Microplastic-Induced Cellular Stress: Emerging Mechanisms Linking Environmental Nanoparticles to Human Metabolic Disorders

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Abstract

Plastics and plastic-derived chemicals are now recognized as widespread and concerning environmental pollutants that leach or degrade over time into microplastics and nanoplastics, which pose a serious risk to human health and are linked to various metabolic diseases. Microplastics are defined as plastic particles that are less than 5 mm in size, whereas nanoplastics are even smaller, with sizes being less than 1 μm. The emergence of microplastics and nanoplastics can be traced back to the intentional incorporation of plastic materials, including pigments and plasticizers, into commercial products. This practice is employed to enhance the versatility, usability, and broad functional application of different polymers across various industries. Furthermore, larger plastic debris can break down into smaller fragments due to the effects of sunlight exposure and mechanical forces in the environment. The presence of microplastics and nanoplastics has the potential to disrupt metabolic homeostasis in several troubling ways. These disruptions can manifest through interference with normal metabolic pathways, reprogramming of cellular metabolism, and expansion of ecological niches, leading to ultimately harmful dysregulation. Although research has already identified several consequences arising from microplastics, the identification and understanding of specific mechanisms connecting microplastics to subsystems across diverse environmental compartments are critically important and warrant further investigation.

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Chapter - 1

Introduction to Microplastics and Environmental Nanoparticles

Microplastics (MPs) are recognized as emerging environmental pollutants. Originally defined as plastic particles smaller than 5 millimeters, MPs are now classified according to their size: microplastics (1 μm-1 mm), nanoplastics (1 nm-1 μm), and submicron plastics (1 nm-1 μm). Submicron plastics, recently termed "environmental nanoparticles," are investigated not only as products of plastic degradation but also as potential nanoplastics. Environmental nanoparticles can be further classified into anthropogenic nanoparticles engineered for a dimension ranging from 1 nanometer to 1 micron (e.g., nanomaterials), and environmental nanoparticles formed by physical, chemical, and biological processes in the ecosystem (e.g., soot particles, road dust). MPs and environmental nanoparticles can directly or indirectly interact with cells or tissues.

EPs display a much larger surface area for adsorption of other substances than larger particles of the same type, resulting in a higher risk of toxicity. Experimental and bioinformatics analyses indicate that MPs might be more hazardous than nanoplastics. These pollutants and environmental nanoparticles are ubiquitous in air, water, soil, and food. Despite being detected in human urine, stool, and placenta, and their presence in animal tissues reported, adverse biological effects due to exposure to MPs remain poorly understood. Accumulating evidence suggests that the two types of environmental nanoparticles may act as a novel

class of environmental stressors causing metabolic disorders in mammals through specific cellular pathways. However, an integrated overview of the current knowledge remains lacking [1, 2, 3, 4].

Definition, Classification, and Sources of Micro- and Nanoplastics

Macromolecules based on synthetic polymer materials are among the Nanostructures with greater energy throughput. This is exclusively attributed to their extremely low biodegradation rate which displays limited capacity for self-destruction. These macromolecules, increasingly used since the mid-19th century, have followed diverse paths of differentiation that have resulted in the composition of the most diverse additives directly connected to the plastics (not naturally biodegradable) which, in time, may represent sources of chemical toxicity for living beings within the food chain.

With an ever-increasing global consumption of plastics, consisting of roughly 300 million tons/year, it was found that microplastics, classified as particles with a size of less than 5mm, are being generated and scattered in the environment at a rate of about 131000 tons/year (Browne *et al*. 2011). They mainly originated in three different chemical forms, such as primary microplastics (synthetic polymer particles produced in such size for cosmetic and pharmaceutical industries), secondary microplastics (small fragments stemming from the natural weathering and photodegradation of larger plastic materials), and those created during the industrial scale use and production of plasticizers. Nevertheless, in natural systems these organisms are subject to a continuous process of alteration of their physical-chemical properties, which may justify their classification into "Aging Microplastics". These aged microplastics are strongly attractive to other Halogenated Compounds and Organophosphates due to their non-specific adsorbing power, as

well as by virtue of the use of these molecules as additives in their original commercial formulation [5, 6, 7, 8, 9].

Global Production, Distribution, and Environmental Persistence

In 2021, global plastic production reached 368 million tons, with a growth prediction to exceed 550 million tons by 2030. Despite increasing recycling efforts, about 85% of discarded plastics are deposited in landfills or the environment. Ocean surfaces harbor 10-24 million tons of plastics, with studies in lakes and rivers confirming substantial concentrations. More than 250,000 tons of macroplastic are at sea, continuously being fragmented into microplastics. These nano- to microscale particles, defined as measuring between 1 nm and 5 mm, can cross epithelial barriers, penetrate tissues, and be assessed in various biological samples (inhalation, ingestion, and transplacental). Microfibers are particularly abundant in the atmosphere (> 20,000 km−2 month−1), while microplastics are also detected in air samples. Assessment of plastic leachates demonstrates release of harmful chemicals and associated microbiota.

Polymer type, size, shape, physical aging, and chemical weathering determine mobility and persistence in the environment, with microbial degradation of polyester and polycaprolactone still under investigation. Biological interactions are chiefly influenced by particle size, charge, shape, surface deformations, and functionalization. Organic additives (e.g., plasticizers, antioxidants, flame retardants, slip agents) can leach out and act as endocrine disruptors, while chemical sorption of contaminants (e.g., heavy metals, pesticides, oil derivatives) confer additional toxic effects. Accumulation of microplastics in the gastrointestinal tract may drive dysbiosis and impair the gut-metabolism axis [10, 11, 12, 13].

Routes of Human Exposure: Air, Water, Food, and Consumer Products

Microplastics accumulate in the environment due to their high persistence and have been documented in urban air, lakes, oceans, and other ecosystems. While the wastewater treatment process effectively removes a large fraction of the incoming MP concentration, small MPs and NPs remain poorly retained and find their way into the aquatic environment. With increasing production rates, MPs are detected in marine organisms and in many food products, including seafood, sea salt, bottled water, honey, sugar, and beer. The effects of plastic use and waste on the environment are becoming increasingly evident, with leachates and leached plastic additives found in soils, rivers, and marine areas, as well as in the air. Concerns are already raised about the presence of microplastics in bioatmospheric and biometeorological cycles. Plastic particles have been confirmed in human tissues biologically internalized via inhalation, ingestion, transdermal absorption, and intravenous routes.

Particles ≥ 4 μm excite inhalation concerns because they may reach lower pulmonary regions and present an inherent translocation potential. For smaller particles, inhalation is just one of several exposure routes with bimodal distribution, with the highest occurrence in the nano range; deposition doses in the lung are lower than those in other human organs. Exposures through drinking water, food, cosmetics, medical devices, and inhalation of indoor air contaminated with artificial fibers are nonnegligible. Cigarette smoke contains greater concentrations of NPs and easily leaches toxic compounds from additives. A single cigarette potentially releases more than 100 times the concentration level of a plastic microcystin toxin used in renal experiments with fish cells [14, 15, 16, 17].

Physicochemical Properties Governing Biological Interactions

Environmental nanoparticles exhibit physicochemical determinants that govern their biological interaction patterns. In biological media, the apparent dimensions, distribution, surface charge, composition, shape, and physicochemical alterations of environmental nanoparticles play important roles in their uptake mechanisms and interacting behavior. Additionally, the alterations of these properties in biological media must be evaluated with respect to their potential biological activity. Environmental nanoparticles can exhibit dynamic adsorption of various soluble molecules from biological media that may facilitate or inhibit their endocytic uptake processes, modulate their catalytic activity, and affect their subsequent cellular responses.

Environmental particles can initiate catalytic reactions and act as reactive agents during interactions with cells and tissues, while the role of such particle-catalyzed reactions during biological interactions remains poorly understood. Another important aspect is the recognition of environmental nanoparticles as bioactive particles that might directly influence biological functions. Based on these aspects, it is crucial to consider the apparent dimensions, distribution, and surface charge of environmental nanoparticles in biological media, as well as the catalytic activity and bioactivity of these particles, when investigating their probable interactions with cells [18, 19, 20].

Current Knowledge Gaps and the Need for Cellular-Level Research

Despite the growing awareness of microplastics as a new class of environmental pollutants, their hazardous potential at higher doses has not been reflected in environmental and health regulations. Notably, the lack of knowledge about the cellular

effects of unmodified microplastics, especially smaller nanoplastics, constitutes a large knowledge gap that hinders risk assessment. Traditional toxicology studies have focused on the effects of bulk microplastics, often using millimeter- or micron-sized particles, with nanoplastic effects being less explored. Investigation of microplastics smaller than 1 μm, as well as the smallest subset of environmental pollutants that have escaped the filtration capabilities of wastewater treatment facilities, is crucial considering that the majority of bioavailable airborne-sized primary microplastics worldwide is nanometric and smaller. The limited evidence of microplastic-induced biological effects at the cellular level calls for integrated approaches using cell culture models to generate a comprehensive effects database that will facilitate the construction of predictive in silico models.

A growing body of literature has demonstrated that environmental micro- and nanoplastics induce oxidative stress, inflammation, mitochondrial dysfunction, endoplasmic reticulum (ER) stress, immune dysregulation, endocrine disruption, and associated epigenetic alterations at exposure concentrations that may realistically occur in the environment. Such cellular responses are further linked to metabolic disorders such as insulin resistance, dyslipidemia, atherosclerosis, obesity, hypertension, and diabetes-associated cardiovascular risks. It is now vital to clarify and integrate the implicated mechanisms across all of these pathways and diseases in order to provide a better understanding of the health effects of environmental micro-and nanoplastics and to identify cellular targets for therapeutic intervention [21, 22, 23].

Chapter - 2

Cellular Uptake Pathways of Micro and Nanoplastics

Plastic particles smaller than 5 mm are classified as microplastics, while those smaller than 1 micron are termed nanoplastics. Microplastics can be ingested, inhaled, or injected through parenteral medical procedures. Deposition in various tissues and organs has been demonstrated in animal experiments using microplastic concentrations commonly detected in environmental samples. Tissue concentration studies confirm potential allergens in lungs. Plastic residue leaching into water, food, and beverages can trigger an inflammatory response or oxidative stress and promote disease development. Plastic particles or chemical additives are incorporated into healthy cells, causing cellular dysfunction. As a consequence, microplastics interact with mammalian cells, including oral, respiratory, intestinal, liver, and blood vessel endothelial cells.

Microplastics enter cells through endocytosis, phagocytosis, and macropinocytosis; uptake pathways vary by particle size and shape. Particles >10 μm typically enter through phagocytosis. Endocytosed particles are transported into the cytoplasm, where they provoke oxidative stress and activate apoptotic regulatory factors. Alterations in endosome morphology and transport impair exosome secretion, and congestion in the endoplasmic reticulum interferes with lysosomal function. Transport across epithelial and endothelial barriers occurs via paracellular routes of small particles and transcytosis of larger particles. Within the

cytoplasm, internalized microplastics are trafficked to lysosomes, mitochondria, or the Golgi apparatus. Imaging techniques, including confocal microscopy and high-angle annular dark field scanning transmission electron microscopy, have been employed to characterize internalization pathways [24, 25, 26, 27].

Endocytosis, Phagocytosis, and Macropinocytosis Mechanisms

Uptake of micro- and nanoplastics by cells occurs through three main pathways: clathrin-mediated endocytosis, phagocytosis, and macropinocytosis. Endocytosis denotes a class of processes whereby extracellular material is internally sequestered within cytoplasmic vesicles formed as a result of membrane invagination. The most widely studied pathway of endocytosis is mediated by the small enzyme clathrin. Many endocytic receptors, located in well-defined membrane regions termed coated pits, bind their specific ligands associated with clathrin adaptors, triggering invagination. Clathrin-and actin-dependent scission takes place at the coated pits membrane, leading to the formation of endocytic vesicles.

Clathrin-independent endocytic pathways have also been reported, although their specific physiological functions are not well understood. These pathways operate through caveolae or lipid rafts and require the function of dynamin, an important component of intracellular membrane fission. Phagocytosis is a specialized actin-dependent endocytic mechanism used primarily by phagocytic cells of the immune system to engulf larger particles such as microbes or apoptotic cells. Upon initiation, membrane protrusions become extended around the particle, followed by membrane fusion to fully internalize the deposited cargo within a phagosome. Macropinocytosis is initiated through the actin-coordinated formation of macropinosomes, large

membrane-bound vacuoles capable of engulfing bulk extracellular fluids as well as solutes and particles. The hallmark of this process is the actin-dependent ruffling of the plasma membrane [28, 29, 30, 31].

Role of Particle Size, Shape, and Surface Charge

While microplastics pose health hazards, all aspects of their cellular biological interplay are poorly understood, especially those linked to internalization. Four major endocytic processes—endocytosis, phagocytosis, macropinocytosis—are chiefly responsible for cellular uptake. Internalization routes depend on particle size, shape, and surface charge, which often dictate nanoparticle internalization pathways in other settings, although data specifically addressing microplastics are sparse. Moreover, the biological environment affects physiological functions in complex combinations, requiring consideration of physically and chemically heterogeneous particle mixtures. Alternative transport mechanisms can also bypass endocytosis, targeting intercellular junctions for paracellular passage or utilizing active transcytosis. After internalization, microplastics enter early endosomes that fuse with lysosomes or advance along the recycling pathway.

Endocytosis refers to the uptake of materials into the cell surrounded by a patch of plasma membrane, resulting in the internalization of a small membrane-bound vesicle called an endosome. The two best-characterized endocytic pathways are Clathrin-mediated endocytosis (CME) and Clathrin-independent endocytosis (CIE). CME is mainly triggered by specific ligands recognized by cluster-dynamic receptors in membrane valleys enriched with Clathrin-coated pits. CIE is initiated by actin cytoskeleton remodeling and involves membrane protrusions and caveolae rafts. Phagocytosis is defined as the cell membrane extending cytoplasmic protrusions called pseudopodia that engulf larger solid particles (0.25-20 μm) to form large

phagosomes. Mammalian phagocytic cells, including macrophages, neutrophils, dendritic cells, and some epithelial cells in the lungs, intestines, and reproductive tract, have developed this mechanism to eliminate pathogens and cellular debris [32, 33, 34].

Transport across Epithelial and Endothelial Barriers

Epithelial and endothelial cells represent the first line of defense against pollutants, serving as essential barriers of the lung, gastrointestinal tract, and blood vessel walls. These polarized monolayers facilitate ion, small molecular, and fluid transport by transcellular or paracellular routes. Transcellular transport occurs through selective channels and transporters, while paracellular transport is regulated by intercellular junction proteins that maintain a size- and charge-selective barrier for solutes. Upon reaching these barriers, micro- and nanoplastics may translocate into sensitive tissues by transcytosis or transcellular passage, potentially inducing cell stress or triggering other pathogenic cascades.

The participation of epithelial and endothelial cells in the internalization of micro- and nanoplastics has been documented and organ-specific differences identified. For example, normal human bronchial epithelial cells exposed to polyethylene (100-nm) and polystyrene (500-nm) nanoplastics elicited increased expression of the small GTPase Rap1A involved in cytoskeletal dynamics during apical-to-basolateral transcytosis. Transcytosis was also confirmed in bronchial epithelial cells transfected with shRNA against Rap1A, notwithstanding a detrimental effect on cell viability and barrier integrity. Additional studies showed that polystyrene nanoplastics (50 and 220 nm) traverse human lung adenocarcinoma A549 and human intestinal Caco-2 monolayers via clathrin- and caveolae-mediated endocytosis, with 50-nm particles also employing macropinocytosis [24, 35, 36, 37].

Intracellular Trafficking and Organelle Targeting

After particle internalization, microplastics can potentially move to various intracellular compartments (Fig. 10). Below are representative data for lysosomal localization reflecting the presence of acidic vesicles. Organelle-targeting studies account for distribution in organelles such as endosomes, mitochondria, Golgi apparatus, and endoplasmic reticulum, and make use of colocalization⁄ co-localization with organelle-specific and membrane-permeable fluorescent probes⁄ff ⁄. Emerging techniques enable tracking of intracellular microplastic dynamics in live cells, revealing the effects of microplastic exposure on intracellular organelles of epithelial cells lining the lungs, gut, and blood vessels.

Ultrasmall nanoplastics administred in drinking water readily penetrate the epithelial barrier and reach deeper lung tissue, raising concerns for lung and systemic health. Gram-positive bacterial peptidoglycan sacculi, structural macromolecules constituting the cell wall of most bacteria in nature, appear to be efficient nanovectors for the translocation of conjugated molecules into human cells. Once encapsulated and internalized by human intestinal epithelial Caco-2 cells, sacculus-shortened polypeptide conjugates transmit the attached cargo into the Caco-2 cells cytosol, generating a higher level of optical signal in comparison with unconjugated cargo. After the endocytotic uptake, polypeptide and hapten molecules travel through the endosomal/lysosomal pathway, initially colocalizing in the acidic compartment before being released from the shorted peptidoglycan sacculus carrier into the cytosol. Further support for efficient cellular delivery is provided by the detection of a humoral immune response against the conjugates in animal modelsfollowed by two-photon microscopy imaging of the intestine, lymph nodes, and visceral organs [38, 39, 40, 41].

Methods for Imaging and Tracking Microplastic Internalization

Continued investigation of cellular interactions with MP/NP will provide the fundamental knowledge needed to address their health issues adequately. Several methodologies can be utilized to demonstrate the uptake of micro- and nanoplastics by different cell types. Light and confocal microscopy, electron microscopy, flow cytometry, atomic absorption, and Raman and transmission electron spectroscopies all provide valuable information on internalization and distribution. These methods are being increasingly enhanced with state-of-the-art techniques and powerful imaging equipment, allowing for real-time observation of MP/NP endocytosis and organelle targeting. Synthetic biology-based advances enable the monitoring of MPs' and NPs' internalization in living organisms.

Confocal microscopy, using fluorescent message or probes (e.g., lipophilic dyes and inclusion tags), provides the finest spatial resolution for quantitative imaging at a high depth of penetration. The use of fluorescently labeled MPs or NPs makes confocal microscopy suitable for studying uptake kinetics and MP/NP localization in primary cells. Flow cytometry is a rapid and sensitive tool for quantifying the internalization and biodistribution of fluorescently labeled particles at a single-cell level. Other approaches involve the quantification of additives leaching from plastics or the detection of the elemental composition of MPs and NPs in biological samples using inductively coupled plasma optical emission spectrometry with sector field mass spectrometry (ICP-OES-SFMS), Fourier transform infrared microspectroscopy (FT-IR/μ-FT-IR), scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDX), and Raman spectroscopy [42, 43, 44].

Chapter - 3

Oxidative Stress Mechanisms Triggered by Microplastics

Microplastic exposure induces ROS formation and triggers cellular oxidative stress. Increased ROS levels overwhelm the antioxidant defense, manifesting as lipid peroxidation, DNA oxidative damage, and redox imbalance in exposed cells. Microplastics may generate ROS via different pathways, with mitochondria being considered the major source under prolonged exposure. Impaired mitochondrial function exacerbates ROS production, thereby contributing to cellular oxidative damage. Such damage is often accompanied by impaired DNA repair and considered as a hallmark of both DNA damage and aging.

In toxicity studies, elevated ROS levels and disrupted activity of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase are widely used as indicative biomarkers of redox imbalance in microplastic-exposed cells. ROS-induced lipid peroxidation can also be evaluated by measuring the levels of malondialdehyde, 4-hydroxy-2-nonenal, acrolein, and lipid peroxidation adducts. Other biomarkers include PTEN-induced putative kinase 1, chemokine ligand 2, superoxide enozidase 1, nuclear factor erythroid 2-related factor 2, heme oxygenase-1, 8-hydroxydeoxyguanosine levels, and radical cation probes. Cellular repair responses can be assessed by determining γH2AX focus formation and expression levels of DNA repair-related genes [45, 46, 47, 48].

ROS Generation and Antioxidant System Disruption

Extensive research has demonstrated that microplastics and

their additives can induce cellular oxidative stress and mitochondrial dysfunction. Microplastic particles act as catalysts of redox-active transition metals or generate reactive oxygen species (ROS) via mitochondrial electron transport chain (ETC) impairment, xanthine oxidase activation, and NADPH oxidase stimulation, leading to redox imbalance. Functional studies reveal that microplastic-induced mitochondrial ROS production drives necrosis and pyrototic cell death through activation of the RIPK1/RIPK3 signaling pathway. In addition, excess lipid peroxides can overwhelm antioxidant defenses and compromise cellular-redox homeostasis and polyunsaturated-fatty-acid metabolism. Microplastic-induced ROS production also impairs DNA-repair capacity by hampering homologous recombination, whereas hydrogen peroxide and lipid-peroxidation products activate nuclear factor erythroid 2-related factor 2 (Nrf2) transcriptional activity and cytoprotective pathways in microplastic-exposed cells.

The generation of inflammatory ROS from innate immune sensors and elevated mitochondrial ROS levels can further promote microplastic-triggered apoptosis and pyroptosis. Prolonged activation of antioxidant defense mechanisms in response to excess oxidative stress can ultimately lead to redox-related metabolic disorders and harmful effects at the organism level. To ensure the fidelity of data derived from microplastic-related oxidative studies, researchers recommend routinely monitoring the levels of superoxide anion, lipid peroxidation products, and glutathione, as well as conducting cellular functional assays for polyunsaturated-fatty-acid metabolism and homologous recombination in cells exposed to these environmental pollutants [49, 50, 51, 52].

Mitochondrial ROS vs. Extracellular ROS Pathways

In addition to ROS generation by mitochondria, microplastics

can induce oxidative stress through the production of extracellular ROS. For example, Huang *et al*. showed that polystyrene nanoplastics interact with metal ions (Fe^{2+} , Co^{2+} , and Cu^{2+}) in the environment, thereby facilitating the Fenton reaction and subsequent generation of highly toxic hydroxyl radicals. These radicals can decompose mitochondrial DNA and RNA, leading to organelle dysfunction and apoptosis. Chen *et al*. demonstrated that polystyrene microplastics promote the generation of extracellular ROS in THP-1 macrophages, enabling the formation and release of pro-inflammatory factors and cytokines (IL-6 and IL-1β) via NLRP3 activation. Zhang *et al*. reported that polystyrene microplastics stimulate RAW264.7 macrophages to release significant levels of extracellular ROS, while the application of reduced glutathione mitigates lipid peroxidation and the subsequent activation of the NLRP3/caspase-1 pathway.

Alterations in the redox status of the cell also influence the activity of antioxidant enzymes. Pal *et al*. found that polystyrene nanoplastics triggered a significant reduction in the activity of superoxide dismutase, catalase, and glutathione peroxidase over time, while levels of malondialdehyde - a marker of lipid peroxidation - increased. Their study, conducted in a gut epithelial cell line, suggests that oxidative stress might contribute to microplastic-initiated intestinal barrier disruption and microbe translocation. Furthermore, Zhang *et al*. reported that polystyrene nano- and microspheres induced cytotoxicity and DNA damage in A549 cells, likely due to significant lipid peroxidation and inhibition of superoxide dismutase and glutathione peroxidase. A recent systematic review concluded that microplastics can alter the redox status and antioxidant responses in different cell types, although the specific mechanisms remain poorly characterized [53, 54, 55, 56].

Lipid Peroxidation and Membrane Damage

Aberrant accumulation of ROS initiates diverse downstream

signaling pathways and cellular dysfunctions in response to microplastics. Cellular membranes are highly susceptible to oxidative damage, mostly via lipid peroxidation of polyunsaturated fatty acyl moieties in phospholipids. Besides directly serving as reactive substrates, lipid peroxides induce cytotoxicity by forming highly reactive secondary products, including 4-hydroxynonenal, which dysfunctionally regulate transmembrane proteins and ion channels. Emerging evidence shows that microplastics can induce lipid peroxidation and resultant membrane damage in different cell types, with implications for their biological responses.

Lipid peroxidation from oxidative stress has recently been recognized as a hallmark of microplastic exposure. The sensor protein "sweet taste receptor" (T1R) senses lipid peroxides and activates PKCδ, leading to NLRP3 inflammasome activation in a TLR4-independent manner. During exposure to polystyrene particles, cytotoxic secondary aldehydes are released and exacerbate airway inflammation. Moreover, various NPs, including silver, titanium, and silica NPs, induce peroxidative damage in the nuclear membrane. When cells are incubated with NP- and/or virus-loaded serum, damage to the viral envelope and cellular membrane enhances infectivity. Collectively, membrane lipid peroxidation presents a crucial mechanism of microplastic stimulation [57, 58, 59, 60].

DNA Oxidative Damage and Repair Inhibition

Microplastic exposure leads to significant oxidative stress, resulting in DNA damage and the inhibition of DNA repair mechanisms. Increased levels of 8-hydroxy-deoxyguanosine (8-OHdG)—a marker of oxidative damage to DNA—and decreases in 8-oxoguanine-DNA glycosylase (OGG1) expression associated with minor DNA repair activity are observed in exposed organisms. 8-OHdG levels correlate positively with

markers of lipid peroxidation and negatively with antioxidant enzyme activity, supporting the assessment of DNA oxidative damage in environmental samples.

Microplastics upregulate cyclooxygenase 2 (COX2) and hinder the expression of key proteins involved in DNA repair and replication, including OGG1, proliferating cell nuclear antigen (PCNA), and RAD51. OGG1 directly participates in the repair of 8-OHdG, while changes in COX2 are linked to the modulation of the cell cycle. The biota compromised by microplastic exposure exhibits clear signs of DNA distortion and potential methylation disturbance. Microplastics enhance the levels of DNA strand breaks in exposed fish, cats, rodents, and Xenopus laevis embryos and intercalate into the DNA of HepG2 cells *in vitro*. These effects may ultimately determine the risk of genetic mutations and cytotoxicity [61, 62, 63, 64].

Cellular Biomarkers of Redox Imbalance

Microplastics play an essential role in cellular redox imbalance, which is the disequilibrium between the production of reactive species and the clearance ability of antioxidant systems. Microplastics can induce redox dysregulation mainly through oxidative stress initiation or exacerbation, although other perturbations (e.g. via mitochondrial dysfunction) also contribute to cellular redox disturbances.

The main indicators of the imbalance are the increased levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS), alterations in the antioxidant system, lipid peroxidation, oxidative damage to DNA, and the impairment of DNA repair mechanisms. A variety of molecular probes have measured the disturbance by monitoring the levels of these species or their effects on other biomolecules. Specific gene expression patterns can also serve as cellular signatures of redox dysregulation or, more narrowly, oxidative stress [65, 66, 67].

Chapter - 4

Inflammation and Immune Dysregulation

Microplastics initiate robust inflammatory responses in various cell types, tissues, and animal models, resembling a cytokine storm and chronic low-grade inflammation. Although the engaged inflammatory and immune responses play a critical role in protecting against microbial invasion, persistent activation of innate immune sensors such as Toll-like receptors (TLR2, TLR4) and the NLRP3 inflammasome can lead to immune dysregulation and serve as a bridge linking inflammation with numerous metabolic disorders. Moreover, the rapid uptake of microplastics by macrophages can overload the cells' capacity to eliminate them, resulting in dysfunctional and immunosuppressive phenotypes, release of pro-inflammatory mediators, and crosstalk with metabolic pathways. Microplastics can also act as sorbents of toxic chemicals, pathogens, and their secreted virulence factors, which further activate innate immune responses and promote inflammation-associated metabolic dysfunction.

Microplastic-associated toxic chemicals such as bisphenol A, phthalates, and per- and polyfluoroalkyl substances (PFAS) possess adjuvant effects that can foster the development of food allergy, asthma, and autoimmune disease. Furthermore, non-chemical aspects of microplastics, such as size, shape, surface features, and biofilm formation, are also acknowledged determinants of innate immune activation. As the immune and metabolic systems are closely interconnected, it is essential to

elucidate the potential crosstalk that underpins such interactions, as this could provide novel therapeutic avenues to curb the metabolic diseases promoted by microplastic exposure [68, 69, 70, 71].

Activation of Innate Immune Sensors (TLRs, NLRP3 Inflammasome)

Innate immune sensors recognize microplastics and activate the immune system. Endosomal toll-like receptors (TLRs), especially TLR3, TLR7, and TLR9, recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) and are upregulated in cell line studies and upon exposure in mice and humans. Plastic, organic pollutant, and pathogen leaching from microplastics increases Toll-like receptor messenger RNA and protein levels. Clearing agents targeting TLR3 and TLR9 confer strong protection against microplastics, implicating endosomal TLRs in eliciting microplastic toxicity. Other sensors, such as stimulator of interferon genes (STING) and the nucleotide-binding domain and leucine-rich-repeat-containing protein 3 (NLRP3) inflammasome, are also activated, triggering pro-inflammatory cytokine production.

The long-term sustained activation of African green monkey kidney (Vero), murine macrophage (RAW264.7), mouse bone marrow-derived dendritic (BMDC), and human peripheral blood mononuclear (PBMC) cells by shopping-bag plastic was addressed in a 14-day exposure study. Plastics activate aggrecan expression via toll-like receptors and nuclear factor kappa B in chondrocytes. Exosomes derived from microplastic-exposed Vero cells induce inflammatory responses, colonic injury, and tumorigenesis in mice. Further investigation in live zebrafish confirmed that microplastic-derived exosomes exert pro-inflammatory and cytotoxic effects. Together, these findings

highlight innate immune mechanisms of microplastic toxicity [72, 73, 74].

Cytokine Storms and Chronic Low-Grade Inflammation

Microplastic exposure can elicit a wide range of pro-inflammatory cytokines across different cell types, leading to local inflammation that resembles a cytokine storm. Many cytokines, such as IL-1, TNF-α, IL-6, IL-8, and the type I interferons, contribute to the initiation and development of atherosclerosis. Clinical studies have linked increased cytokine levels with high PL concentration, suggesting that chronic low-grade inflammation may serve as a pathway through which PL exposure induces metabolic disorders.

Research into the interactions between endotoxins and PL is limited. However, the existing literature indicates microplastics as carriers for gut bacteria and their toxins. Additionally, direct exposure to PL may dysregulate gut homeostasis, resulting in microbiota dysbiosis, increased gut permeability, and bacterial translocation into the circulation, which induce an inflammatory response. Disruption of the gut barrier may also enhance the translocation of bacteria and their toxins via the circulatory system, further disturbing immune and metabolic homeostasis [13, 75, 76, 77].

Immune Cell Dysfunction and Immunosuppressive Effects

Two lines of evidence support microplastics as modulators of immune cell function, with an emphasis on their immunosuppressive role. Firstly, the innate immune response is characterized by increased expression of pro-inflammatory cytokines and chemokines, which recruit and activate platelets and leukocytes. No alterations are observed in the adaptive immune response, as the production of IgG and IgM antibodies remains unperturbed during microplastic exposure. Secondly, in addition to their capacity to elicit innate sensor activation,

microplastics emerging as modulators of immune cell function owing to their surface chemistry. At high concentrations, they induce cytotoxicity and apoptosis, while lower doses impair the phagocytic ability of macrophages and the migration response of neutrophils. Indeed, nephrotoxicity and an alteration in macrophage polarization status during microplastic exposure highlight the importance of evaluating immune responses.

The role of immune cells in response to microplastics is therefore not limited to innate sensors. They are also regulated by the interactions of microplastics with the glycan surface, modulating the activation of Toll-like receptors or promoting an immunosuppressive milieu. Microplastics can also dysregulate the functional response of immune cells. Macrophage and monocyte polarization kinetics may be altered after exposure to microplastics, leading to impaired phagocytosis and altered oxidative burst response. Disturbances of these functions may consequently affect physiological responses, such as tissue homeostasis, angiogenesis, and acute immune responses, and promote the development of chronic diseases [78, 79, 80, 81].

Crosstalk between Immune and Metabolic Pathways

Crosstalk between the immune system and metabolism has gained research interest due to the capacity of innate immune cells to secrete bioactive molecules that influence multiple metabolic processes in obesity and T2DM. For instance, pathogen-associated molecular patterns from infectious agent recognition by innate receptors such as Toll-like receptors (TLRs) can directly activate insulin receptor substrate 1 (IRS-1) serine phosphorylation in human skeletal muscle, inducing insulin resistance and contributing to type 2 diabetes development.

Similarly, metabolic syndrome risk factors can stimulate immune cells to release proinflammatory cytokines such as

interleukin (IL)-6 and tumor necrosis factor-alpha (TNF-α), and in turn, these immune mediators contribute to systemic low-grade inflammation, augmenting metabolic disorders. Conversely, microplastics can also concentrate numerous pathogenic agents and chemical pollutants capable of inducing viral, bacterial and parasitic infections, and thus promote immune activation and imbalances. Long-term and chronic systemic dysregulation of both immune and metabolic pathways might contribute to increasing metabolic syndrome records worldwide, and in particular those diseases related to adult obesity and associated comorbidities.

Role of Microplastics as Carriers for Toxic Chemicals and Pathogens

Microplastics and nanoplastics are environmental carriers of toxic compounds, loaded with persistent organic pollutants (POPs) and heavy metals from the nearby environment or adsorbing/orignating pathogens or toxic bacteria. These harmful additives can leach into body fluids, tissues, and organs, where they can modulate hormonal functions and induce inflammatory responses. Microplastic and nanoplastic-persistent organic pollutants (POP) interactions in animal models further confirm that these particles can carry and deliver POPs into animals and humans.

Plastics are not only susceptible to absorb toxic chemicals, but can also be colonised by pathogenic agents, exerting further hazards to human and animal models. Bacteria such as Escherichia coli and others, isolated from microplastics, can cause deleterious effects on the exposed organisms. Alteration of gut microbiota harmonics also increase the toxic impacts of microplastics. Shewanella spp. are bacteria with the capacity to transfer and propagate resistance or virulence gene into other members of the microbiota, causing a higher damaging degree on mammals. These interactions are important because changes in

microbiota composition and functions may also enhance the overall harmful effects induced by microplastics on mammals [82, 83, 81, 84].

Chapter - 5

Mitochondrial Dysfunction-The Energy Crisis

Compromised mitochondrial activity and energy depletion represent key hallmarks of cellular stress triggered by microplastic exposure. Mitochondria are double-membraned organelles found in nearly all eukaryotic cells involved in the intracellular production of adenosine triphosphate, acting as the main source of chemical energy for cellular homeostasis. Mitochondria generate energy through the synthesis of ATP in a process called oxidative phosphorylation (OXPHOS). This process occurs chiefly in the inner mitochondrial membrane and is fueled by an electrochemical gradient generated with the reduction of oxygen at the end of the electron transport chain (ETC), the key metabolic pathway for OXPHOS. Mitochondria also provide numerous other essential cellular functions, including redox homeostasis, thermogenesis, biosynthesis of central metabolic intermediates, and regulation of apoptosis. Given the multitude of actions played by mitochondria, it is difficult to identify a specific mitochondrial function that has been selectively modulated in response to microplastic exposure.

Experimental studies have demonstrated that microplastic exposure impairs the capacity of mitochondria to generate ATP. Several groups have defined diminished ATP levels (directly or indirectly) as a consequence of exposure to microplastics and nanoplastics. Particularly, Huang *et al*. showed that exposure to nanoplastics derived from polystyrene resulted in a downregulation of both ATP production and ATP levels in the Caco-2 intestinal epithelial cells. Decreased ATP levels

associated with mitochondrial dysfunction have been observed in cellular models of colorectal and breast cancer exposed to nanoplastics [85, 86, 87, 88].

Disruption of Electron Transport Chain Function

Mitochondrial dysfunction is often defined as the impairment of organelle integrity or bioenergetics and can begin with electron transport chain (ETC) failure. An increasing number of studies report altered expression of key mitochondrial components and major respiratory complexes in cells treated with microplastics. The initiation of mitochondrial dysfunction was first detected in a high glucose-treated HK-2 cell line and subsequently confirmed in human primary macrophages exposed to polystyrene, natural-source and oxidized polystyrene nanoplastics. Notably, the expression levels of 40 mitochondrial-related proteins were significantly altered, as revealed by a proteomics approach. Losses in the levels of mitochondrial proteins that participate in the ETC and oxidative phosphorylation were specifically linked to mitochondrial dysfunction, as validated using a Seahorse XF Analyzer. Both siRNA transfection and the application of the metabolic modulator metformin confirmed the essential role of mitochondrial dysfunction in polystyrene and microplastic-induced insulin resistance in human umbilical vein endothelial cells.

Nanoplastic-induced ETC disruption has been implicated in hUVC-induced cytotoxicity, wherein metabolic damage progressed regardless of continued nanoplastic exposure. In the absence of inefficient autophagic degradation, suboptimal adaptive thermogenic responses suppressed nitric oxide production and insulin resistance in 3T3-L1 adipocytes treated with 350 nm polystyrene nanoplastics. During senescence, unexplained decreases in body weight coincided with disrupted

temperature homeostasis and drainage of brown adipose tissue, and behavioral assays suggested dysregulated thermoregulation in trials employing 3D-printed polystyrene microplastic beads. Persistent small intestine exposure in a zebrafish model was also sufficient for subsequent reduction of heart, skeletal muscle, and liver fatty acid oxidation, a deficit attributed to accumulated lipid droplets in the muscle and small intestine.

Altered ATP Production and Bioenergetic Collapse

Mitochondrial dysfunction leads to a decrease in ATP production and an energy crisis in cells. Changes in mitochondrial function are mirrored by altered cellular bioenergetics. A drop in the ATP/ADP ratio triggers a compensatory increase in glycolysis via activation of the hypoxia-inducible transcription factor (HIF). Microplastics can cause changes in bioenergetics through their mitochondrial effects. In primary cultured murine cardiac microvascular endothelial cells exposed to PS beads, the ATP content was diminished, while control cells showed a HIF1-α-dependent increase in glycolysis. This dysfunction was attributed both to alterations in the mitochondrial membrane potential and to the impairment of the glycolytic enzyme phosphofructokinase-1 (PFK-1). A study in cardiac myocytes reported a reduced ATP/ADP ratio and a lower glycolytic capacity, with downregulation of hexokinase-2 (HK2) and PFK-2.

Dynamic mitochondrial balance—including fission, fusion, and mitophagy—is crucial for preserving bioenergetics and cell function. PS nanoparticles cause an imbalance favoring mitochondrial fragmentation, contributing to metabolic dysfunction. PS nanoplastics induce glucose metabolism changes, including reduced ATP/ADP ratios, impaired glycolysis, and decreased glucose reserves, through suppression of HK2, PFK-1, and glycogen phosphorylase. In primary rat

cardiomyocytes, PS particle exposure results in suppression of glycolysis, attributed to inhibition of HK2 and PFK-1 activity. Adjustment of bioenergetics—isolation of lipid droplets through the peroxisomal system—is altered upon PS bead exposure in macrophages.

Mitochondrial DNA Mutations and Stress Responses

A wide spectrum of mitochondrial DNA (mtDNA) lesions facilitates the response of cells to oxidative and environmental stresses. Notably, mtDNA mutations and changes in mitochondrial gene expression induced by exogenous stimuli provoke a failure of net replication and can lead to mitochondrial dysfunction and cellular transformations associated with aging. Radicals generated by environmental stresses, including exposure to microplastics, directly and dynamically alter mitochondrial epigenetics, whereas mtDNA mutagenesis may trigger additional mtDNA modifications and dysfunctions. The variety of mtDNA lesions induces distinct uncoordinated and co-regulatory aberrations of Sp1, TFAM, and MTFP1 involved in mitochondrial activity and maintenance.

Environmental stimuli associated with the presence of microplastics and other pollutants can lead to excessive production of reactive oxygen species (ROS), which are involved in the dynamics of cellular life and death. ROS are also implicated in pathological alterations in the cellular microenvironment and can exert both damaging and regulatory effects on biological processes, including those involved in tumor promotion. Radicals generated upon environmental stimuli can directly damage mitochondrial genomes, leading to mitochondrial DNA (mtDNA) mutations. The nature of mtDNA lesions influences the net replication rate and expression of mitochondrial genes, and these alterations can stimulate mitochondrial maintenance responses. However, persistent

environmental damage may induce net mtDNA loss and affect cellular homeostasis. A wide spectrum of mtDNA lesions can indeed facilitate the response of cells to oxidative and environmental stresses [85, 89, 90].

Mitophagy Impairment and Dynamics Imbalance

Aberrant mitophagy induces dysfunctional mitochondria accumulation and promotes metabolic disorders in response to external stressors. Mitophagy is the selective removal of damaged mitochondria via autophagic mechanisms and is crucial in maintaining mitochondrial homeostasis. In addition to mitochondrial quality control, normal mitochondrial dynamics and a balanced fission-fusion process are necessary for maintaining mitochondrial function. Mitochondrial fission mediates the division of mitochondria into smaller organelles and is involved in several cellular processes such as apoptosis, cell cycle progression, and distribution of mitochondria during mitosis. Mitochondrial fusion promotes the mixing of mitochondrial contents and functions to maintain normal mitochondrial membrane potential, redistribute mitochondrial DNA, and promote ATP production, which are essential for cells under energy stress or apoptosis. Importantly, disruption of normal mitochondrial dynamics is closely related to various metabolic disorders.

Compromised mitophagy has been implicated in alterations of lipid metabolism and obesity. Reduced Parkin expression leads to the accumulation of dysfunctional mitochondria in mouse adipocytes, resulting in reduced cellular free fatty acid uptake, decreased differentiation, and altered lipolysis. The crosstalk between damaged mitochondria and methyl palmitate has been revealed in 3T3-L1 cells, where methyl palmitate-induced inhibition of mitophagy contributes to dysfunctional mitochondria accumulation, leading to lipotoxicity and insulin

resistance. Furthermore, the decrease of Sirt3, a protein that plays a critical role in oxidative metabolism, activates the aberrant accumulation of damaged mitochondria in adipose tissue, promoting inflammation and dyslipidemia, as well as exacerbating the insulin resistance process in mice. Ectopic fat deposition acts as an age-related risk factor for obesity-associated insulin resistance by disrupting mitochondrial homeostasis. Long-term excess free fatty acid accumulation disturbs mitochondrial dynamics and consequently activates the UPRmt pathway; however, the imbalance of mitochondrial dynamics counteracts the protective effect of the UPRmt pathway and contributes to insulin resistance. Lastly, the environmental endocrine disruptors bisphenol A and bisphenol S induce abnormal lipid metabolism in 3T3-L1 adipocytes via changes in mitochondrial dynamics and aberrant autophagy [91, 92, 93].

Mitochondria-Driven Metabolic Reprogramming

The mitochondrial connection with metabolic disorders is of crucial importance, as oxidative phosphorylation is the main ATP-producing pathway used by most differentiated cells and tissues. Thus, mitochondrial dysfunction is linked to a variety of metabolic diseases, including diabetes. Microplastics can induce mitochondrial dysfunction through several mechanisms, including inhibition of chemiosmotic coupling, alteration of mitochondrial dynamics, and transcription of mtDNA, among others. Such dysfunction may ultimately contribute to impaired insulin secretion and the development of insulin resistance.

Microplastic exposure triggers accumulation of mitochondrial mass and fragmentation. Imbalances in the rates of mitophagy and mitochondrial biogenesis underlie such accumulation, which is commonly observed in aged tissues and in conditions of energy deficit. In these situations, cells compensate for the loss of mitochondrial function by increasing

mitochondrial numbers. Nonetheless, higher mitochondrial numbers do not prevent respiratory dysfunction, as supporting mitochondrial operation becomes an excessive energy burden leading to progressive proteotoxic and bioenergetic stress [94, 85, 95, 96].

Chapter - 6

ER Stress and Unfolded Protein Response (UPR)

An accumulated burden of misfolded proteins due to exposure to environmental contaminants, coupled with an overload of damaged organelles, overwhelms the ability of the cellular machinery to restore balance, resulting in disruption of proteostasis control. While UPR activation initially helps restore normal protein homeostasis through concerted action of the three branches, persistent ER stress leads to cellular dysfunction, and ultimately culminates in cell death. Under such conditions, cross-talk with different signaling pathways such as the oxidative stress pathway acts as a signaling amplifier and accelerates cellular demise.

During metabolic syndrome development, UPR is frequently associated with altered protein expression of the three branches as well as with dysregulated genes associated with autophagy and with the generation of ROS. Both dysregulation of the UPR and disruption of lipogenesis and lipolysis hallmark metabolic disease. As a potent constituent of the UPR, the IRE1α-XBP-1 axis is essential for glucose homeostasis and the absence of IRE1α aggravates insulin resistance, associated with impaired autophagy and dysregulated expression of inflammation-related genes in adipose tissues. Modulation of UPR activity may represent a unifying principle to counteract cellular aging and the development of age-related diseases [97, 98, 99, 100].

Interference with Protein Folding and Quality Control

Microplastics can disrupt the normal folding of proteins,

triggering cellular responses similar to those seen in the accumulation of misfolded proteins associated with neurodegenerative diseases. The cytosolic transmembrane protein Hsp70 interacts with the endoplasmic reticulum (ER)-localized transmembrane protein Grp78/BiP to maintain proteostasis in the ER while preventing the aggregation of denatured proteins in the cytosol. In response to microplastic-induced cellular stress, these scaffolding proteins relocalize to distinct cytoplasmic foci or mislocalize to cytosol-enriched subcellular factions where the expression level of their respective encoding genes is enhanced. The activation of the protein kinase R-like ER kinase (PERK) pathway, along with diminished protein disulfide isomerase (PDI) activity, further supports the speculation that microplastics interfere with protein folding and endoplasmic reticulum quality control activity. Levels of HSP70- mediated assembly and disassembly of the HSF1 transcription factor complex reflect the cellular redox environment, and its translocation can be perturbed by strong oxidative stress. Proteins that require reductants for correct folding, such as PDI and some substrates of the thiosulfate reductase, are potential targets of redox imbalances. These observations place microplastics as disrupting cellular protein folding and quality control and thereby advancing a risk assessment strategy for transgenerational disturbances in protists that challenge human health.

Disruption of the disulfide bonds formed during the oxidative folding of secretory and surface proteins causes a loss of protein folding competence in the ER and contributes to increased susceptibility to oxidative stress. Endoplasmic reticulum (ER) stress and the activation of the unfolded protein response (UPR) signal many forms of the response to redox imbalance. Upon exposure to microplastics, the dysregulation of the ER-localized, thiol- dependent protein chaperone Hsp47 indicates a

perturbed redox environment that might drive an imbalance of protein-folding capacity. Microplastics can drive the divergence of the UPR signal toward a cell death program. Relocalization of the redox- sensitive chaperone Grp78/BiP from the ER lumen to non- ER cellular compartments, accompanied by the spatial coordination of different cellular chaperones, supports their involvement in regulating the balance of the redox environment. Continued exposure of cells to elevated levels of hydrogen peroxide accelerates the aggregation of oxidatively damaged proteins into insoluble deposits [94, 101, 102, 103].

Activation of PERK, ATF6, and IRE1α Pathways

The aim of the unfolded protein response (UPR) is to restore endoplasmic reticulum (ER) homeostasis. While the acute response is beneficial, prolonged activation has detrimental consequences. Accumulating evidence indicates that exposure to micro- and nanoplastics triggers ER stress, activating all three UPR pathways. The inositol-requiring enzyme-1α (IRE1α) pathway is generally considered the most evolutionarily conserved, although the PKR-like ER kinase (PERK) and the activating transcription factor 6 (ATF6) pathways also play crucial roles in vertebrates. The PERK pathway phosphorylates eukaryotic translation initiation factor 2α (eIF2α), inhibiting global protein synthesis while enhancing selective translation of transcription factor ATF4, which induces the expression of pro-apoptotic CHOP and transactivator of amino acid biosynthetic enzymes. The transcription factors downstream of IRE1α perform various functions, including apoptosis, X-box binding protein 1 (Xbp1) mRNA splicing, and new ER membrane biogenesis, whereas the ATF6 pathway regulates chaperone expression and autophagy.

Microplastic-induced ER stress is involved in various human diseases. Mitochondrial dysfunction is a common hallmark of

UPR activation. The subcellular compartments are communicating organelles with established crosstalk, and experimental evidence indicates that micro- or nanoplastic exposure affects interorganellar interactions—contributing, directly or indirectly, to UPR and mtUPR amplifications—resulting in a proteostasis crisis. Impaired selective autophagy also participates in the dysfunction of both organelles and expands the proteotoxic burden in the UPR context. Finally, microplastics play a role in the development of metabolic syndrome through the UPR pathway [104, 105, 106, 94].

ER-Mitochondria Crosstalk in Stress Amplification

Mitochondria and the ER communicate extensively through distinct domains that serve as gateways for lipid transport, Ca2+ homeostasis, and metabolic signaling. Mitochondrial respiration generates protein synthesis by modulating cellular energy status, while the ER provides quality control. Mitochondria and the ER are also involved in the biosynthesis of several major compounds critical for cellular function. Under stress, proper ER function is crucial for preventing the accumulation of misfolded proteins in the secretory pathway. Conversely, dysfunctional mitochondria induce ER stress by compromising mitochondrial metabolism, depleting cellular ATP levels, altering the cytosolic redox state, releasing apoptosis-inducing factor, and activating the NLRP3 inflammasome. Microplastics interfere with mitochondrial function, thus disrupting an important signal that promotes optimal ER activity. Changes in the mitochondrial-ER interface can also initiate signaling pathways that promote the UPR, amplifying ER stress. Mitochondrial Ca2+ deficiency, resulting from the reduction of the mitochondrial Ca2+ uptake rate, disturbs the calcium gradient between the ER and mitochondria, leading to dysfunctional calcium release and mitochondrial damage. The impaired mitochondrial metabolism further increases ERand UPR-related markers, culminating in the

accumulation of misfolded proteins. Microplastics may affect mitochondrial activity and result in dysfunctional mitophagy, which, together with perturbed calcium signaling, activates PERK, IRE1, and ATF6, as well as ER stress.

Chronic ER stress can lead to an impaired ability to maintain protein homeostasis and, consequently, to the activation of the pro-apoptotic branch of the UPR and apoptosis. Persistent UPR activation, coupled with an inability to restore normal ER homeostasis, can also cause excessive mitochondrial fission, impair mitochondrial dynamics, and disrupt proteostasis. The interplay between ER stress and mitophagy has emerged as a key regulator of proteostasis, with impaired mitophagy leading to compromised mitochondrial function and global ATP depletion. Conditioning of the UPR, in particular of the ATF6 branch, optimizes the capacity for mitochondrial quality control by increasing the capacity of the mitophagic pathway. The two organelles strive to maintain proteostasis and prevent cell death in response to acute stress, and their interplay serves as a safety mechanism to counteract minor initial disturbances. When both compartments become compromised, however, the functional interaction tips from protection to pathological activation of apoptosis [107, 108, 109, 110].

Impaired Autophagy and Proteostasis Failure

In addition to the ER-mitochondria crosstalk, plastic-associated ER stress inhibits autophagic flux in cells. Zhang *et al*. demonstrated that polystyrene nanoplastics (PS-NPs) inhibit autophagic-lysosomal degradation of the unfolded overexpressed protein of the hepatocyte-selective transcription factor hepatocyte nuclear factor 4α. The authors suggested that microplastic-induced ER stress interferes with the protein degradation process during the unfolded protein response. Lin *et al*. reported that polystyrene nanoplastics (PS-NPs) inhibit autophagic flux by decreasing autophagosome-lysosome fusion

capacity in glial cells, and this impairment contributes to further neurotoxicity, hinting at the interaction between microplastic-induced ER stress and impaired autophagic process. The dysregulation of autophagy may lead to proteostasis failure.

In addition, the above studies have found that an overall naïve characteristic of PS-NPs increases the ER stress levels. A similar conclusion was made by Huang *et al*., who suggested that the overall particle naïve characteristic of PS-NPs could lead to the aggravation of ER stress in endometrial epithelial cells. Indeed, these characteristics determine the ER stress state of the cells. Active cellular ER stress is thought to be either a protective or an aggravating factor for the cellular toxicity of microplastics; nevertheless, its ultimate effects fall under the influence of multiple auxiliary factors, especially on the tissue or organ level [111, 112, 113, 105].

Role of ER Stress in Metabolic Syndrome Development

Accumulation of developing and fully folded polyproteins can occur in the ER lumen when the protein influx into the ER lumen surpasses the capacity of the cellular folding mechanisms. Furthermore, the induction of ERAD, under the control of the UPR, can occur when even a fully folded protein is misfolded. Therefore, microplastic exposure may lead to the accumulation of misfolded proteins and unassembled protein complexes, which should be considered as general ER stress responses. Human metabolic syndrome is a multifactorial disease characterized by a group of metabolic disorders, such as dyslipidemia, hypertension, insulin resistance, and fatty liver. Its occurrence not only has a close connection with genetic and environmental risk factors, but also is closely related to the onset of other diseases.

Consequently, the main risk factors for the onset of metabolic syndrome include age, sex, diet, obesity, inflammation,

endocrine dysfunction, high levels of free fatty acids, increased levels of oxidative stress, and the active expression of pro-inflammatory cytokines. Recently, the UPR has been reported to participate and play an important role in the pathogenesis of metabolic syndrome. With the continuous deepening of research, it is becoming increasingly clear that ER stress and disorders in ER homeostasis are associated with high levels of oxidative stress, the inflammatory response, dyslipidemia, insulin resistance, obesity, and its relevance. In turn, these factors can affect the stability of the ER and cause ER stress [50, 114, 115, 116].

Chapter - 7

Microplastics and Endocrine Disruption

Microplastic particles are complex environmental pollutants composed of a wide variety of polymers and additives—many of which are known endocrine-disrupting chemicals (EDCs)—with the capacity to leach into the environment and, like other plastic materials, can persist and accumulate within ecosystems. A selection of EDCs that have been frequently identified as leachates from plastic materials includes bisphenol A (BPA), phthalates, poly- and perfluoroalkyl substances (PFASs), brominated flame retardants (BFRs), polychlorinated biphenyls (PCBs), per- and polyfluoroalkyl substances (PFASs), dioxins, and heavy metals. Exposure to microplastics through air, drinking water, and the food chain has been shown to induce an elevated risk of endocrine-disrupting disease. A wealth of evidence also supports the important role of endocrine disruption in metabolic syndrome development and related diseases. Nonetheless, direct insights into the association between micro- and nanoplastic exposure and changes in endocrine hormone levels are still scarce. Furthermore, the expression levels of hormones and hormone receptors from different glands and systems also appear to be minimally explored.

More than 450 endocrine disruptors have been documented, and while guidance values for the majority of classes of these compounds remain absent, the European Chemicals Agency (ECHA) has classified a total of 62 substances as CMRs (carcinogenic, mutagenic and/or toxic for reproduction). ECHA

has also recommended that microplastics are classified as endocrine disruptors for human health and/or the environment. Given the important roles of thyroid hormones in metabolic homeostasis, abnormal levels of these hormones, receptors and synthesizing proteins can result in metabolic disturbances such as changes in mitochondrial thermogenesis, glucogenesis and gluconeogenesis, reduced lipogenesis or lipolysis in adipose tissues, and dysregulation of blood glucose and lipid metabolism [117, 118, 119, 120, 121].

Leaching of Additives: BPA, Phthalates, PFAS

Plastic materials are chemically complex because they often contain numerous additives (i.e., plasticizers, flame retardants, stabilizers, lubricants, fillers, colorants, and antimicrobials). The properties of plastics are tuned for specific purposes; for example, additives such as bisphenols, phthalates, organotins, and tris(2-butoxyethyl) phosphate confer plasticity, durability, flame resistance, and thermal resistance, respectively. PVC compounds contain more than 30% by weight of plasticizers such as di-(2-ethylhexyl) phthalate (DEHP) and dibutyl phthalate. Plasticizers are leachates that can interact with numerous cellular growth and differentiation processes. Many of these additives have obesogenic properties that may induce metabolic dysregulation via endocrine-disrupting (ED) actions.

BPA is one of the most known plastic additives, added to polymeric matrices in the cell to improve thermal stability and impact resistance. BPA is successfully incorporated not only into polycarbonate and poly(acrylonitrile-butadiene-styrene) but also into other plastics. BPA leaching was reported from baby bottles, municipal drinking waters, and thermal receipt paper. BPA is widely present in human urine and breast milk, and xenoestrogenic effects have been documented in a variety of vertebrates. It has been suggested that the possible association

between BPA exposure and obesity could be mediated by obesity-associated dysbiosis. In general, the U.S. Environmental Protection Agency (EPA) concluded that "current evidence is inconclusive for the carcinogenicity of BPA in humans." However, these volume scores do not include recent investigations indicating that the lifetime exposure of zebrafish to low-dose BPA results in metabolic diseases, obesity, reproductive system diseases, precursor lesions, and tumors by 29.3%, 22.7%, 23.8%, 25.2%, and 6.1% of the exposed fishes, respectively [122, 123, 124, 125].

Interference with Hormone Receptors and Signaling

Environmental nanoparticles have been shown to impact the endocrine system and disrupt hormone cellular pathways. The detection of such compounds in human tissues, blood and urine suggests their capacity to act as endocrine disruptors, as they are able to hit specially sensitive targets involved in hormonal signaling in endocrine glands, peripheral neurons and their projections. Microplastics can directly induce endocrine dysregulation at the tissue level and can also be associated with changes in peripheral hormonal levels, anatomical modifications in food-related endocrine glands, and effects on food intake via interference with hormones involved in appetite regulation, linking plastic exposure and appetite changes.

Human tissues may be exposed to microplastics released from consumer goods and products applied to the skin. Several receptors that are actively modulated during metabolic syndrome-related alterations are sensitive to plastic components such as bisphenol A, BPA analogs, and phthalates, and emerging effects on receptors such as the bile acid receptor (i.e., farnesoid X receptor, FXR) and the free fatty acid receptors (FFA1, FFA2, and FFA3) can be associated with changes in bile acid flux and sensing of dietary free fatty acid levels [126, 127, 128].

Disruption of Thyroid, Reproductive, and Adrenal Hormones

Endocrine disruptors are exogenous compounds that give rise to adverse effects in organisms through modifications in the functions of the hormonal system. Examples of such compounds include substance produced by phthalates, polyfluoroalkyl substances (PFAS), bisphenol A (BPA), and others. Leaching of endocrine disruptors from plastic particles during degradation can immensely exercise an effect on the entire endocrine system. The thyroid is controlled by the hypothalamic-pituitary-thyroid (HPT) axis, and the thyroid hormones T3 (liothyronine) and T4 (thyroxine) regulate metabolism, growth, and tissue differentiation. Substantial evidence exists that exposure to endocrine-disrupting chemicals like BPA can cause abnormal levels of T3 and T4 in different organisms. Lipid droplets are the chief storage form of energy in the vertebrate body, participating in metabolism, energy homeostasis, hormone synthesis, and many other functions. The active reuptake of liposomes by various cells suggests that liposomes could serve as a natural energy source for cells.

Adipose tissue is a high-storied place of lipids that plays an essential role in metabolic homeostasis. It also acts as an active organ that secretes various bioactive substances, called adipokines, which play vital roles in appetite control, energy balance, vascular homeostasis, and reproduction. Disruption in endocrine function has been reported to modify the interaction of adipokines with their receptors, thus changing food appetite. Transient administration of the plasticizer dibutyl phthalate (DBP) during the critical period of sexual differentiation in rats affects the development of the hypothalamic-pituitary-gonadal axis and leads to abnormal hormone levels in the adult period. It has also been hypothesized that faulty or abnormal interaction of DBP with nuclear receptors downregulates the expression of

anorexigenic factors of the CNS like α-MSH, CART, or thyroid hormones [129, 130, 131, 129, 130, 131, 132].

Endocrine-Driven Metabolic Dysfunction

Endocrine-regulating pathways are prime targets for microplastic exposure, which is associated with metabolic impairment in humans and animal models. Many plastic products release chemical additives during their entire life cycle and after disposal. Growth and endocrine-disruptive compounds such as bisphenols, phthalates, perfluoroalkyl substances (PFAS), organochlorines, and peroxisome proliferator-activated receptor (PPAR) agonists are known to leach out from plastics. Leaked chemicals can interfere with sexual, thyroid, and adrenal hormone levels and signaling mechanisms, modifying the endocrine system; for instance, the glucocorticoid receptor is a potential target of combinations of chemicals released from plastics, affecting glucose homeostasis. Prenatal exposure to endocrine disruptors can trigger adult obesity and metabolic syndrome.

Several epidemiological studies have begun to highlight the correlation between concentrations of plastics or plastic-related endocrine disruptors in biological samples (urine, serum, and cervicovaginal lavage) and increased markers of obesity and metabolic syndrome. Bisphenol A, phthalates, and PPAR agonists have been connected to a higher incidence of insulin resistance, hyperglycemia, reduced HDL cholesterol, and increased waist circumference. Bisphenol S is associated with elevated blood pressure in adolescents. In a Danish birth cohort, combined prenatal exposure to DEHP and BPA was correlated with alterations in body composition.

Biomonitoring and Risk Assessment

The accumulation of Microplastic (MP) and Nanoplastic(NP) particles in the human body has been investigated in many

studies, and their presence in breast milk, placenta, amniotic fluid and meconium strongly supports the need for human biomonitoring. As a result of the associated health risks, the European Food Safety Authority (EFSA) and the European Chemicals Agency (ECHA) have included MP and NP in the community action plan on endocrine disruptors and are considering new methods for assessing human exposure, together with the need for preventive measures.

Despite the important regulatory advancements, gap in knowledge remain as the current human risk assessment approaches are limited to potential leaching of additives from larger MP embedded in food or cosmetic products. A more comprehensive health risk assessment that considers both human exposure routes (inhalation, ingestion and dermal) and MP/NPs properties (particle size, shape, surface silane density, chemical nature or functional groups) is still needed, especially in relation to their role as carriers of toxic chemicals and pathogens [133, 134, 135, 136].

Chapter - 8

Epigenetic Alterations Induced by Microplastics

Microplastic pollution is increasing globally, yet current knowledge of its effects on human health remains incomplete. A growing body of evidence highlights epigenetic alterations as a mechanism through which microplastics may contribute to metabolic disorders. Epigenetic modifications play a crucial role in gene regulation without affecting DNA sequence information, and changes in DNA methylation patterns, histone modifications, and microRNA expression have been associated with exposure to microplastics and nanoplastics.

DNA methylation is critical to gene regulation, and abnormal patterns can result in gene silencing or activation. Numerous DNA regions are differentially methylated following microplastic exposure, affecting genes controlling proliferation, metabolism, and insulin signal transmission. These aberrant methylation patterns are not merely a byproduct of exposure, as they can be confirmed in tissues of exposed organisms.

Individual genes are also subject to abnormal methylation modifications. In the context of metabolic diseases, distinct alterations in DNA methylation patterns are recognized as potential signatures for developing diagnostic or therapeutic tools [137, 138, 139, 140].

DNA Methylation Changes and Gene Silencing

In several human cell lines and animal models, the presence of microplastics has been associated with altered DNA methylation patterns. Milesi *et al*. highlighted that chronic

exposure of human embryonic kidney cells to 1.5 µm polystyrene beads resulted in the differential expression of genes involved in cytoskeletal organization and exocytosis. Pathway analysis revealed that genes involved in epigenetic modifications were the most significantly affected category, along with genes in the HSP family. The effect on DNA methylation was further confirmed by patterns of H3K9 acetylation, an epigenetic mark associated with active transcription. Overexpression of the chromatin-remodeling factor HMGA2 and/or depletion of the DNA methyltransferases DNMT1/DNMT3B restored the expression of the silenced hypomethylated genes.

DNA methylation alterations have also been demonstrated after exposure to polyethylene glycol modified polystyrene nanosized particles. In human bronchial epithelial cells, the transcript expression of a number of genes was notably altered by NP treatment. Ingenuity Pathway Analysis indicated that EP300 and RB1 were involved in the prediction of upregulated transcripts. Consistently, ChIP-seq analysis of H3K27ac-enriched regions suggested enhancer activation at the EP300 site. A subsequent reduction in DNA methylation levels was validated at the promoter regions of five of the upregulated genes after NP treatment in two human airway epithelial cell lines. A similar mechanism might also be involved in lung cancer cells exposed to ultrafine microplastics. In this case, differentially expressed genes were closely associated with DNA repair, methylation, and transcriptional regulation. Furthermore, gene silencing combined with DNA methylation detection revealed that DLGAP4 downregulation following exposure to UMP may occur via DNA demethylation [141, 142, 143].

Dysregulated Histone Modifications

Nuclear proteins known as histones bind to the genomic DNA and maintain chromatin structure. Their post-translational

modification enhances or represses gene transcription, thereby regulating various cellular functions. Histones can also undergo specific modifications independent of methylation, MS, acetylation, ubiquitination, and ADP-ribylation. Various reports indicate that these modifications play a key role in the modulation of the cellular mechanisms associated with different exposure conditions. One of the main patterns associated is changes in histone acetylation levels followed by down-regulation of acetylated histones H3 and H4. An increase in global mono-nucleosome acetylated histones H3 and H4 levels, and a subsequent persistent reduction in histone deacetylase (HDAC) activity, are also observed in exposed cells. Other studies report an increase in histone H3 acetylation in SKBR3 human breast cancer cells treated with PDA. Dephosphorylation of histone H3 at sites known to promote transcription, as well as deacetylation of H4 by elevated class II HDAC activity, are implicated in the sterile inflammatory response developed by THP-1- derived macrophages under the same stimulation conditions. In neuronal cells exposed to L. monocytogenes-induced exogenous mitochondrial stress, survivin and Bim expression is rebalanced through acetylation and deacetylation of H3 and H4 histones.

Excessive mitochondrial ROS levels drive aberrant histone lysine acetylation/double methylation balance in Col10a1-expressing chondrocytes. Histone activation has been correlated with increased levels of tumor necrosis factor (TNF) and interleukin- 1β (IL- 1β). Also in the context of Peripheral arterial disease, acetylation of histone H3 associated with up-regulation of IL-32 expression is promoted by plastic particle exposure. An intricate interplay between serine ADP-ribosylation and lysine acetylation also has been identified in the development of lung inflammation. Regulation of histone H3 lysine and arginine methylation peak in colonic epithelia upon

Mycobacterium avium subspecies paratuberculosis infection. Infection with Pancu-1 pancreatic adenocarcinoma cells altered Oct4 localization through modulation of histone acetylation. Finally, epigenomic alterations in 5- ethynyl- 2′- deoxyuridine- labeled neurons induced by maternal high- fat diet were linked to BMI changes, a key risk factor for the development of obesity or other metabolic disorders in later life. Therefore, although some histone modifications exert opposing effects, increased knowledge about these changes in Microplastic-exposed cells can help to clarify epigenetic alterations upon specific exposures [144, 145].

MicroRNA and Non-coding RNA Perturbations

The dysregulation of microRNAs (miRNAs) and long non-coding RNAs may be implicated in microplastic-induced metabolic impairment. miRNAs are short (≈22 nucleotide), non-coding RNA molecules that post-transcriptionally silence messenger RNAs (mRNAs) and modulate diverse cellular and metabolic pathways by acting as "fine-tuners" of gene expression. The miRNA regulatory network is essential for maintaining metabolic homeostasis. Loss or gain of cargo/molecule transporters or enzymes needed to maintain lipid and glucose homeostasis leads to upstream or downstream metabolic disturbance. Due to the roles of miRNAs in controlling metabolic pathways, it is not surprising that dysregulated miRNA expression profiles have been demonstrated in several obesity-associated diseases.

Aberrant miRNA expression profiles are also considered potential epigenetic biomarkers of microplastic exposure. Nontargeted miRNA sequencing revealed 66 dysregulated miRNAs in C57BL/6J mice exposed to microplastics via drinking water. Targeted investigations in other animal models and *in vitro* systems have revealed additional microplastic-

induced miRNA alterations. Functional miRNA enrichment analysis has shown a possible involvement of these microRNA perturbations in the regulation of the insulin signaling pathway, inflammatory response, glycolipid metabolism, CCR7 signaling pathway, and factors associated with viral infection.

Non-coding RNAs are also involved in the regulation of multiple metabolic pathways during microplastic exposure. N6-methyladenosine (m6A) methylation, the most prevalent internal modification of eukaryotic mRNA, modulates gene expression, mRNA stability, and alternative splicing of genes encoding proteins related to lipid accumulation. m6A methylation disorder is implicated in the pathogenesis of various metabolic diseases. Transcriptomic analysis of Huatuo mouse models of microplastic exposure has indicated that genes related to obesity, fatty acid metabolism, and small-molecule transport are among the most dysregulated. Targeted RNA-sequencing analysis has revealed that m6A methyltransferases (METTL3, METTL14) are significantly upregulated in C57BL/6J mice after microplastic exposure. These changes may contribute to changes in the m6A methylation level of the lipid metabolism gene, fatty acid synthase, thus dysregulating lipid metabolism [146, 147, 148].

Transgenerational Effects and Developmental Toxicity

Experimental studies have reported several epigenetic effects of NP exposure, including changes in DNA methylation and transcription profiles of key genes controlling development, apoptosis, senescence, and xenobiotic metabolism in exposed organisms and their descendants. Therefore, the role of in utero exposure on the epigenome offers a unique window of susceptibility during development for the dysregulation of non-coding RNAs and potential transgenerational impacts. In a zebrafish (Danio rerio) model, chemically different NPs were used: polystyrene NPs decorated with polyethylene glycol (PS-

PEG, 200 nm) and silica NPs (SiO2, 50 nm). Drugs commonly used in human therapy were selected: diazepam, progesterone, and ibuprofen. NPs and drugs were exposed to embryos in single or mixture exposure. The main focus was to evaluate transcriptional and epigenetic disruption of exposed embryos and potential transgenerational neurological alteration.

Alterations in gene expression and transgenerational effects that generate alterations in neuronal development and behavior represent a relevant concern. Age-dependent and specific behaviors are modified in the offspring from the second generation. The possible presence of DNA damage and gene expression alterations in descendants was addressed. Polystyrene and silica NPs, as well as drug combinations, were detected in tissues at early stages. The amount of SiO2 found in descendants raises serious concerns about the potential transgenerational effects of NPs as epigenetic modifiers [149, 150, 151, 152].

Epigenetic Biomarkers for Exposure Assessment

Microplastic (MP) contamination in the environment is a global issue. Human biomonitoring studies have detected MP particles in blood, lungs, urine, amniotic fluid, and breast milk. In addition to direct exposure through inhalation and ingestion, MPs can cross the placental barrier and reach the fetus. Recent research provides evidence that MPs can translocate into the circulatory system and interact with various tissues and organs. The cytotoxicity profile and effects on functional pathways share similarities with those of other toxicological stressors, such as heavy metals, air pollution, and endocrine disruptors. Pan-omic investigations have pointed to crosstalk among different stress pathways, supporting the idea of an integrated biological response to exposure-related factors. Persistent exposure to MPs may thus increase the risk of developing metabolic disorders and associated diseases. As shown in animal models, MPs

accumulate in the gastrointestinal tract, disrupt gut microbiota composition, and induce metabolic dysregulation.

Microplastics are also associated with alterations in the expression of microRNAs involved in metabolic dysfunction and epigenetic changes associated with exposure. Microplastic exposure is linked to activation of the unfolded protein response and a late-onset metabolic syndrome in mice. Despite the detection of MPs in human faeces, the identity of particles in biological samples remains speculative, and changes in miRNA expression levels are considered to reflect exposure rather than a direct effect. Nevertheless, the effects observed suggest that transgenerational impacts of MPs and their leachates on human health merit further investigation. Although technology detection and quantification remain challenging, investigating exposure-induced epigenetic variations affords a viable route for future cellular-level studies [153, 154, 155, 156].

Chapter - 9

Gut Microbiota Dysbiosis and Gut-Metabolic Axis

The gut microbiota of humans and other mammals changes over time, shaped by numerous factors, including age, physiology, genetics, diet, medications, and environmental exposure. The gut microbiome is composed of trillions of microorganisms residing in the gastrointestinal tract and has a role in gut health and diseases, metabolism, drug distribution, immune response, development, and protection against pathogens. Dysbiosis, or the pathological disturbance of gut microbial communities, is identified as a potential cause of conditions like metabolic syndrome, diabetes, obesity, and inflammatory diseases. Nanoplastics, similar to other pollutants, can accumulate in the gastrointestinal tract and dysregulate gut microbiota.

Plastic debris in the environment is often populated by pathogenic bacteria or toxins that can colonize the host, trigger inflammatory responses, and cause gut microbiota dysbiosis. Microplastics also serve as vectors for such pathogens, and these can accumulate in the intestines of exposed organisms. Microplastic leaching products, like plastic-associated toxins or adsorbates, target gut commensal microorganisms, affecting microbial composition and metabolic functions, leading to dysbiosis during concomitant exposure to heavy metals, glucose, or fructose. Dysbiosis driven by chronic microplastic exposure can be correlated with aberrations in metabolic homeostasis and insulin sensitivity, and microplastic-induced elevation of blood branched-chain amino acids indicates interference with the host gut-metabolic axis.

Impaired gut microbiota-host axis crosstalk as a result of microplastic exposure may lead to nonalcoholic fatty liver disease, increased liver fat deposition, and other metabolic disturbances. Microplastic-induced alteration of gut microbiota can partly account for dietary-toxic compound-induced metabolomic perturbations. The microbiota can mediate alterations in major metabolic signaling pathways correlating with steatosis and lipid homeostasis. A meta-analysis demonstrates a significant association between the presence of microplastics in the human gastrointestinal tract and changes in gut microbial composition [157, 158, 159, 160].

Microplastic Accumulation in the Gastrointestinal Tract

Microplastic particles have been detected in human stool and breast milk, underscoring their presence in the gastrointestinal tract. The mouth, esophagus, stomach, and intestines are exposed to food and drinks that may contain micro- and nanosized plastic particles (MNPs). Food and drink contaminated with MNPs can enter the gastrointestinal tract, resulting in the accumulation of MNPs in various segments. Recent studies demonstrated the detection of both micro- and nanosized plastic particles (MNPs) in the feces of adults, teenagers, and infants, highlighting the exposure of vulnerable individuals.

MNPs can deposit at different anatomical locations of the gastrointestinal tract following ingestion. In experimental models, MNPs are observed in the stomach, intestines, and fecal samples following administration. Moreover, alterations in gastric histology, including inflammatory infiltration and an increased thickness of the muscle layer, have been noticed after exposure to MNPs, suggesting the development of pathological changes. MNPs can be dynamically accumulated in the gastrointestinal tract. The levels of accumulated MNPs in different segments are variable, depending on the gut

compartment and treatment mode. Together, these findings lay the foundation for future studies aimed at functionally elucidating the impacts of MNPs in the gastrointestinal tract and beyond as well as in evaluating their role in shaping gut-microbiome interactions [161, 162, 163].

Changes in Microbial Diversity and Metabolites

A growing number of studies have demonstrated that the gut microbiota may serve as a central hub for both pro- and anti-T2DM mechanisms through the regulation of various metabolites. Inert particle accumulation in the gastrointestinal tract of mice has been shown to induce gut dysbiosis and microbial metabolite dysregulation, resulting in increased abnormal glucose tolerance and serum insulin levels. At the phylum level, the relative abundances of Bacteroidetes and Firmicutes, and the ratio of Bacteroidetes to Firmicutes, were correlated with increased blood glucose levels. Notably, dysbiosis of the gut microbiota has also been linked to obesity, metabolic endotoxemia, and T2DM. These findings support the notion that microplastics may interfere with the gut-microbiota-metabolite axis, leading to further insulin resistance.

Microbial communities in the gut play an important role in maintaining glucose homeostasis in hosts by regulating fat metabolism, producing short-chain fatty acids (SCFAs), and preventing the generation of circulating lipopolysaccharides (LPS) or LPS-like substances. The abundance of SCFA-producing bacteria is negatively correlated with LPS-producing bacteria. Microplastics have been shown to induce lower relative abundances of SCFA-producing bacteria, suggesting potential roles for SCFAs in glucose homeostasis. Feeding mice with SCFAs could ameliorate their enhanced glucose intolerance but further investigations are required to fully elucidate the mechanism [164, 165, 166, 167].

Intestinal Inflammation and Barrier Dysfunction

Microplastic accumulation in the gastrointestinal tract can cause intestinal inflammation and barrier dysfunction, contributing to gut-metabolic dysbiosis and affecting host metabolism. Dysbiosis of the gut microbiota disrupts the gut-metabolic axis and is associated with metabolic syndrome. Changes in microbial composition can impede metabolism of probiotics, bile acids, or even dietary residues, with consequences for host metabolism. Evidence is accumulating that microbiota-host cross-talk participates in the development of metabolic disorders. In particular, alterations in bile acid reabsorption via the gut-liver axis. Cytokines released from the gut-intestinal epithelial barrier modulate glucose homeostasis by affecting insulin or insulin-resistance signaling in peripheral organs. Several bacterial genera and species have been implicated in the modulation of metabolic syndrome or obesity.

Clinical and epidemiological data suggest an association between microplastics and metabolic syndrome, diabetes, obesity, and obesity-related factors (e.g., hypertension, high levels of cholesterol and triglycerides, nonalcoholic fatty liver). Microplastics have been detected in human stool and may therefore be ingested. Indeed, fish and shellfish, two commodities of high economic and food value, have been found contaminated. Also highlight alterations in diversity, diversity, and composition of gut microbiota in response to microplastics and nanoplastics. Alterations of the gut microbiota and mucus layer induced by nanoplastics can lead to gut dysbiosis and metabolic dysfunctions. Exposure of gestating mice or mice of the F1 generation to microplastics induces gut dysbiosis. Experimental evidence confirms that implantation or oral intake of microplastics or nanoplastics induces intestinal inflammation, dysbiosis [168, 169, 170, 171].

Microbiota-Driven Insulin Resistance and Obesity

Accumulation of microplastics in the gastrointestinal tract induces dysbiosis by altering microbial composition and reduces microbial metabolites critical for host energy homeostasis, contributing to a low-grade inflammatory state and metabolic disorders. These changes activate proinflammatory molecules in the gut, including monosaccharide-binding lectin 2 (MCL 2) and macrophage galactose-binding lectin, leading to translocation of endotoxins, such as lipopolysaccharides, into the bloodstream. These activate toll-like receptors (TLRs), nuclear factor Kappa B (NF-κB), and associated signaling pathways, resulting in chronic low-grade inflammation and insulin resistance. In addition to these microbiota-related mechanisms, other changes due to microplastic exposure speed the emergence of obesity. Microplastics increase appetite by stimulating central inflammation in the hypothalamus, which secretes neuropeptide Y (NPY) in the arched nucleus and downregulates α-melanocyte-stimulating hormone (α-MSH), which plays a key role in appetite control. Changes in brown and white adipose tissue, impaired energy homeostasis, and increased plastic-borne chemical risks add to food-safety concerns and support epidemiological reports correlating plastic exposure with human obesity.

Several studies have reported alterations of the gut microbiota in animals exposed to microplastics, indicating the potential of microplastics to disrupt the gut-microbiota axis. Indeed, these changes enable gut dysbiosis and inflammation, promoting the entry of bacteria and their products into the bloodstream and resulting in the stimulation of TLRs. In mice, microplastic exposure reduces gut microbiome diversity and increases the abundance of harmful bacteria, while decreasing the production of beneficial components, such as short-chain fatty acids and tryptophan. Metabolomic and transcriptomic analyses confirm dysregulated metabolic pathways, including the

tryptophan metabolism, citric acid cycle, amino acid synthesis, and pyrimidine metabolism pathways. Additionally, microplastics may upregulate inflammatory factors, including tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). Infection with Enterotoxigenic Escherichia coli (ETEC) exacerbates the inflammatory response, triggering gut dysbiosis, inducing insulin resistance, and ultimately disrupting glucose homeostasis [164, 172, 173].

Interactions with Dietary Nutrients and Xenobiotics

Microplastics interact with dietary nutrients, xenobiotics, and other environmental factors in ways that may exacerbate metabolic disturbances associated with exposure. An *in vivo* study examining the interaction of a high-fat diet with polystyrene NPs showed exacerbated fat deposition, inflammatory response, and oxidative stress in the liver as well as decreased inhibition of insulin signaling-associated pathways and the maintenance of glucose homeostasis in mice exposed to NPs compared to controls fed a normal diet, leading the authors to conclude that this interaction may promote insulin resistance and correlate with obesity-related metabolic disorders. A recent *in vitro* investigation suggested that polystyrene NPs can enhance pyrene bioaccumulation and toxicity levels in the edible marine bivalve Mytilus edulis by facilitating the transport of the extremely hydrophobic polycyclic aromatic hydrocarbon in the digestive gland and might compromise P450-mediated detoxification in M. edulis. Folic acid dysfunction is also suggested to play an important role in mediating the adverse effects of microplastics on the health of bivalves.

Nutritional exposure to heavy metals can also aggravate the adverse effects of MPs on edible bivalves. The joint exposure of Mytilus galloprovincialis to a high-fat diet and MPs has been shown to disrupt gut homeostasis and induce neurotoxic effects via the gut-brain axis. Co-exposure can accelerate senescence

and seriously inhibit cytoskeleton dynamics, resulting in intracellular space enlargement, mitochondrial fragmentation, and reduced secretion of antifungal and antibacterial proteins by hepatopancreatic cells of the freshwater fish Carassius auratus. The detrimental effects of MPs on fish skeletal muscle tissues can be aggravated by cadmium co-exposure [174, 175, 176, 177].

Chapter - 10

Microplastics and Lipid Metabolism Disorders

Microplastics have been reported to trigger dysregulation of lipid metabolism in exposed organisms. In particular, lipidomic studies demonstrated that exposure interferes with the regulation of both lipogenesis and lipolysis. Adipose tissue homeostasis is crucial for maintaining normal whole-body lipid metabolism, and dysfunction of adipocytes, especially adipocyte hypertrophy, leads to an increase in the risk of obesity, type 2 diabetes, and atherosclerosis. Microplastic-induced dyslipidemia or atherosclerosis is involved in toxicity mechanisms spanning several species, from fishes to mammals, indicating such effects are not exclusively linked to rodents. Disruption of peroxisome function and fatty acid oxidation has also been described. Lipidomics-based approaches are considered promising tools for assessing exposure to micro- and nanoplastics.

Microplastic-derived leachate and chemical additives have been linked to alterations of the lipid-metabolizing homeostasis in mammals. Enhanced lipogenesis and reduced lipolysis in hepatic tissues of female mice exposed to microplastics in drinking water, as well as inhibition of lipolysis in 3T3-L1 adipocytes exposed to nanoplastics, provide additional support to the involvement of microplastics in lipid metabolism disorders. Specifically, the expression levels of lipogenic-related genes and key adipokines, including adiponectin and resistin, were affected in adipose tissues of mice receiving drinking water containing microplastics. In addition, circulating lipid levels were altered in

mice exposed to microplastics or nanoplastics. Disruption of the dynamic balance between lipogenesis and lipolysis is also considered a key risk factor for the incidence of atherosclerosis [178, 179, 180].

Alterations in Lipogenesis and Lipolysis

Dysregulated lipid metabolism is a key mechanism linking microplastics to cardiovascular and metabolic diseases. Current evidence reveals that micro- and nanoplastics can perturb both lipogenesis and lipolysis, although lipidomic analyses are scarce. Exogenous surplus lipids, indicated by increased concentrations of triglycerides, cholesterol esters, or non-esterified fatty acids, suggest impaired lipolysis. Conversely, upregulation of lipogenic rate-limiting enzymes (FASN, ACLY, ACC1, SREBP-1) signals stimulated lipogenesis. Several metals associated with plastics (e.g., Mn, Pb) compromise lipid homeostasis by modulating the expression of lipogenic and lipolytic genes.

Microplastics have been shown to upregulate FAC lipid synthesis (LPCAT1, FASN), downregulate oxidation (ACADL), and stimulate FA import (FAT/CD36), thereby increasing cellular surplus lipid pools (triglycerides, cholesteryl esters). Similarly, *in vitro* stimulation of lipid accumulation, increased transcript levels of key adipogenic regulators (PPARγ, CEBPα, SREBP1), and diminished lipolysis (ATGL, HSL) have been observed. In the context of adipose tissue, plastic-induced impairment of lipogenesis and lipolysis has been linked to metabolic disorders. Elevated levels of circulating free FA following microplastic exposure can further disrupt metabolic homeostasis and glucose balance, thereby heightening the risk for chronic diseases such as diabetes and atherosclerosis [181, 182, 183, 184].

Effects on Adipocyte Function and Adipokine Secretion

Microplastics may influence adipocyte biology through two

main mechanisms: the release of plastic-derived compounds capable of modulating adipogenesis and adipokine secretion via receptor-mediated pathways, and the direct action of micro- and nanoplastics on adipocytes. A limited number of *in vitro* studies have demonstrated an effect on adipogenesis, and increased concentrations of proinflammatory cytokines such as IL6 and TNFα following co-stimulation with microplastics and lipopolysaccharides (LPS). Microplastic exposure disrupts the normal pattern of secreted adipokines such as IL6, IL8, IL10 and leptin and affects lipid metabolism in the adipose tissue, leading to dyslipidemia. Additional lines of research have highlighted potential endocrine disruptions caused by the leaching of endocrine active additives in food and beverage packaging. The combination of internal exposure to food additives and external exposure to airborne chemicals may thus represent a risk factor for promoting human obesity, generating a cumulative effect on the adipose tissue.

Increased levels of pro-inflammatory cytokines released from microplastics and plastic-derived chemicals in the circulation might also trigger local inflammation and oxidative stress in adipose tissue, thus impairing the normal metabolic function of adipocytes. It therefore remains important to better establish the contributions of direct stimulation of adipocytes by microplastics, and the chronic low-grade inflammation triggered by other internal organs, to alterations in adipocyte function and adipokine secretion [185, 181, 186].

Dyslipidemia and Atherosclerosis Mechanisms

Microplastics may directly disrupt lipid metabolism, promoting dyslipidemia and atherosclerosis, with additional indirect effects via gut microbiota dysbiosis or cutaneous metabolism regulation. *In vitro* studies showed microplastics or nanoplastics upregulate lipogenesis and downregulate lipolysis

and fatty acid oxidation in liver and adipose cells, respectively. Impairments in adipocyte function and dysregulated secretion of adipokines such as leptin and adiponectin then contribute to metabolic disorders. Control of lipid metabolism is further affected *in vivo*, as dietary exposure to microplastics alters blood lipid levels and induces dyslipidemia in mouse models. Multispecies gut microbiota transplantation and the associated dysbiosis itself can also induce dyslipidemia. Moreover, atherosclerosis may result from the combined action of microplastics on lipid metabolism, the gastrointestinal tract, and gut-metabolic axis disorders.

The potential action of endocrine-disruptive chemicals leaching from plastics, including bisphenol A, phthalates, polychlorinated biphenyls, dioxins, per- and polyfluoroalkyl substances, and other sources, was not examined here. Epidemiological studies also highlight a causal relationship between heavy metals—especially cadmium, lead, and mercury—and the risk of atherosclerosis; thus, industrial pollution may modulate cardiovascular risk via microplastics acting as vectors for such metal burdens. Confirming a link between microplastic exposure and dyslipidemic indices demands further evidence from well-designed, large-scale, long-term epidemiological studies. Lipidomics strategies represent powerful tools for statistical correlation of exposure status with host-microbe co-metabolism in association with dyslipidaemia [187, 188, 189, 190].

Disruption of Peroxisome Function and Fatty Acid Oxidation

Emerging evidence indicates that microplastics may disrupt peroxisomal function, thereby affecting lipid metabolism and predisposing to dyslipidemia. Accumulation in peroxisomes has been reported for diverse environmental nanoparticles, but this finding remains unexplored in the case of microplastics. Nanodebris derived from a polyamide tea bag has also been

found to hamper fatty acid β-oxidation in both mice and human hepatocytes. Increased liver triglyceride levels, altered histological features, and upregulated lipogenic biomarkers suggest a dysregulation of hepatic lipid metabolism. The mechanism likely involves reduced recruitment of fatty acid translocase by peroxisome proliferator-activated receptor-α, leading to decreased mitochondrial fatty acid uptake and blame, and lowered β-oxidation in mitochondria.

Collectively, these results suggest dysregulated lipid metabolism after microplastic exposure and highlight the roles of peroxisomes and mitochondria in microplastic-induced lipid metabolic disorders. Ongoing lipidomic examinations will further characterize the lipidomic landscape and identify microplastics-induced differentially expressed lipid species. At the cellular level, plastic leachate upregulates the expression of key genes involved in lipogenesis while downregulating lipid catabolic genes and reducing the secretion of Apolipoprotein A1. The alterations in fatty acid metabolism are not limited to the liver. Within the gastrointestinal tract, altered organoid morphology and membrane lipid peroxidation are manifested when exposed to PS nanoplastics and polystyrene nanofibers, presenting the possibility of intestinal lipid disturbances [191, 192, 181, 104].

Lipidomics Approaches for Assessing Exposure

Emerging lipidomic technologies enable direct assessment of microplastic exposure. Lipidomics aims to profile the holistic lipid repertoire of a biological system by identifying and quantifying lipid species using mass spectrometry. Microplastic-carrying alterations in lipid storage, transport, and metabolism have been demonstrated, and such perturbations may be detected via lipidomic profiling. In order to directly assess the involvement of microplastics in metabolic syndrome-related

changes, the targeted application of lipidomics is proposed.

Lipidomic techniques broadly include qualitative and quantitative mass spectrometry-based approaches. Quantitative lipidomic approaches typically employ multiple-reaction monitoring in tandem mass spectrometry to enable high sensitivity but usually lack qualitative information. Targeted lipidomic methods can be combined with liquid chromatography, facilitating simultaneous quantification of many lipid classes. Current nuclear magnetic resonance-based lipidomic methods are low-throughput but provide precise quantification of visceral lipid depots while enabling classification of molecular structure and stereochemistry. The utility of lipidomics for mapping metabolic disorders symptoms and elucidating pathological mechanisms is increasingly recognized. Lipidomic signatures of disease risk or the underlying effects of therapeutic interventions can be detected. Lipidomic analyses are being applied to investigate numerous diseases, including obesity and complexity-related food disorders [193, 194, 195, 196].

Chapter - 11

Microplastic-Induced Insulin Resistance

Impaired insulin signaling pathways are well known to be disturbed by microplastic exposure. Such interference is found to correlate with oxidative stress and inflammation, two cellular response pathways that are also triggered by microplastics. Experimental studies confirmed that exposure to microplastics causes pancreatic β-cell dysfunction, glucose transport dysregulation, and GLUT4 modulation. Animal models also reveal that microplastics can compromise glucose homeostasis. Notably, these effects are not limited to mammals, as zebrafish models converge on similar metabolic impairments.

The insulin receptor substrate (IRS) plays crucial roles in the initiation and transduction of insulin signaling pathways. In response to insulin stimulation, although no significant changes are detected in the expression level of protein IRS-1, a concomitant upregulation of CDC42 and downregulation of PAK1 are observed. These two proteins are known to associate with IRS-1 and inhibit IRS-l-dependent signaling in other models, indicating a potential mechanism for the inhibition of insulin signaling. Microplastics may also alter the expression of other components in the adipose tissue insulin signaling pathway [197, 185, 198, 199].

Impaired Insulin Signaling Pathways (IRS/AKT)

The insulin receptor substrate (IRS) and protein kinase B (AKT) pathways mediate metabolic actions through extracellular signal-regulated kinases (ERK) 1 and 2. Activation of these

pathways promotes glucose transport through glucose transporter 4 (GLUT4) and the expression of lipoprotein lipase (LPL) and perilipin, which are associated with lipogenesis, energy storage, and insulin action. Alteration of lipopolysaccharide (LPS)-induced AKT signaling in fish has been linked to metabolic syndrome. Microplastic mixtures containing LPS, a Gram-negative bacteria component often leached during the manufacturing process, activated the CD14 receptor to induce inflammation and downregulate IRS1 and AKT expression and phosphorylation. The presence of LPS or microplastic-stimulated LPS increased the release of IL-6 and IL-1β and decreased the expression of glycogen synthase 2 (GSK2) and glucose-6-phosphate dehydrogenase. In another study, exposure of the liver hepatocyte cell line (HepG2) to polystyrene nanoplastics impairing ATP production inhibited AKT phosphorylation and reduced membrane translocation of GLUT4.

In summary, the IRS/AKT signaling pathway is disrupted by microplastics and nanoplastics in multiple species, contributing to insulin resistance and pathological conditions [200].

Crosstalk with Inflammation and Oxidative Stress

Crosstalk between insulin resistance and concurrent inflammation and oxidative stress represents a potential link between microplastic exposure and metabolic disease. The insulin receptor signaling pathway is exceedingly complex and dysregulated insulin signal transduction is involved in various forms of metabolic syndrome. Level alterations in substrates and other associations from microplastic exposure have been found in *in vitro* systems and animal studies, with loss of receptor function, downregulation of insulin-responsive glucose transporter GLUT4, insulin-release impairment of pancreatic β-cells, and unmetabolized glucose release discovered. All implicated functions are interconnected. These studies support the conclusion of microplastics facilitating insulin resistance.

Insulin resistance has been associated with chronic oxidative stress, as the transcription and activation of genes involved in the formation of various oxidant species are influenced through the forkhead transcription factor FOXO3. Excessive production of both mitochondrial and cytosolic reactive oxygen species together with internal superoxide dismutase depletion also seems to activate c-Jun NH2-terminal protein kinase-c-Jun signaling. Nuclear factor κB-mediated activation is subsequently implicated in the synthesis of proinflammatory cytokines such as tumor necrosis factor-α, interleukin-6, and monocyte chemotactic protein-1, which in turn can negatively regulate insulin receptors and insulin receptor substrate 1. Mitochondrial dysfunction also contributes to oxidative stress and alters insulin signaling. Notably, such remark is supported by the ability of various antioxidants to alleviate insulin resistance [201, 202, 115, 203].

Pancreatic β-Cell Dysfunction

In vitro and animal studies have reported that microplastics can induce dysfunction of pancreatic β-cells, which secrete insulin essential for normal glucose homeostasis. In a co-culture model of human pancreatic β-cells and monocytic cells exposed to polystyrene nanoplastics, pro-inflammatory cytokines were released from the monocytic cells, which then inhibited insulin secretion and cell viability of co-cultured β-cells. Such effects may be mediated by the NLRP3 inflammasome and downstream release of IL-1β. Exposure to other environmental nanoplastics has also exacerbated high-fat diet-induced impairment of pancreatic β-cell function. Following polysulfide nanoplastics exposure, glucose-stimulated insulin secretion and mitochondrial membrane potential were disrupted via oxidative stress, leading to mitochondrial dysfunction and bioenergetic collapse. Such experimental findings indicate that microplastics can trigger insulin secretion defects in pancreatic β-cells, enhancing the risk of type 2 diabetes mellitus.

In vitro and animal models have suggested that exposure to microplastics may cause dysglycemia via organ-independent mechanisms, including disruption of the insulin signaling pathway and down-regulation of glucose-transporter subunit 4. Indeed, injection with polyethylene terephthalate nanofibers increased serum glucose in mice. Furthermore, BAMA particles, polyethylene nanoplastics, and aged polystyrene nanoplastics disrupted glucose homeostasis, inhibited glucose transport in 3T3-L1 adipocytes, and reduced glucose uptake. Impaired insulin signaling in muscle and adipose tissues and down-regulation of Glut4 mRNA further illustrated the potential for microplastics to induce insulin resistance [204, 205, 184].

Disrupted Glucose Transport and GLUT4 Regulation

Microplastics induce impaired insulin signaling and transport processes, decreasing glucose uptake in different cell types. Elevation of endoplasmic reticulum (ER) stress, caused by microplastic exposure, directly contributes to decreased glucose uptake by lowering the expression of glucose transporter GLUT4, the primary transporter for glucose uptake in insulin-responsive tissues. Microplastic-induced ER stress also mediates the reduction of glucose transport into the pancreas, critical for maintaining glucose homeostasis since pancreatic β-cell dysfunction results in decreased insulin secretion. Furthermore, the effect of microplastics on glucose uptake dysregulates GLUT4 translocation and transmembrane particle transportation, representing an important mechanism underlying insulin resistance, type 2 diabetes, and subsequent metabolic syndrome. An experiment demonstrated that the 100-nm microplastics directly suppress palmitic acid-stimulated glucose uptake and translocation of GLUT4 to the plasma membrane in adipocytes and cardiomyocytes.

Another study provided cumulative evidence that

microplastics directly inhibit glucose uptake and transmembrane transport in L6 skeletal muscle cells. Lipopolysaccharide pretreatment magnified the inhibitory effect of microplastics and downregulated the expression of IRS-1, IRS-2, PI3K, and Akt in L6 myotubes, suggesting that microplastics exert an inhibitory influence on the translocation of glucose transporters to the plasma membrane through the lipopolysaccharide-mediated inflammatory pathway. The evidence indicates that microplastics disturb glucose homeostasis, triggering insulin resistance in the systemic metabolism associated with T2DM development [197, 204, 206].

Animal and *In vitro* Models of Metabolic Impairment

Recent *in vivo* rodent studies have highlighted metabolic links between plastic exposure and human cardiometabolic disorders. Adverse effects in animal models include increased fat accumulation, insulin resistance, dyslipidemia, and hypertension. Nalbant *et al*. reported increased abdominal and liver fat in adult female rats exposed to 20 nm carboxylated polystyrene (CPS) particles via drinking water. They hypothesized that CPS-accelerated obesity may occur through gut microbiota dysbiosis; they confirmed an increase in the abundance of pathogenic bacteria (Enterobacteriaceae family) and a decrease in beneficial genera (Akkermansia and Lactobacillus), along with decreased intestinal barrier integrity and elevated lipopolysaccharide levels. Wu *et al*. detected 40 nm sulfonated polystyrene particles in the livers, lungs, and blood of adult mice given 30 nm sulfonated polystyrene particles by oral gavage for 28 days. These nanoparticles caused glucose intolerance, aggravated insulin resistance, and induced pancreatic β-cell dysfunction, possibly through reactive oxidative species production and DNA damage.

In vitro studies with adipose tissue have further defined mechanisms of plastic-induced metabolic disturbance. Zhang *et*

al. reported that exposure of 3T3-L1 adipocytes to polystyrene (PS) microplastics stimulates lipid accumulation and promotes nuclear factor (NF)-κB activation and interleukin 6 (IL-6) secretion. Chen *et al*. found that aberrant lipolysis and dysregulated lipogenic-related gene expression are induced in 3T3-L1 adipocytes by low-dose PS particle exposure via increased intracellular oxidative stress levels. Co-exposure of 3T3-L1 cells with PS microplastics and saturated fatty acids has been shown to potentiate activation of NF-κB signaling and aggravate lipotoxicity by disrupting glucose homeostasis [207, 208, 209, 210].

Chapter - 12

Nanoplastics and Obesity-Associated Mechanisms

Over the last four decades, plastic use has expanded exponentially, with plastic production having surpassed 400 million tons yearly in recent years. In parallel, obesity rates have steadily increased, with approximate global prevalence rates of 41% in adults and 19% in children. Children suffering from substantial obesity are at a much higher risk than those without obesity for additional obesity-associated disorders, including hypertension, type 2 diabetes, non-alcoholic fatty liver disease, asthma, and mental disorders, and those who remain obese into adulthood are prone to various health problems and a generally less healthy life when compared with others.

Environmental pollutants that are linked to obesity include sustained-release per- and poly-fluoroalkyl substances (PFAS), organochlorine pesticides (e.g., dichloro-diphenyl-trichloroethane; DDT), bisphenols, polycyclic aromatic hydrocarbons, brominated flame retardants, phthalate esters, and polychlorinated biphenyls. The association between artificial temperature changes and obesity rates in a US population has been recognized, and Taiwan has reported associations between annual temperature changes (seasonal averages) and the prevalence of childhood obesity [211, 212, 213, 214].

Hypothalamic Inflammation and Appetite Regulation

Nanoplastics, as well as microplastics, may be associated with deleterious hypothalamic effects. Munciwalkar *et al*. reported that polystyrene nanoplastics induced inflammation in

hypothalamic neurons that regulate appetite. Male 5-week-old Kunming mice were treated with saline or 500 μg/kg/day polystyrene nanoplastics via oral gavage for 18 days, after which RNA sequencing was performed on the hypothalamic tissue. The results showed 154 significantly upregulated and 238 downregulated genes. Gene set enrichment analysis revealed most dysregulated genes were involved in the regulation of neuroinflammatory responses. The authors proposed that transcriptional changes described the inflammatory responses of hypothalamic neurons and were involved in appetite regulation during polystyrene nanoplastic exposure. Such results suggest polystyrene nanoplastics may contribute to obesity via hypothalamic inflammation.

Kantor *et al*. reported on the effects of polystyrene (PS) nanoplastics on the expression of neuropeptide Y (NPY) and proopiomelanocortin (POMC) in the hypothalamus of male mice. Following oral administration of a single dose of 200 μg of 20 nm PS nanoplastics in 10 μL, male mice were euthanized 12, 24, or 48 h post exposure. The findings indicated a transient upregulation of NPY mRNA after 12 h but no change in POMC. These results suggest dynamics of NPY and POMC in the hypothalamus may be involved in the regulation of energy homeostasis following exposure to PS nanoplastics [215, 216, 217].

Effects on Brown and White Adipose Tissue

Recent studies indicate that nanoplastics may modulate adipocyte metabolism, with both direct and indirect effects. On the direct level, exposure to nanoplastics stimulates the function of white adipocytes, leading to the secretion of the proinflammatory factor IL-6, which is presumed to be involved in the development of neurodegenerative disorders. On the indirect level, the presence of nanoplastics in the central nervous system may trigger a neuroinflammatory response that modulates

food intake, suggesting a prevention of neuroinflammation and a restoration of appetite regulation could help ameliorate nanoplastic-induced changes in brown adipose tissue. Together, these findings provide new insights into the effects of nanoplastics on adipocyte metabolism. Still, whether nanoplastics can elicit direct alterations in the function of brown adipose tissue remains to be elucidated.

Nanoplastics inhibit fish muscle cell proliferation while inducing cell death. Fathead minnow skeletal muscle cells treated with nanoplastics displayed decreased metabolic activity, as indicated by the MTT assay, increased apoptosis, and a higher percentage of apoptotic cells in the early and late stages. Aberrant expression of apoptosis marker genes, including caspase 9, caspase 3, and bcl-2, was also observed. Mechanically, stimulation of the glucocorticoid signaling pathway may partly participate in the process. These results reveal that nanoplastics have detrimental effects on fish skeletal muscle cells and act as potential endocrine disruptors by inducing apoptosis via the glucocorticoid signaling pathway [218, 219, 220, 221].

Disruption of Energy Homeostasis and Thermogenesis

Adverse effects on adipose tissue are increasingly recognized as potential mechanisms linking microplastics to obesity. These nanoparticles can accumulate in brown and white adipose tissue and dysregulate lipid and glucose homeostasis in both cell cultures and rodent models. Thermogenesis function and sympathetic activation of lipolysis in brown adipocytes are inhibited, whereas pro-inflammatory markers are upregulated in both adipose depots. Obese mice fed a high-fat di*et al*so exhibit increased body weight, inguinal fat mass, and blood glucose levels upon oral exposure to nanoplastics. Moreover, chemicals leaching from exposed polymers have obesogenic potential and human epidemiological studies suggest a link between plastic

consumption and obesity. Since these processes appear to sensitize the host to obesity, they constitute another important pathway linking microplastic exposure to metabolic disease.

The hypothalamus is the master regulator of energy homeostasis, coordinating the autonomic nervous system and neuroendocrine system to control food intake, energy expenditure, and thermogenesis. Dysregulation of hypothalamic function and inflammatory processes may therefore contribute to obesity and have been observed in animals exposed to plastic waste. Direct exposure of primary hypothalamic neurons from a zebrafish model to ambient leachate from plastic waste revealed apoptosis and elevated expression of metal-responsive elements and cytokines, changes that could promote metabolic dysregulation in exposed animals [222, 223, 224, 225].

Obesogenic Chemicals Released From Plastics

Certain plastic materials such as polyvinyl chloride (PVC) or polystyrene can be considered positive sources of chemicals associated with obesity. PVC is produced with phthalates, which may leach during the use period. Phthalates function as disruptors of the endocrine system and may affect metabolic processes. Some phthalates are considered obesogenic, moderate the expression of genes related to the fatty-acid metabolism in adipocyte cultures, and are associated with increased body mass index among adults and children.

The chronic exposition of mice to post-consumer polystyrene microspheres induces an increase in body weight and fat deposition. Such obesity-related phenomena involve a lower energy expenditure and a reduction in brown-adipose-tissue function. The observed alterations in expression and local production of leptin and adiponectin are negatively correlated with the levels of post-consumer polystyrene microspheres. Moreover, food-poisoning bacteria associated with polystyrene

containers and foams can possibly produce toxins that independently modify central appetite regulation. In humans, a significant association between BMI and the concentration of bisphenols, phthalates, or dibutylphthalate urinary metabolite was detected. Moreover, a positive association with body fat mass and a negative correlation with serum adiponectin for the sum of four phthalate metabolites was apparent [226, 227, 228, 229].

Epidemiological Trends Linking Plastics to Obesity

Recent epidemiological findings indicate a significant association between plastic pollution and the worldwide obesity epidemic. Notably, in China, both obesity rates and plastic waste generation have surged. Furthermore, time-series analyses reveal strong correlations between the volume of plastic waste and obesity prevalence across different nations. A study encompassing adult populations from 26 countries has linked the consumption of packed food and drinks with increased body mass index, waist circumference, and obesity risk, independent of sociodemographic factors. Subsequently, it has been demonstrated that inhalation exposure to microplastics can induce obesity-related symptoms in a murine model. Elevated levels of airborne microplastics have also been measured in urban areas of China, coinciding with rising obesity trends, while an epidemiological assessment has connected air microplastic pollution with obesity-related changes in humans.

Emerging evidence suggests that exposure to microplastics can trigger various mechanisms associated with obesity, including hypothalamic inflammation, dysregulation of brown and white adipose tissues, and other disturbances in energy homeostasis. Nonetheless, further epidemiological investigations are warranted to establish solid causal links between microplastic exposure and the obesity epidemic [230, 127, 198, 205].

Chapter - 13

Cardiometabolic Risks from Microplastic Exposure

Accumulating evidence from animal studies suggests associations between microplastic exposure and cardiovascular and metabolic dysfunctions. Hypertension and vascular endothelial damage have been observed after exposure to air, drinking water, or food contaminated with microplastics. Cardiovascular alterations have also been related to exposure to plastic particles through the respiratory tract. The potentially synergistic effects of air pollutants, such as particulate matter, and microplastics on cardiovascular health need further investigation. Microplastics may serve as vectors for microbial colonization, contributing to cardiovascular and metabolic disturbances. New data point toward platelet activation resulting from microplastic exposure and suggest destabilization of the vascular-coagulation homeostasis. A possible association between microplastics and impaired cardiovascular physiology has been proposed.

Epidemiological studies show an alarming upward trend in the prevalence of obesity. Microplastics and their leachates may have a fundamental role in this process and represent an associated risk factor for the development of obesity-related metabolic disorders. Furthermore, the detected activation of hypertensive mechanisms related to microplastic exposure points to an imminent cardiovascular risk. Hypertension, a historically underestimated risk factor, is now recognized as a major cause of cardiovascular disease. Accumulating evidence indicates the potential of microplastics to disrupt lipid and glucose

homeostasis, and further studies are essential to dissect the cardiovascular and metabolic consequences of microplastic exposure and the possible synergism induced by specific chemical leachates [127, 186, 185, 231].

Hypertension and Vascular Endothelial Dysfunction

Microplastic exposure has been associated with various cardiometabolic disorders, including arterial hypertension. This relationship may arise from crosstalk with systemic metabolism or cardiovascular-targeted toxicity. Vascular endothelial dysfunction, another recognized effect of microplastics, contributes to the risk of cardiovascular diseases such as atherosclerosis, thrombosis, coronary artery disease, heart failure, and stroke. Recent findings support the impact of microplastics on hypertension and endothelial function, highlighting potential mechanisms underlying these effects.

Epidemiological studies show an association between preeclampsia, gestational hypertension, and elevated levels of environmental plasticizers in urine. Blood-plastic-exposed mice also develop hypertension, which is alleviated by intestinal microbiota depletion. While direct blood concentration data remains scarce, and intra-arterial exposure has not been assessed, the role of micro- and nanoplastics in hypertension is regarded as a priority research topic. Indeed, nanoplastics are often internally administered to mimic exposure in animals.

An emerging dimension involves microplastics' contribution to vascular endothelial dysfunction, a recognized human health effect and one of the first abnormal states in the cardiovascular continuum. Vascular endothelial dysfunction permeates conditions such as atherosclerosis, thrombosis, coronary artery disease, heart failure, and stroke. A recent study indicates that microplastics trigger an endothelial inflammatory response characterized by the release of proinflammatory cytokines and

chemokines and the upregulation of adhesion molecules. Notably, structural alterations in endothelial cells are reminiscent of an inflammatory status.

Cardiac Remodeling and Arrhythmogenic Effects

Research on conventional cardiotoxicity is maturing, and the links between cardiac anomalies (arrhythmias, hypertrophy, heart failure) and SCPs have begun to be understood. However, contributing factors beyond direct toxicity remain largely unexplored. In cardiac tissue, metabolic and hormonal dysregulation, alongside disturbances in lipid, glucose, and coagulation homeostasis, constitute additional infection-independent risk factors. Epidemiological studies also indicate that the effects of SCPs on these pathways can jointly amplify cardiovascular risk.

These facts have only recently yielded results revealing that microplastic exposure can precipitate cardiac remodeling or arrhythmias in rodents and cardiomyocyte cell lines. Further, the effect of cardiosphere-derived cells and indole-administered probiotics on the diabetic heart is reduced in those exposed orally to SCPs, possibly indicating that the structure and function of the cardiac and circulatory systems are becoming impaired whether by direct particle exposure or through internal metabolic relationships.

Cardiovascular toxicity has been correlated with exposure to SCPs and other air-environmental stimuli. Long term exposure to sewage and ambient air containing mixtures of SCPs can lead to heart function dysfunction. Ingestion of food-derived particles can impair the hyperglycemic effect by acting on both endocrine and non-endocrine pancreatic β cells, and increase the glycemia-raising action of epinephrine at β2-adrenoceptors present in the liver [232, 233, 234, 235, 232, 233, 234, 235].

Platelet Activation and Coagulation Imbalance

Keywords: microplastics, exposure, cardiovascular system, cardiometabolic disease, arrhythmia, hypertension, cardiovascular toxicity

Microplastics induce platelet activation and coagulation imbalance. Microplastic exposure is associated with the activation of all elements of the hemostatic pathway, including the fibrinogen-fibrin conversion process. Experimental studies characterize the interaction between platelets and microplastics, demonstrating that human platelets exposed to microplastics exhibit membrane density reductions and an increase in phosphatidylserine residues. The human endothelial cell line HUVEC and the bacterial toxin lipopolysaccharide LPS coexist to mimic the analysis of vascular dysregulation triggered by microplastics, which contribute to apoptosis and dysfunction. The vascular cardiotoxicity of microplastics is correlated with the impact on dyslipidemia and glucose stability, and these factors are further integrated with platelet activation and coagulation.

Microplastics may act as the circulating carriers of other infectious pathogens, and their interaction with spores of various fungi initiates Steinernema-crossroad polymer-induced activation of circulating hemocytes, resulting in the synthesis of foam cells. The presence of these foam cells suggests the transcription of a variety of functional genes that assist in cellular immune removal during microbial infections centered on Glomerella leaf spot. The acute-phase response is strongly triggered by microplastic exposure, with markedly elevated concentrations detected in the plasma of fish exposed to microplastics. Animals exposed to the pollutants commonly exhibit sequestering damage to gills and skin, although elongated tails may also be a characteristic response. Moreover, adult zebrafish exposed to polyethylene microplastics experience hypothalamic inflammation and exhibit behavioral adaptations.

Microplastic-Induced Lipid and Glucose Instability

Accumulating evidence from epidemiological and *in vivo* studies indicates an association between microplastic exposure and dysregulated lipid metabolism. Lipidomics studies have revealed changes in the levels of lipid species involved in membrane formation and energy storage, increased abundance of fatty acids and bile acids, and decreased phospholipid and sphingomyelin levels in animals exposed to microplastics through inhalation or enteral routes. The involved mechanisms include modulation of lipogenesis and lipolysis, alterations of adipocyte function and adipokine secretion, and development of dyslipidemia and atherosclerosis. Notably, the potential role of dyslipidemia in microplastic-mediated cardiometabolic impairment has been recognized. Microplastics have the capacity to disturb peroxisome function and fatty acid oxidation, and lipidomics approaches may contribute to the evaluation of microplastics and nanoplastics exposure.

Epidemiological data indicate a potential link between plastic pollution and cardiometabolic diseases. According to a recent population study on blood lipid profiles, cumulative concentrations of microplastics in air, drinking water, and food were positively correlated with triglyceride levels. Animal exposure models have provided supporting evidence, revealing associations with increased total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol. Dyslipidemia largely contributes to the development of cardiovascular diseases and atherosclerosis, and emerging findings point to a role for microplastics in these processes. The dysregulation of lipid metabolism may be mediated by cell signaling pathways such as peroxisome proliferator-activated receptors and SREBP (sterol regulatory element-binding protein) as well as crosstalk with inflammatory signaling. In addition to dyslipidemia, other microplastic-induced mechanisms associated

with lipid metabolism disorders require further investigation [236, 237, 238, 239].

Integration of Cardiovascular-Metabolic Toxicity

Notably, hypertension may indeed represent a prominent health risk following microparticle exposure. In both population studies and toxicological investigations, increased blood pressure has been directly linked to microplastic particles and their nanometre-sized degradation products. Nevertheless, clinical observations associating microplastics with metabolic syndrome, obesity, and diabetes are also emerging. These findings open the possibility that microparticles may induce or aggravate key metabolic disorders triggering cardiovascular disease. The established impact of microplastics, nanoplastics, and their chemical leachates on the insulin signalling pathway, control of lipid homeostasis, and gut microbiota perturbations affecting intestinal-metabolic health are all potential contributors to microplastic-associated cardiometabolic dysfunction. The hypothesis that augmented cardiovascular toxicity is a secondary consequence of microplastic-related metabolic syndrome merits further investigation. Such a relationship could guide development of targeted solutions to ameliorate the cardiovascular risk of exposed populations.

Historically, associations between troubled metabolic processes and cardiovascular alteration have been predominantly clinical observations, supported by independent investigations focused on either the heart or metabolic pathways. More specifically, hyperglycaemia and hypolipidaemia, key components of cardiometabolic disease, have been linked to environmental exposure to endocrine-disrupting chemicals retarding peripheral glucose transport and altering lipid metabolism and storage. Although these studies have not addressed potential direct effects on cardiovascular function,

recent findings indicate that environmental plastic nanoparticles can impair cardiovascular function through induction of hypertension. Development of a generalised model to investigate environmentally linked metabolic disruption and subsequent cardiotoxicity now seems timely. Initial consideration of potential links from disturbed metabolism to altered cardiovascular function is given here, along with an overview of the concomitant cardiotoxic effects of other environmental contaminants [240, 241, 242].

Chapter - 14

Detection, Quantification, and Analytical Methods

A wide array of chemical, spectroscopic, and microscopic techniques exists for detecting and analysing microplastics, nanoplastics, and their leached additives, and these can be applied to biological samples as well as environmental samples. Plastic particles in an environmental or exposed biological background can also be quantified by density floatation and visualized through fluorescence, scanning electron microscopy (SEM), or transmission electron microscopy (TEM). The multiplicity of possible approaches for analysis of microplastics and nanoplastics, however, highlights the need for standardization.

Synchronous fluorescence spectroscopy has shown validity in the identification of microplastic particles and their leachates in environmental and biological samples. Different sets of chromatographic methods have been developed for the identification of plastic additives, such as bisphenols, phthalates, per- and polyfluoroalkyl substances (PFAS), organophosphate esters (OPE), organotin compounds, and dimethylfumarate. Spectroscopic and chromatographic methods are being integrated into a coherent analytical workflow for biological and environmental samples. Among the *in vitro* and *in vivo* models used to study microplastic exposure, zebrafish, mice, rats, and rodents have been employed. However, the detection and quantification of nanoplastics (i.e. plastic particles below 100 nm, NP) remains challenging, and significant work is still needed in this area.

Recent avenues of study in this emerging research field include risk assessment and analytical methodology standardization, especially regarding exposure by inhalation and ingestion routes. The relevance of these techniques for the study of plastic pollution-associated human disorders is supported by the intensified detection of microplastics in a wide range of human tissues, especially the lung, placenta, and gastrointestinal tract. Future research efforts should adopt a predictive modeling approach for the study of microplastic interactions with the main cellular pathways involved in metabolic syndrome and metabolic-associated disorders. System-level modulators capable of alleviating or reversing specific pathway dysfunctions induced by microplastics and related plastic additives might also be established [243, 244, 245, 246].

Spectroscopic and Microscopic Approaches

A range of spectroscopic and microscopic techniques can detect and quantify microplastics and nanoplastics in environmental, biological, and food samples. Fourier transform infrared (FT-IR) spectroscopy and Raman spectroscopy are frequently employed methods for polymer identification, both in bulk and in single-particle analysis by mapping two-dimensional images. These techniques are useful for organic polymer identification, while secondary ion mass spectrometry (SIMS) can also detect non-polymeric fillings and pigments that could serve as biomarkers in environmental sample studies. Scanning electron microscopy (SEM) provides surface morphology information for metals, oxides, and silicates but cannot identify organic poly-crystalline materials.

The Fourier transform infrared microspectroscopy imaging technique has successfully detected microplastic particles with sizes in the micrometer and submicrometer range for environmental samples, tissue analysis, and transmission

electron-microscopy sample preparation. In addition to infrared and Raman spectroscopy, novel mass-spectrometry imaging can automatically visualize styrenes, polycyclic aromatic hydrocarbons, and their sulfur- and nitrogen-containing derivatives within a single analysis.

As the number of publications on microplastics detection methods grows, so does the diversity of materials being studied. In addition to those derived from plastics, other samples such as anthropogenic fibers, paper pulp, road markings, paints, roofing materials, cosmetics, and textiles have already become subjects of dedicated detection methods [247, 248, 249].

Chromatographic Identification of Additives

Microplastics are often complex mixtures due to their environmental aging, degradation, and use as carriages for toxic substances (e.g., persistent organic pollutants, heavy metals, pathogens, plasticizers, fire-retardants), which represent a serious threat to human health. Adverse biological effects are closely associated with leaching of toxic components, including plasticizers and stabilizers. Furthermore, these additives can be found at high concentrations in environmental matrices and living organisms, offering good indicators of plastic contamination. However, identifying plasticizers and other additives in contaminated samples is methodologically challenging because of the large excess of microplastic particles.

Chromatography techniques, such as gas chromatography and liquid chromatography, have been extensively used for analyzing small organic molecules (typically below 1000 Da), including phthalates, alkylphenols, brominated flame retardants, and nonylphenol. Analyses of plasticizers and plastic-related additives in complex environmental matrices (e.g., sediment, wastewater, soil) commonly employ small-molecule-selective LC-MS techniques such as ultra-high-performance liquid

chromatography coupled to quadrupole time-of-flight mass spectrometry. Non-targeted methods can also identify non-exhaustive phthalate plasticizers leached from plastic pellets into sediment. The obstacles posed by complex sample backgrounds can be overcome through clean-up by adsorbent-gel chromatography prior to strong cation-exchange LC-MS/MS [250, 251, 252, 253].

Workflow for Environmental and Biological Sampling

Environmental occurrence and biological effects are the two main aspects investigated for microplastic research. Extensive efforts have been devoted to environmental sampling and analysis. Microplastic sampling from atmospheric deposition and air, surface water, river sediment, soil, and marine, freshwater, and wastewater has been reported. Environmental detection mandates standardized sampling and quantification. For air, total suspended particles are collected using a filter suitable for further visual inspection by optical microscopes or Fourier-transform infrared spectroscopy; quantification is performed using thermal desorption/gas chromatography or thermal desorption/gas chromatography-tandem mass spectrometry. Surface waters are filtered through a microfilter/membrane; morphologies, concentrations, and polymeric composition are identified using light/fluorescence microscopy, Fourier-transform infrared spectroscopy, or Raman spectroscopy. River sediments can be isolated with a modified density separation technique. Wind tunnel-based models have been proposed for airborne transport of microplastics embedded with synthetic fluorescent microspheres. Freshwater, marine, and wastewater studies use collection nets simulating microplastic settlement; concentrations are correlated with pH, total dissolved solids, and electrical conductivity. Microplastics are also recovered from mussel, clam, and fish, along with insight into deposition and feeding-associated concentration.

To elucidate the hazards and risks for human health, studies on cellular, tissue, and *in vivo* models are necessary; such examples have reported colon cells and organoid models, mouse subcutaneous tissue, and the blood, lungs, and liver of aerosol-exposed mice maintained for five weeks. Tissues and fluids have been suggested as suitable samples, and detection patterns can lead toward predictive evidence for risk determination. An *in vivo* experimental framework investigating exposure specificity, court, or route has been presented for microplastics with natural fluorescent features. For *in vitro* studies, microplastics labeled for detection, and complex exposure-dosage and timing-response relationships with lipid metabolism and cumulation in the gut of mice, combined with resident microbiota, have been evaluated. Overall exposure is respiratory and/or gastrointestinal canal; the use of mature samples is preferred [254, 255, 256, 257].

In vitro and *In vivo* Exposure Models

In vitro and *in vivo* exposure models are crucial for elucidating the biological effects of micro- and nanoplastics on living organisms and establishing a cause-effect relationship. Several *in vitro* cell culture systems have been employed to explore particle toxicity and cellular responses because of their lower cost, ease of use, reduced ethical concerns, and the potential for high-throughput screening. The choice of cell types depends on the intended target tissues and routes of entry. For instance, human lung alveolar epithelial cells (e.g., A549), bronchial epithelial cells (e.g., BEAS-2B), or primary cells from healthy donors have been widely used to investigate inhalation-associated effects. Human intestinal enterocytes (e.g., Caco-2) have been studied to assess ingestion-related endocytosis and transcytosis events. Immune-related pathways have been characterized mainly in phagocytic immune cells, such as macrophages (e.g., THP-1, RAW264.7). Other cell types, including vascular endothelial, liver, and placental cells, have

also been utilized to study the interaction of microplastics with additional human systems.

Whole-animal models are essential for examining the integrated organism-level effects of environmental exposure to microplastics and providing information on tissue distribution, bioaccumulation, and adverse outcomes after the challenge of an entire organism. *In vivo* studies employing mammals, such as mice, rats, guinea pigs, and squirrels, are limited in number because of restricted accessibility, high husbandry costs, and lengthy research periods. However, they yield valuable insights that cannot be obtained using simplified organisms. Nevertheless, several study models in the zebrafish embryo and fish systems Yang *et al*. 2021; Zuo *et al*. 2021; Yang *et al*. 2022; Fei *et al*. 2023) C. elegans Jiang *et al*. 2023), the nematode, and Drosophila Wang *et al*. 2022a) have been established due to their rapid life cycle and behavioral and physiological similarities with mammals. Besides possessing transparent embryos that facilitate direct monitoring, these models are easy to expose to both particulate and dissolved mixtures in water and food [258, 259, 260, 261].

Standardization and Challenges in Nanoplastic Detection

Advancing understanding of nanopolymers requires development of standard protocols for their characterization and quantification. Such protocols should enable determination of size, shape, surface charge, and chemical fingerprint, and ultimately facilitate quantification in air, water, soil, and impacted species. These efforts, moreover, demand an integrated research approach coupling experimental studies with predictive modeling. The challenge presented by nanoplastics and associated toxic effects on organisms requires no less.

Despite current analytical advances, the different size distribution of nanoplastics and the much wider nanoplastics'

overlap with background environmental concentrations and other environmental contaminants render non-standardized signal separation and extraction an unmanageable task in routine analysis. For instance, partly due to their tiny size and sometimes complex nature, challenges arise in the detection of nanoplastics in environmental samples and in the quantification of ocular fluids or tissue samples collected from ocular exposure models, necessitating rigorous signal extraction and corner case management during the proposed non-standardized signal detection workflow. Further improvements in separating the target signal from background signals originating from environmental matrices require concerted research efforts guided by predictive modeling approaches [262, 263, 264].

Chapter - 15

Mitigation Strategies and Future Perspectives

In order to safeguard human health, it is imperative to reduce microplastic exposure at the source. Since the largest proportion of microplastics is released from the atmospheric and marine environment into the food chain, the immediate objective is to concentrate on environmental policies that limit microplastic pollution. Multiple international organizations have called for strict actions to reduce plastic waste and microplastic emissions. Countries, including Canada and France, have introduced laws that limit the use of plastic bags, plastic cutlery, and microbeads, while the European Union has prohibited over-the-counter wet wipes containing plastic fibers and placed restrictions on the sale of single-use cutlery, plates, and straws made from plastic. Plastic and microplastic waste in the oceans can be reduced through elimination, remediating hot spots, using biocatalysts, and deployment of booms for collecting floating debris in targeted areas. Biodegradable materials and alternative packaging that are naturally biodegradable or made from natural fibers can prevent microplastics in the first place. Microplastics leaching harmful additives can be avoided by employing green nanotechnology for the next-generation products.

Although the concentration of microplastics is extremely low in biological systems, they cause significant effects, probably due to their high surface area to volume ratio, release of toxic chemicals, and co-contamination with hazardous pollutants. Therefore, answering the question of how microplastics affect biological systems is of global importance. Furthermore, when

trying to identify the precise mechanisms, a cellular approach is preferred over the whole-organism level. The effects can be elucidated more precisely, and the interplay with existing cellular problems, such as obesity, metabolic disorder, cancer, and others, can be studied more holistically. Apart from identifying the mechanistic pathways, the aim is to explore whether similar pathways are also altered with other environmental agents, such as heavy metals and airborne ultrafine nanoparticles in smaller sizes and much higher concentrations. Predictive modeling can be done specifically focusing on microplastics and the metabolic response of the human body. In addition, these findings can assist in predicting future concerns regarding human health, with a priority on endocrine disruption, epigenetic alteration, gut microbiota dysbiosis, and infectious disease susceptibility [265, 266, 267].

Reducing Human Exposure through Environmental Policies

Major efforts are required to control environmental pollution. Initiatives should focus on altering the production process and developing biodegradable alternatives, such that waste is managed appropriately during its life cycle. Enhancing the recycling systems is essential, as is shifting consumption towards sustainable alternatives. Although continued application pressure is necessary, predictions suggest global consumption reduction in future decades.

Many governments have already started to impose restrictions on a range of plastic products, such as single-use cutlery and containers, plastic bags and straws, bottles, and other packaging. For these regulations to be effective, countries with recycling capability and technology must provide alternative solutions to countries without such capacity. In addition, stronger policies should be introduced to prevent single-use materials from being mismanaged before they can be recycled. Other policies directed at major emitters, such as the fashion industry,

should be urgently enacted, and companies should be subject to greater accountability throughout the life cycle of their products. Disposing of microplastics directly into the environment and the marine ecosystem should be prohibited. Adequate sewage treatment infrastructure is required, particularly in developing nations. Plastic waste should not enter the ocean or coastal ecosystems [268, 269, 270, 271].

Biodegradable Materials and Green Nanotechnology

For effective risk management, research aimed at reducing human exposure to microplastics must focus on establishing proper environmental policies. Absolute prevention of microplastic contamination is currently impossible, as microplastics have been identified in remote wilderness and oceanic zones. However, implementation of stringent waste management and reduction strategies can bring a notable decline. Decreased input from the five largest rivers around the globe would result in diminished microplastic concentration in the ocean and a corresponding reduction in atmospheric deposition. Global warming is contributing to the mobilization of historical reservoirs of plastic debris, and therefore limiting anthropogenic climate change can help reduce the dispersal of microplastics to pristine areas. Furthermore, the introduction of regulations limiting leaching of plastic additives in food has been correlated with reduced concentration of plastics in foodstuffs as well as reduced disease incidence. Incorporation of renewable energy sources in the production of polyesters can significantly reduce the overall plastic footprint.

The development of biodegradable materials that can replace petroleum-based plastics would significantly lower the impact of microplastic accumulation in the environment and the human body. Biodegradable plastics made from renewable biomass such as proteins, polysaccharides, starch, poly (lactic) acid, poly (glycolic) acid, poly (butylene succinate), and poly

(hydroxyalkanoates) are gradually being commercialized. The rise of a microbial biosphere capable of degrading these polymers through the activity of specific enzymes and/or whole cells is of great interest. Thwarting the enzymatic degradation of nanoparticles is a promising strategy to mitigate the harmful effects of non-degradable plastics. Research aimed at green nanotechnology is focused on developing more sustainable alternatives for nanoplastics. Environmental exposure to nanoplastics produced by the natural processes of erosion is inevitable, but understanding their potential effects on human health provides a basis for risk management [272, 273, 274].

Cellular Targets for Therapeutic Intervention

Therapeutic strategies targeting the biochemical mechanisms of microplastic-induced cellular stress may lower cardiometabolic risk associated with exposure. For example, administrating compounds with antioxidant activity—including melatonin, curcumin, and resveratrol—may ameliorate oxidative stress. Related approaches, designed to attenuate inflammation or cellular senescence, have shown similar potential. Nanoparticle-induced mitochondrial dysfunction may be confronted with therapeutic reperfusion, with the selective mitochondrial ATP-sensitive potassium channel opener diazoxide acting protectively in exposed animals. Furthermore, inhibiting exaggerated TRPC6 activity and downstream Ca2+, CAMKII, and Akt signaling have alleviated microplastic-related cardiac damage. Promoting neuronal autophagic flux has diminished neurotoxic effects connected to microplastics. Considering the pleiotropic cardiometabolic effects of exercise training—including anti-inflammatory and antioxidative properties, support of mitochondrial homeostasis and mitophagy, and abatement of senescence—nutritional or physical interventions with global health benefits may also lessen the adverse effects of microplastic pollution.

Building direct links between toxicological or metabolomic effects linked with microplastics and cardiovascular or metabolic disease will be an important advance. This may be achieved by evaluating established risk factors affected by microplastics, analyzing susceptible populations, or combining untargeted high-throughput analyses with epidemiological investigations. The application of network-based approaches may help elucidate relationships between plastic exposure and cardiometabolic disease. Predictive modeling of microplastic-induced alterations within key cardiometabolic pathways could also provide valuable insights, facilitating future hypothesis testing in human populations [184, 198, 275].

Predictive Modeling of Microplastic-Metabolic Interactions

Computational tools are becoming increasingly popular for predicting potential associations between external factors and metabolic disorders. An innovative exposome-wide association study identified 1246 environmental factors that correlated with human metabolic syndrome, thereby establishing a reliable reference list for risk association analyses. This large-scale exposome-metabolome association analysis enabled the prediction of environmental risk factors for metabolic impairment in the general population, with subsequent experimental validation showing that nine of the ten predicted factors tested had an adverse effect on glucose homeostasis. Inert polymer nanoplastics (NPL) emerged as a potential risk factor for metabolic disorders, and bioinformatics predicted a positive association between NPL exposure and dyslipidemia. NPL exposure reduces free fatty acid concentration and activates fatty acid synthase expression during cell experiments, thus supporting the hypothesis that PUFA may serve as a restorative agent for NPL-induced metabolic defects.

Together with numerous other studies, these analytical predictions provide a valuable basis for elucidating the

association between microplastic exposure and metabolic syndrome. With a consistently rising trend in the production of these pollutants and the UN Global Assessment Report on Biodiversity declaring that these particles have already reached the deepest parts of the oceans and the highest points of the Himalayas, nanoparticles of plastic origin represent a severely underestimated but emerging health risk. Integrative modeling confirms the prediction that exposure to NPL can lead to metabolic dysfunction, with *in vivo* and *in vitro* studies revealing compromised regulation of glucose metabolism and dyslipidemia in NPL-exposed subjects [184, 185, 276].

Future Research Directions and Global Health Implications

Future studies should focus on establishing environmental exposure dose-response relationships, critically examining either increasingly sophisticated multiexposure risk-factor models or univariate assessments. Innovative modeling approaches predicting direct concentration-response relationships between microplastic concentration and prevalence/incidence of associated health disorders might also help provide solutions. Finally, novel plastic shapes, materials, or additives able to escape the pattern of industrial-university patents/devices-to-prediction, offering - from invention to endorsement - either truly biodegradable or biocompatible and bioeliminable exposure-free alternatives may further mitigate risks to public health and ecosystem integrity.

In April 2021, the WHO's Special Advisory Group on Antimicrobial Resistance stated for the first time that "the human health impact of plastic pollution - particularly plastic waste leaking into waterways and coastal environments - is primarily through bio-ha-zards associated with an increased human risk of infections or exposure to chemical contaminants". Toxicity-based, ecosystem-approach to pill-popping integrative

multitarget-multisite multipath-stimulus toxicology had already identified similar direct but poorly documented crosstalks during metabolic syndrome/bacterial vaginosis interaction networks. Integrative epidemiological insights linking the exponential boost of plastic pollution to world prevalence and incidence curves of overweight (2021) or patient number curves of type 2 diabetes, hypertension, coronary artery disease and/or other related cardiovascular-to-cerea-vascular impairments, might in turn provide semiquantitative supporting factual evidence for any future development able either to remedy the risk or to help decisively prevent such plastic-free alternatives from being strategically produced.

Conclusion

Experimental and epidemiological evidence to date suggests that microplastics may be a novel class of environmental pollutants with far-reaching effects on human health. Studies have shown that high concentrations of microplastics can induce a variety of metabolic diseases in animal experiments, operating via mechanisms pursued by metabolic mediators. A growing body of evidence confirms these pollution-related diseases in the human population, showing an increasing risk of obesity, diabetes, and hypertension associated with environmental nanoplastics. Nevertheless, significant knowledge gaps remain, particularly with respect to cellular-level interactions and pathways.

The literature survey presented here integrates evidence from *in vivo* and *in vitro* studies to outline pathways through which microplastic exposure may disrupt cellular homeostasis, providing new insights into mechanisms linking environmental nanoplastics to human metabolic disorders. The activated cellular responses integrate crosstalk among inflammation, oxidative stress, mitochondrial dysregulation, endoplasmic reticulum stress, alteration of endocrine pathways, and epigenetic modifications—pathways associated with immunity and metabolism. Ultimately, these pathways culminate in broad cellular dysfunction associated with human metabolic disorders. Interference with other cellular processes, such as those involved in lipid homeostasis, insulin signaling, pancreas function, gut-microbiota composition, cardiometabolic homeostasis, and obesity, respectively, may contribute further to underlying mechanisms.

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