.H. & J.W..; methodology, M.J. & J.W.; software, M.J. & J.K.N.; formal analysis, M.J.; investigation, A.M.A., M.J., K.K., A.M.B &, J.B.; resources, J.W.; writing—original draft preparation, A.M.A., M.J. & J.W.; writing—review and editing, K.K., A.M.B., J.B., A.D., J.G., E.M., J.K.N. & K.H.H.; visualization, A.M.A.; supervision, M.J. & J.W.; project administration, M.J.; funding acquisition, J.W. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest statement

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Semaglutide as a promising treatment for metabolic dysfunction-associated steatotic liver disease

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ABSTRACT

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most prevalent liver disorder globally, and if left untreated, it may progress to liver cirrhosis and even hepatocellular carcinoma. As of 2024, the European Association for the Study of the Liver Guidelines have recommended resmetirom as the only pharmacological treatment for adults with non-cirrhotic MASLD who have significant fibrosis (stage ≥ 2). However, lifestyle interventions and management of comorbidities, such as type 2 diabetes and obesity, remain the cornerstone of treatment. Glucagon-like peptide-1 receptor agonists, particularly semaglutide, have shown emerging promise in treating MASLD. Notably, the Phase III ESSENCE trial, presented in late 2024, demonstrated semaglutide's potential in improving liver fibrosis, confirmed through histological evaluation, marking a possible breakthrough for MASLD management. This review aims to synthesise current evidence on the efficacy of semaglutide in treating MASLD, highlighting its potential to fill a significant gap in the therapeutic options available for this growing global health concern.

MASLD

Definition and epidemiology

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most prevalent liver disease globally, affecting approximately 30% of the population, with rates continuing to rise [1]. In 2023, a new consensus published by several international societies emphasised the link between metabolic disorders and nonalcoholic fatty liver disease, introducing the term MASLD to describe liver steatosis associated with cardiometabolic risk factors. A more severe form of MASLD called metabolic dysfunction-associated steatohepatitis (MASH), is characterised by lobu-

lar inflammation and hepatocyte ballooning and can progress to hepatic fibrosis, cirrhosis, and hepatocellular carcinoma [2]. MASH is currently the fastest-growing cause of liver cancer among individuals who are potential candidates for liver transplantation [3].

Aetiology

MASLD is a multifactorial disease primarily driven by lipotoxicity, insulin resistance, and activating inflammatory and immune pathways. It is strongly associated with metabolic conditions, particularly type 2 diabetes (T2DM) and obesity [4]. The presence of T2DM and its related comorbidities, such as visceral obesity, hypertension, and dyslipidemia, can accelerate the progression from MASLD to MASH and cirrhosis. On the contrary, MASLD can impair hepatic insulin sensitivity, which may worsen glucometabolic control [4,5].

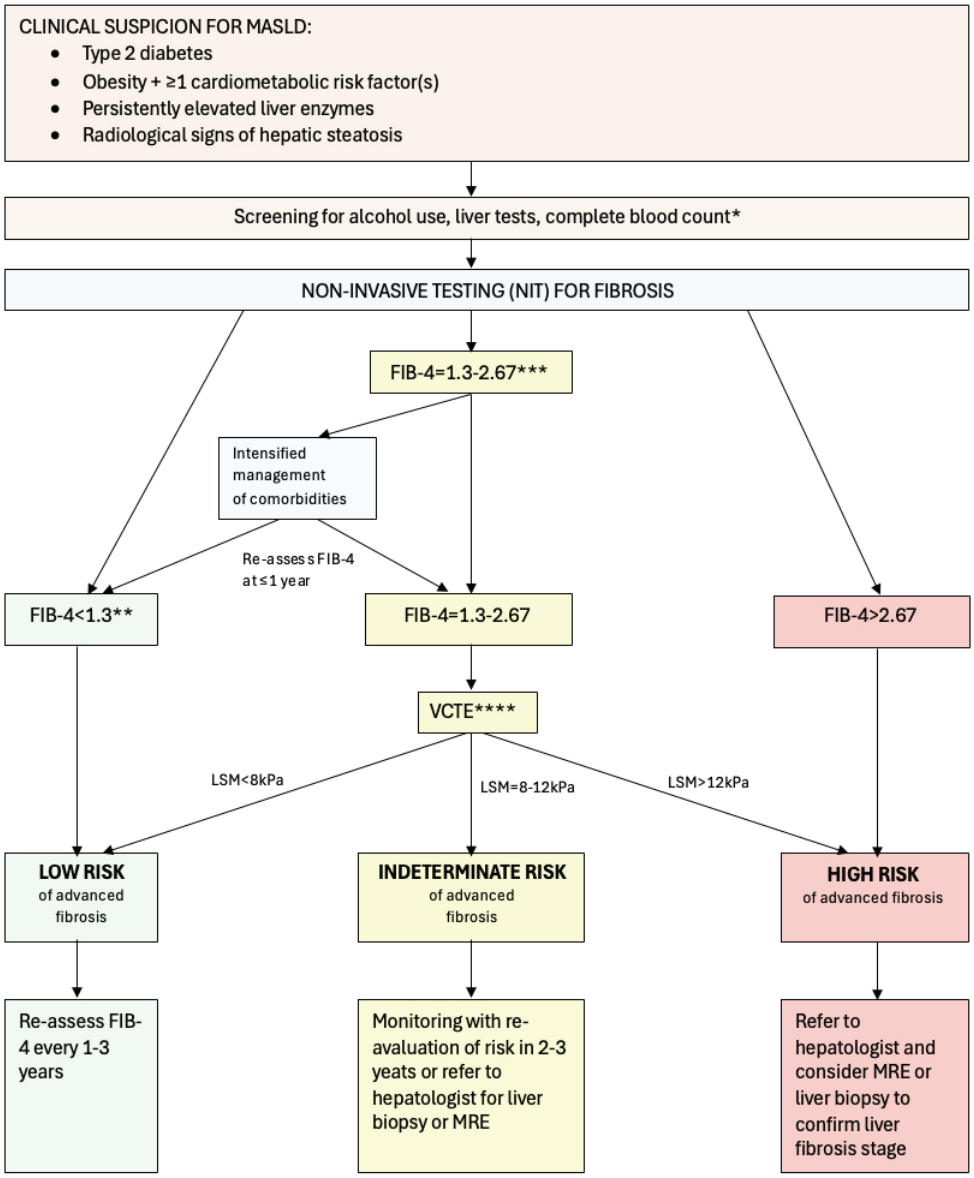

Figure 1. The recommended strategy for MASLD risk stratification [7,8]. *These initial laboratory tests can also identify patients with elevated aminotransferases, all of whom should be evaluated for other chronic liver and biliary diseases. ** The FIB-4 thresholds apply to individuals aged 65 years or younger (for those older than 65, a lower FIB-4 cut-off of 2.0 is used). *** Management of patients with FIB-4 scores between 1.3 and 2.67 depends on medical history, clinical context, and local resources. ****or alternative test, e.g. magnetic resonance elastography (MRE), shear wave elastography (SWE), enhanced liver fibrosis (ELF), with adapted thresholds

Diagnostic process

The diagnosis of MASLD requires the presence of at least one cardiometabolic risk factor in an individual with documented steatosis. Non-invasive screening for steatosis should be considered for patients with obesity/T2DM or raised liver enzymes. B-mode ultrasonography (US) and MR-based techniques such as MRI proton density fat fraction (MRI-PDFF) or proton magnetic resonance spectroscopy (1H-MRS) may be used to assess liver lipid content. Assessing liver fibrosis is essential in MASLD patients, as it is a critical indicator of liver-related outcomes. A multi-step approach is recommended, starting with the fibrosis-4 (FIB-4) score, followed by measurements of liver stiffness measurement (LSM) (vibration-controlled transient elastography [VCTE] or magnetic resonance elastography [MRE]) to assess advanced fibrosis risk. This strategy helps with risk stratification and guides interventions, including speciality referral, for those at intermediate or high risk [6–8] (**Figure 1**).

Treatment

The primary goal of MASLD treatment is to prevent cirrhosis progression to hepatocellular carcinoma and liver failure. Evaluating cardiovascular risk and implementing a multidisciplinary approach is crucial for comprehensive care. First-line therapy for MASLD focuses on lifestyle changes to achieve safe weight loss; however, long-term compliance is often challenging. Resmetirom is the only MASH-targeting drug with positive results from a registrational phase III clinical trial. The latest recommendations for MASLD treatment are shown in **Figure 2** [6–8].

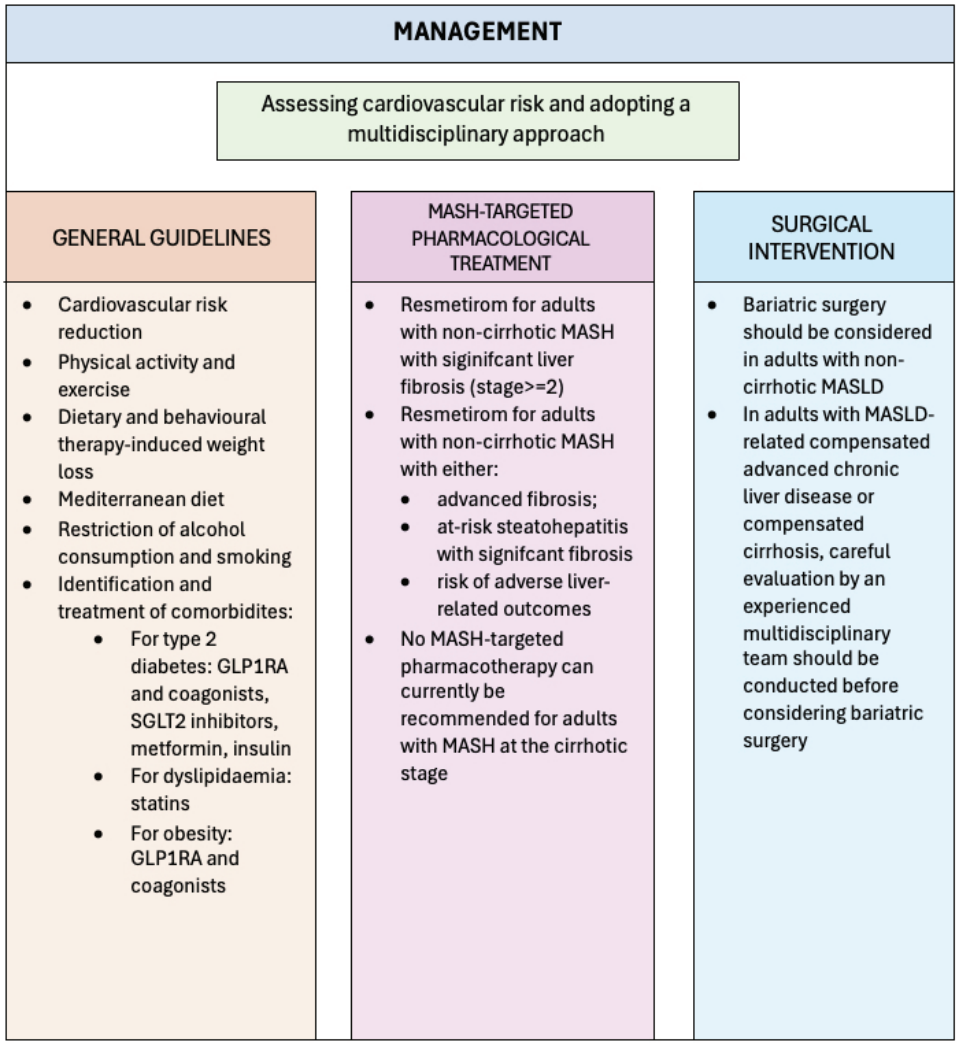

Figure 2. Recommended management of MASLD [6–8]. SGLT2 – sodium/glucose cotransporter 2.

Semaglutide in MASLD treatment

Glucagon-like peptide 1 receptor agonists (GLP-1 RAs), such as semaglutide, are effective glucose-lowering agents for managing T2DM. Treatment with semaglutide leads to weight reduction, decreased cardiovascular risk, and lowered systolic blood pressure, all with minimal incidence of hypoglycemia [9]. In subcutaneous and oral forms, semaglutide demonstrated the greatest glycemic and weight loss benefits from all GLP-1 RAs and has shown reduced rates of major adverse cardiovascular events in patients with T2DM [10,11]. Many recent studies have evaluated GLP-1 RA's potential benefits in MASLD management. GLP-1RAs are thought to provide indirect liver benefits through improved metabolism, weight loss, and their multiorgan effects [12,13]. (**Figure 3**). A recent meta-analysis concluded that daily semaglutide may be the most effective treatment for MASLD and T2DM compared to other GLP-1 RAs [14].

Newsome et al. conducted one of the first trials on semaglutide's efficacy in MASLD patients in 2021 and showed promising results. In the phase IIb study involving 320 patients with MASH and liver fibrosis, daily semaglutide 0.4 mg for 72 weeks led to more patients achieving MASH resolution without worsening fibrosis than placebo. While the trial did not show significant improvements in fibrosis, it was suggested that a more extended treatment period might be needed to evaluate its effectiveness [15] entirely. A 2024 post-hoc analysis using Artificial Intelligence machine learning models found a significant reduction in biopsy slides from the mentioned trial, suggesting it was a more sensitive method than traditional histopathology [16]. Subsequent trials on semaglutide for MASLD have been conducted, and their findings are summarised in **Table 1**.

After showing inconsistent results in improving MASH-related fibrosis, a significant breakthrough occurred at the end of 2024. The first

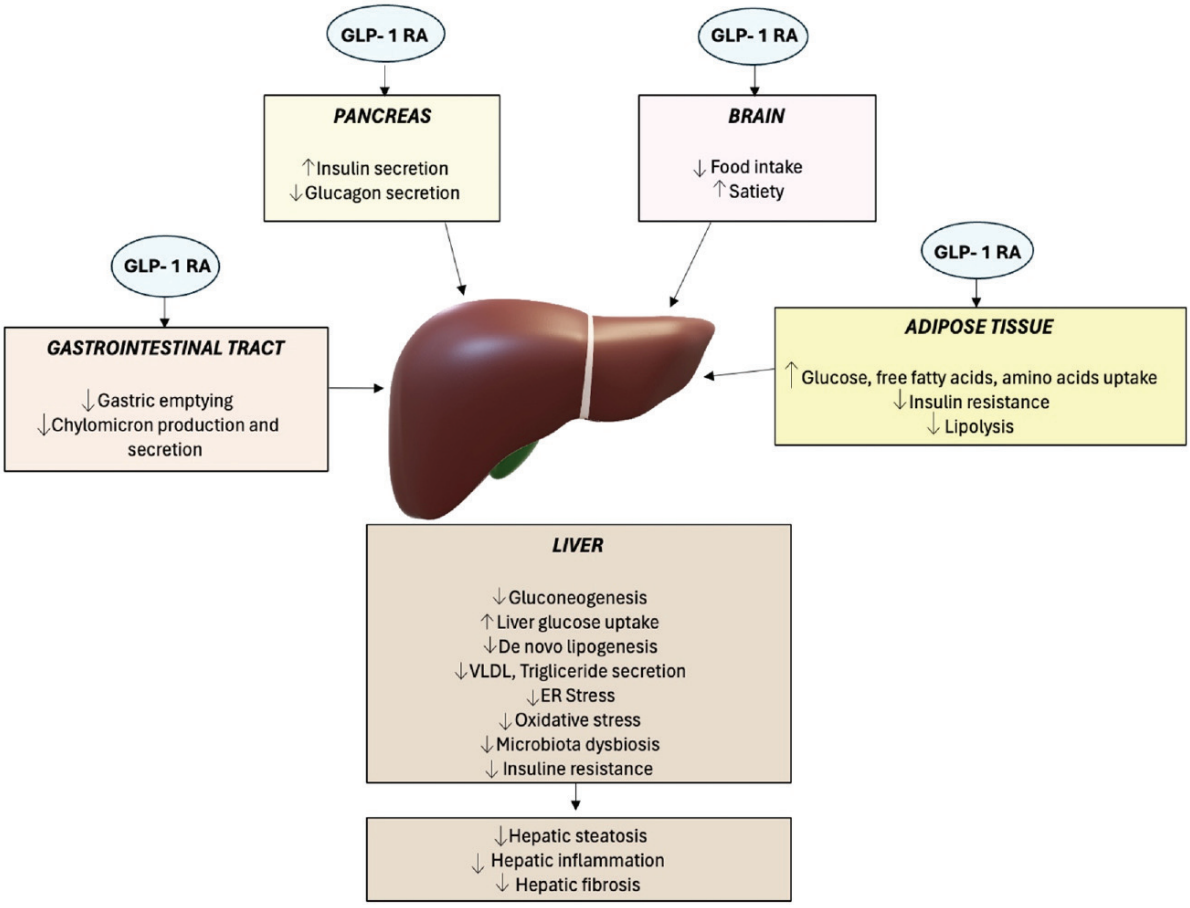

Figure 3. Overview of the pleiotropic effects of GLP-1RAs in managing MASLD/MASH [12,13]. ER – endoplasmic reticulum.

Table 1. The latest publications on the efficacy of semaglutide in MASLD [19–25].

Publication	Methods	Results
Gad et al. 2024	An open-labelled intervention study with 180 patients classified into three parallel groups (1:1:1): – group I received oral semaglutide (up to 14mg) daily – group II patients received injectable semaglutide (up to 2mg) once-weekly – group III received pioglitazone and/or vitamin E	– A substantial improvement in lipid profile, liver enzymes, and body mass index, especially in group II – Only group II showed a consistent increase in HDL – Group II had significantly lower scores of the fibrosis-4 score (FIB-4), liver stiffness measurement (LSM), and controlled attenuation parameter (CAP) at 6 and 12 months ($p < 0.001$)
Loomba et al. 2023	A double-blind, placebo-controlled phase 2 trial with 71 patients with biopsy-confirmed MASH-related cirrhosis and body-mass index (BMI) of 27 kg/m2 or more, who were randomly assigned (2:1) to receive either once-weekly subcutaneous semaglutide 2.4 mg or placebo	– After 48 weeks, there was no statistically significant difference between the two groups in the proportion of patients with an improvement in liver fibrosis of one stage or more without worsening of MASH – There was also no significant difference between groups in the proportion of patients who achieved MASH resolution ($p = 0.29$)
Volpe et al. 2022	A prospective, single-arm, real-life study with 48 patients treated with subcutaneous semaglutide (up to 1mg) once-weekly in add-on to metformin for 52 weeks	– A significant decrease in anthropometric and glucometabolic parameters, insulin resistance, liver enzymes, and laboratory indices of hepatic steatosis during treatment. – Fat mass and visceral adipose tissue (VAT) decreased – Ultrasound-assessed VAT thickness and the 12-point steatosis score also declined at T3 up to T12 – Liver steatosis improved in most patients (70%), showing a reduction by at least one class in the semiquantitative ultrasound staging
Flint et al. 2021	A randomised, double-blind, placebo-controlled trial with 67 patients with liver stiffness 2.50-4.63 kPa by magnetic resonance elastography (MRE) and liver steatosis ≥ 10% by MRI proton density fat fraction (MRI-PDFF), randomised to once-daily subcutaneous semaglutide 0.4 mg (n = 34) or placebo (n = 33).	– Decrease in liver enzymes, body weight and HbA1c with semaglutide – Reductions in liver steatosis were significantly greater with semaglutide (estimated treatment ratios: 0.70 [0.59, 0.84], P = 0.0002; 0.47 [0.36, 0.60], P < 0.0001; and 0.50 [0.39, 0.66], P < 0.0001) and more subjects achieved a ≥ 30% reduction in liver fat content with semaglutide at weeks 24, 48 and 72, (all P < 0.001) – Not signifcant differences in liver stiffness with semaglutide
Kitsunai et al. 2025	– A secondary analysis of a multicenter, retrospective, observational study, analyzing oral semaglutide up to 14 mg once-daily – Subjects with suspected MASLD were placed in an overall group; a subpopulation from an overall group at high risk for hepatic fibrosis was placed in a high-risk group (n = 67); and the remaining subjects were placed in a low-risk group (n = 102)	– Oral semaglutide significantly improved the hepatic steatosis index (from 46.1 to 44.6, $p < 0.001$) and FIB-4 (from 1.04 to 0.96, $p < 0.001$) – Improvement in the FIB-4 index was significantly negatively correlated with the baseline FIB-4 index. – HbA1c, body mass index, systolic blood pressure, and lipid profile decreased in the overall cohort – The mean values of liver enzymes showed a significant improvement
Arai et al. 2022	A single-arm, open-label pilot study with 16 patients receiving semaglutide initiated at a dose of 3 mg once daily, which was sequentially increased to 7 mg at 4 weeks and 14 mg at 8 weeks (maintenance dose)	– Semaglutide decreased body weight, levels of liver-related biochemistry, plasma glucose, HbA1c, HOMA-IR, triglyceride, CAP and liver fibrosis markers (fibrosis-4 index, ferritin, and type IV collagen 7 s) – Changes in body weight were correlated with those in levels of ALT (alanine aminotransferase) and CAP – Semaglutide did not decrease liver stiffness measurement
Alkhouri et al. 2022	A phase II, open-label, proof-of-concept trial involved patients with mild-to-moderate fibrosis due to MASH, who were randomized to three groups: A. Semaglutide 2.4 mg/week (n = 21) B. Semaglutide 2.4 mg/week and once-daily, cilofexor 30 mg (n = 22) C. Semaglutide 2.4 mg/week and once-daily, cilofexor 100 mg (n = 22)	Compared with semaglutide monotherapy, combination treatments resulted in greater improvements in liver steatosis measured by MRI-PDFF (least-squares mean of absolute changes: ranging from −9.8% to −11.0% vs.−8.0%; the difference was statistically significant only between the semaglutide and semaglutide + firsocostat groups) as well as in non-invasive tests of liver fibrosis and liver biochemistry

results of the Phase III ESSENCE trial emerged, which evaluated once-weekly 2.4 mg semaglutide in adults with MASH and moderate to advanced liver fibrosis (F2–F3). At The Liver Meeting 2024, Phil Newsome (London, UK) presented an interim analysis from the 72-week data, highlighting promising results in addressing MASH-related fibrosis. Semaglutide demonstrated significant superiority over placebo on both primary endpoints. A greater proportion of patients receiving semaglutide achieved resolution of steatohepatitis without worsening fibrosis. More notably, 37% of semaglutide-treated patients showed improved fibrosis without worsening steatohepatitis, compared to 22.5% in the placebo group. This study confirmed semaglutide's effectiveness in improving liver fibrosis, with the histological improvements assessed through liver biopsies [17].

Conclusions

The ESSENCE trial and AI-driven liver histology assessments highlight semaglutide's potential to transform MASH-related fibrosis treatment. Promising results show semaglutide outperforms placebo in improving liver histology and fibrosis, offering a new therapeutic option for MASH. Following the positive outcomes of the trial, Novo Nordisk expects to file for regulatory approvals in the US and EU in the first half of 2025. Given these findings, continued investment in clinical trials is essential to explore the long-term benefits of semaglutide in MASLD management. There are ongoing trials combining semaglutide with other therapies, such as luseogliflozin (UMIN000045003) and zalfermin (NCT05016882). Recent studies have also highlighted the effectiveness of novel incretin-based analogs, including dual GLP-1/GIP agonists (tirzepatide), dual GLP-1/glucagon agonists (survodutide, pemvidutide, and cotadutide) and GLP-1/GIP/GCGR agonist (retarutide) in reducing liver fat and fibrosis. These innovative therapies offer promising potential for the future management of MASLD [18].

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