

Article

Time-Dependent Bioaccumulation and Demographic Determinants of Micronanoplastic Concentration in Human Plasma

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Abstract

The pervasive nature of plastic pollution has raised significant concerns regarding human exposure and internal body burden. Micronanoplastics (MNPs) are known to translocate across biological membranes and accumulate in various tissues, with plasma serving as a critical transport and surveillance compartment. This study longitudinally assessed changes in plasma MNP concentrations from 2014 to 2023 and evaluated the influence of sex and Body Mass Index (BMI) on accumulation levels. The longitudinal analysis revealed a statistically significant increase in median plasma MNP accumulation between the Baseline period (2014–2018) and the post-lockdown period (2023) (Wilcoxon $p = 0.0145$), indicating a growing internal body burden. Coinciding with COVID-19, this escalation suggests that the surge in plastic PPE and single-use plastics may have accelerated human exposure, though causality is unproven. A highly significant difference was observed based on sex, with males exhibiting a greater median accumulation (2.09×10^6 NP/ μ L) compared to females (1.58×10^6 NP/ μ L) ($p = 0.0068$). Furthermore, the highest BMI quartile (Q4) showed significantly higher median accumulation (2.01×10^6 NP/ μ L) compared to the lowest quartile (Q1) (1.53×10^6 NP/ μ L) ($p = 0.0173$), supporting the hypothesis that lipophilic plastic particles may be sequestered in adipose tissue reservoirs. Despite an upward overall trend, low individual correlation ($r_s = 0.189$) suggests diverse personal behaviors drove individual trajectories, leaving the precise impact of pandemic habits unclear. These findings underscore the urgent need to understand the long-term health consequences of continuous and accelerating microplastic bioaccumulation.



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This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.**Keywords:** microplastics; nanoplastics; flow cytometry; nanocytometry; human plasma; bioaccumulation

1. Introduction

The relentless increase in global plastic production has led to the widespread ubiquity of MNPs (particles < 5 mm) in virtually every ecosystem, including air, water, soil, and the food chain [1,2]. Recent studies document internal biological contamination from human exposure, specifically within the bloodstream [3–6]. Plasma, the liquid component of blood, acts as the primary transport medium for exogenous and endogenous substances, making

it a critical matrix for monitoring systemic exposure and the trafficking of microplastic particles throughout the body [7–9].

Microplastic particles, often highly lipophilic, are hypothesized to translocate from the gastrointestinal and respiratory tracts into the circulation [10–13]. In the bloodstream, these particles are likely transported by lipoproteins to be deposited in reticuloendothelial organs or sequestered in adipose tissue, causing long-term bioaccumulation [14]. Beyond their physical presence in the bloodstream, internalized MNPs actively trigger pathophysiological cascades across multiple organ systems. Vascular accumulation of MNPs induces endothelial oxidative stress, local inflammatory responses, and atheroma instability, with their presence in carotid plaques directly linked to an elevated risk of major adverse cardiovascular events [15]. In circulation, these lipophilic particles interact with cellular elements to disrupt blood clotting dynamics, predisposing to thrombus formation [9,15]. Ingested MNPs further compromise intestinal integrity by inducing gut dysbiosis, altering metabolic profiles, and downregulating epithelial tight junctions [12]. Additionally, their capacity to act as lipophilic vectors facilitates the systemic distribution and tissue sequestration of plasticizers and endocrine-disrupting chemicals, contributing to broader metabolic and endocrine dysfunction [16].

Global synthetic polymer production now reaches nearly 400 million tonnes annually, with much of it fragmenting into ubiquitous MNPs [17]. This study addresses existing research gaps by using nanocytometry, a rapid, high-sensitivity approach. Previously validated for human blood analysis [18], the method utilizes dual-parameter flow cytometry and fluorescence staining to screen for synthetic particles down to the micrometer scale. This new study applied this method to a nested longitudinal cohort from the GCAT Genomes for Life project. By analyzing 100 longitudinal plasma samples from 50 individuals (17 males, 33 females) at Baseline (2014–2018) versus post-pandemic (2023), the authors provide evidence of time-dependent MNP bioaccumulation in humans. Previous findings of MNP distribution and transplacental transfer in marine predators suggest these particles achieve deep tissue sequestration in mammals rather than remaining transient contaminants [19]. Furthermore, MNP ingestion via beverages and infant foods is ubiquitous regardless of packaging, driving constant internal accumulation [20]. By investigating the roles of biological sex and Body Mass Index (BMI) in this decade-long period of accumulation escalation, the study provides new insights into the kinetics of plastic pollution within the human body.

Although the presence of MNPs in human peripheral blood [21] and other tissues is well established [22], current literature is constrained by several distinct research gaps. First, most existing studies provide only cross-sectional snapshots, leaving a complete absence of longitudinal data to monitor how internal body burdens fluctuate within individuals over time [19]. Second, identifying the NP fraction (<1000 nm) has been hindered by the detection limits of conventional techniques like μ FTIR and Raman spectroscopy, which frequently miss the specific particles most capable of translocating across biological barriers [20]. This vulnerability is underscored by the COVID-19 pandemic, a period marked by an unprecedented ‘plastic surge.’ Mandatory polypropylene-based PPE and increased single-use packaging likely acted as an environmental forcing function, accelerating systemic exposure. We assessed whether this period of heightened dependency acted as an external catalyst, potentially overwhelming physiological clearance mechanisms. By analyzing paired samples collected before and after the global lockdowns, this study aims to determine if this environmental transition manifested as a measurable increase in the transport of MNPs within the circulatory system. Ultimately, this work tracks long-term MNP accumulation within the same cohort over a decade, providing a high-resolution window into the sub-micron-scale standard approaches overlook.

2. Materials and Methods

2.1. Study Design and Sample Collection

This study used a longitudinal paired design with plasma samples from the GCAT (Genomes for Life) cohort. GCAT is a population-based project established in Catalonia (Spain) and managed by the Germans Trias i Pujol Research Institute (IGTP). It includes adult participants aged 40 to 45 years at the time of recruitment, enrolled between 2014 and 2018, with the aim of investigating the genetic and environmental determinants of complex diseases. Additional sampling was conducted through the GCAT-COVICAT follow-up during 2020, 2021, and 2023, incorporating longitudinal data related to the COVID-19 pandemic and its impact on health and exposure patterns. This study included baseline and 2023 samples. The GCAT cohort consists primarily of individuals from urban settings and is characterized by extensive epidemiological, clinical, and biological data collection using well-standardized protocols. The cohort profile has been previously described by Obón-Santacana et al. [23] and in a follow-up study by Kogevinas et al. [24]. To assess the longitudinal “plastic surge” and the impact of the COVID-19 pandemic on human exposure, we analyzed 50 paired plasma samples ($n = 100$ total data points) collected from the same individuals at two distinct time points: a baseline period between 2014 and 2018 and a follow-up in 2023. At baseline, the selected sub-cohort had a median age of 42 years (mean: 42.26 years; range: 40–45 years) and was 66% female and 34% male. In alignment with GCAT’s biobanking protocols, all blood draws were obtained from non-fasting participants. At the time of both sampling intervals, all participants were free from severe chronic medical conditions or active pathologies, establishing a healthy-volunteer baseline. Participants were aged 40 to 45 at baseline and were selected to isolate temporal variables while minimizing environmental noise. Specifically, the sample was restricted to residents of urban settings to ensure geographic homogeneity and reduce the variability associated with different environmental exposure levels. To test the hypothesis that adipose tissue may act as a reservoir for lipophilic plastics, subjects were selected from the extreme quartiles of the Body Mass Index (BMI) distribution. This included 31 individuals from the first quartile (Q1/lower BMI) and 19 individuals from the fourth quartile (Q4/higher BMI), allowing for comparative assessment of plastic pollution trends across different physiological profiles. Ethical approval for this research was obtained from the Germans Trias i Pujol University Hospital Ethics Committee (EC number PI-20–182). All participants provided informed consent and agreed to be contacted for follow-up investigations. All procedures were conducted in strict accordance with the General Data Protection Regulation (GDPR) and applicable international bioethical guidelines, ensuring that the observed increase in MNPs represents a true longitudinal baseline for the general population rather than a reflection of pre-existing pathological conditions.

2.2. Sample Preparation and Staining

All samples were manipulated in a BSL2+ environment. Whole blood (10 mL) was obtained by venipuncture using BD Vacutainer® PST™ (Becton Dickinson, BD, Franklin Lakes, NJ, USA) tubes containing lithium heparin (84 USP units) and thixotropic acrylic polymer gel. Samples were centrifuged and the resulting plasma was collected and stored at $-80\text{ }^{\circ}\text{C}$ until analysis. Beckman Coulter, Inc. (Brea, CA, USA) manufactures the Allegra X-30 centrifuge system utilized for the plasma isolation protocol. Briefly, 20 μL of plasma was incubated in 1% aqueous potassium hydroxide (KOH) (Sigma-Aldrich, #221473, St. Louis, MO, USA) (w/v , 1000 μL final volume) in a thermal block at $60\text{ }^{\circ}\text{C}$ for a minimum of 10 days, to degrade the organic matter. The robustness of the alkaline digestion was tested across varying lipid gradients to establish an analytical window. While human plasma remained stable and fluid for analysis, matrices with high lipid concentrations may

undergo saponification reactions during preparation, requiring careful optimization of digestion protocols [19]. The absence of these reactions in our plasma samples, validated by triplicate monitoring and the use of high-purity 100% biobased PP Eppendorf® tubes (Eppendorf SE, Hamburg, Germany), confirms that the recorded signals are not artifactual products of lipid-KOH interactions. Organic matter was degraded via exhaustive alkaline digestion using 1% KOH at 60 °C for 10 days (pH > 13.0), a process that also eliminated lipophilic biological interference to facilitate the isolation of target particles. The high-pH environment was maintained throughout the incubation period by introducing an excess of KOH, which effectively neutralized acidic degradation byproducts. This treatment hydrolyzed the organic and proteinaceous matrices into soluble peptide fragments and amino acids, preventing both the protein aggregation typically observed at lower alkalinity and non-specific dye precipitation. Following a 1:50 dilution with pyrogen-free water, these liberated peptide fragments served as an endogenous buffer that stabilized the fluorescent signal of the dye. Protocol verification was performed using polystyrene bead controls; the absence of solution turbidity combined with consistent bead recovery verified that the detected optical events represented distinct plastic particles rather than proteinaceous artifacts.

To minimize the risk of exogenous contamination, all plastic consumables underwent rigorous preliminary screening for particle leaching. Only high-quality, CE-IVD certified plasticware was selected, ensuring compliance with strict purity standards required for diagnostic applications. Preliminary testing substituting glass tubes for plastic to prevent polymer leaching revealed that the glass alternatives carried heavy baseline contamination from plastic microparticles and other residues, likely introduced during industrial manufacturing, washing, or sterilization. Consequently, certified high-quality plastics provided a cleaner, more tightly controlled analytical environment for nanoplastic detection [18]. Based on their high thermal stability and resistance against alkaline degradation, 1.5 mL Eppendorf Safe-Lock® tubes (Eppendorf SE, Hamburg, Germany) composed of high-purity polypropylene were selected for this study and pre-tested to confirm they did not contribute to the MNP counts.

For flow cytometric preparation, samples were processed in triplicate by diluting 20 µL of the digestate with pyrogen-free water to a final volume of 1000 µL. Staining was performed by adding 2 µL of a 0.1 mg/mL stock solution of Nile Red (#N1142, Sigma-Aldrich, St. Louis, MO, USA). This highly lipophilic fluorophore selectively partitions into hydrophobic environments, such as the polymer matrices of plastic particles. The designated working concentration ensured efficient particle labeling without inducing fluorophore aggregation or altering original nanoparticle properties. Following dye addition, the mixtures were incubated for 15 min at room temperature in the dark to facilitate uniform dye diffusion and optimal penetration into the particles.

2.3. Flow Cytometry Acquisition Parameters

Data acquisition and initial cytometric processing were performed on an Attune™ NxT Flow Cytometer equipped with Attune Next Software version 5.2 (Thermo Fisher Scientific, Carlsbad, CA, USA). Direct volumetric absolute counting was achieved via the instrument's integrated, syringe-driven positive displacement pump. Samples were analyzed at the minimum fluidic flow rate of 12.5 µL/min while keeping the total event rate below 10,000 events per second to ensure accurate volumetric quantification. The H-pulse parameter was specifically employed during acquisition and analysis.

Submicron nanoplastics were resolved by collecting violet side-scatter (VSSC) signals using the Attune™ NxT Violet Side-Scatter Filter Kit (Thermo Fisher Scientific, Carlsbad, CA, USA). The shorter wavelength of the violet laser (~405 nm) optimizes light-scattering

efficiency for submicron objects, significantly improving small-particle detection. Fluorophore excitation was driven by a 561 nm yellow–green laser, and emitted fluorescence was collected through a 585/16 bandpass filter in the YL1 channel. This targeted excitation–emission configuration maximized the Nile Red fluorescent signal while minimizing spectral interference and background matrix autofluorescence [18,25].

2.4. Protocols for Contamination Control and Cleaning

Between each sample, a thorough rinse with filtered deionized water was carried out. This is an essential washing procedure for minimizing the carry-over contamination so that particles from the previous sample do not falsely contribute to the counts of the subsequent sample. In addition to inter-sample rinsing, a thorough cleaning of the flow cell was performed both prior to and after the entire sampling session using Attune™ NxT Flow Cell Cleaning Solution (Hellmanex® III, Thermo Fisher Scientific, Carlsbad, CA, USA) (Cat# A43635). Such thorough cleaning is essential for the removal of any residual plastic particles or contaminants that might accumulate in the fluidics system. Background signals were dynamically measured for every manufacturing batch via triplicate event counting. If analytical discordances occurred (anomalous event counts or discordant light-scattering profiles), a quadruplicate analysis was executed to validate the baseline. Consistent with prior methodology, durable polyethylene terephthalate (PET) blood collection tubes were used to reduce baseline contamination. Potential variations in Nile Red (NR) fluorescence across different stock solutions were normalized using blank reference beads (15 min at room temperature, light-protected) for calibration. Deionized water and aqueous KOH matrices were systematically pre-tested to ensure negligible background signal. To prevent airborne contamination, all blood processing steps were performed within a certified Biosafety Level 2+ (BSL2+) environment. Under these protocols, background measurements for deionized water, EDTA tubes, and aqueous KOH solutions were consistently restricted to < 71 events/μL. All analytical and fluidic protocols were performed in strict compliance with manufacturer specifications and established good laboratory practices at the Germans Trias i Pujol Research Institute (IGTP).

2.5. Calibration and Controls

The flow cytometer was calibrated using two distinct sets of small particle kits. First, a submicron particle reference kit (Bangs Laboratories, Inc., Fishers, IN, USA) was utilized; this multiplex reagent consists of a single-vial mixture of 0.2 μm, 0.5 μm, and 0.8 μm beads internally dyed with yellow–green (YG) fluorescence (refractive index = 1.59). Second, high-resolution calibration was performed using fluorescent polystyrene nanospheres from Molecular Probes (Molecular Probes, Inc., Thermo Fisher Scientific, Eugene, OR, USA). These included 0.5 μm TetraSpeck™ multi-fluorescent nanospheres (Cat# T7281), 0.175 μm yellow–green fluorescent nanospheres (Cat# P7220 B), and 0.1 μm FluoSpheres™ yellow–green fluorescent carboxylate-modified nanospheres (Cat# F8803) (Molecular Probes, Inc., Thermo Fisher Scientific, Eugene, OR, USA). Pyrogen-free water was used as a negative control in the background calibration. This water is rigorously purified to remove all particles and biological contaminants, and serves as a reference for detecting any instrument background noise or potential reagent contamination, thereby ensuring the specificity and accuracy of the quantification.

2.6. Evaluation and Mitigation of Exogenous Plastic Contamination

To minimize background interference during high-throughput MNP analysis, preliminary screening of consumables was performed. Round-bottom glass tubes (10 × 75 mm; Ref 12802100, Nahita, Spain) cleaned with hexane and acetone were rejected due to persistent particulate contamination and cost-prohibitive, time-consuming maintenance. Stan-

standard microcentrifuge tubes (Ref 200400, Deltalab, S.L., Barcelona, Spain) also exhibited highly variable leaching after 10 days at 60 °C. Consequently, only two specific products met the stringent low-background requirements; flow cytometry acquisition: CE-IVD 5 mL polypropylene (PP) 12 × 75 mm tubes (Ref 400800, Deltalab, S.L., Barcelona, Spain) were used exclusively, providing consistent, low-background counts in triplicate runs. For sample digestion; 1.5 mL Eppendorf® Safe-Lock tubes (100% biobased PP, Ref 0030 120.086, Eppendorf, Germany) were selected for 10-day thermochemical digestion (1% KOH at 60 °C), demonstrating excellent thermostability and leaching resistance due to high-purity composition and secure hinged lids.

2.7. Statistical Analysis

To determine the appropriateness of parametric versus non-parametric tests, the distribution of MNP concentrations at Baseline and the 2023 follow-up was formally evaluated. Normality was assessed using four complementary tests: Anderson–Darling, D’Agostino & Pearson, Shapiro–Wilk, and Kolmogorov–Smirnov. These formal tests were supplemented visually by generating Normal Quantile–Quantile (Q–Q) plots via the automated “Normality and Lognormality Tests” analysis in “Actual vs. Predicted” format. In these plots, observed data quantiles were plotted against expected theoretical Gaussian quantiles, where a linear relationship following the 45-degree reference line indicated normality, and deviations at the extremities identified heavy-tailed distributions or influential outliers. Because significant deviations from normality were confirmed both statistically and visually, non-parametric methods were prioritized for all subsequent comparative analyses.

For longitudinal assessment, a Wilcoxon matched-pairs signed-rank test was utilized to compare paired MNP concentrations between Baseline and the 2023 follow-up. For cross-sectional assessments, a Mann–Whitney U test was employed to compare median plasma MNP concentrations between independent groups. This included a comparison by sex, evaluating males ($n = 17$) versus females ($n = 33$), as well as a comparison by body mass index (BMI). For the BMI analysis, subjects were categorized into quartiles based on measured BMI, and the comparison was conducted between the highest quartile (Q4, $n = 19$) and the lowest quartile (Q1, $n = 31$). All statistical tests were two-tailed, and a p -value < 0.05 was considered statistically significant.

To complement null hypothesis significance testing, non-parametric effect sizes and 95% confidence intervals (CIs) were calculated. For the independent cross-sectional cohorts of sex and stratified BMI, the magnitude of the distribution shift was evaluated using the rank-biserial correlation coefficient (r), and differences were quantified via the Hodges–Lehmann median difference and its 95% CI. For longitudinal tracking, matching pairs were evaluated using the matched-pairs rank-biserial correlation (r), computed as the ratio of the Wilcoxon signed-rank statistic (W) to the total absolute rank sum, alongside the median of paired differences and its 95% CI. Effect sizes (r) were interpreted as small (≥ 0.10), medium (≥ 0.30), or large (≥ 0.50). All statistical analyses and graphical visualizations were performed using GraphPad Prism (version 9.0, Boston, MA, USA).

3. Results

3.1. Longitudinal Trends in Plasma MNP Concentration

To evaluate temporal changes in internal body burden, 50 paired plasma samples were analyzed from a Baseline period (2014–2018) and a follow-up period in 2023. Initial data screening using the Shapiro–Wilk and Anderson–Darling tests indicated that the distributions were non-normal. A visual assessment via Normal Q–Q Plot confirmed these findings, revealing that while the central data points adhered to the diagonal ref-

erence line, several low-value outliers, particularly in the Baseline group, skewed the distribution (Figure 1).

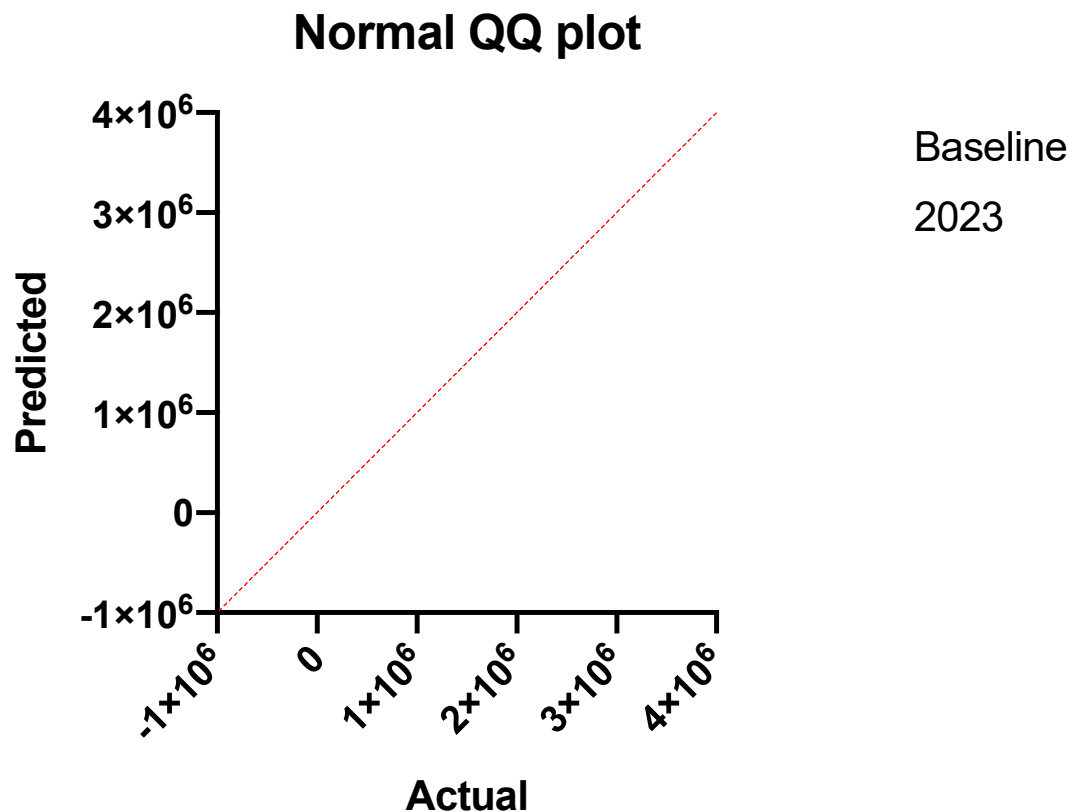


Figure 1. Assessment of Normality for Plasma MNP Concentrations via Normal Q–Q Plot. This plot displays the distribution of MNP concentrations for the Baseline (circles) and 2023 follow-up (squares) cohorts ($n = 50$ pairs). The observed (actual) values are plotted against predicted values from a theoretical Gaussian distribution. This visual assessment, combined with formal testing, justified the use of non-parametric methods, including the Wilcoxon matched-pairs signed-rank and Mann–Whitney U tests, for all subsequent comparative analyses.

Methodologically, this deviation justified non-parametric testing across subsequent comparative analyses. Critically, this distribution profile, coupled with a weak, non-significant pairing correlation ($r_s = 0.1891$, $p = 0.0943$), demonstrates that individual baseline concentrations were not predictive of long-term follow-up burdens. Because of this non-normality, a Wilcoxon matched-pairs signed-rank test was utilized to evaluate temporal shifts. The analysis demonstrated a significant increase in internal MNP accumulation from Baseline to the 2023 follow-up window ($p = 0.0145$, Figure 2). The median of the paired differences between the two periods was 0.4078×10^6 MNP/ μL , with a 95% confidence interval spanning 0.1293×10^6 to 0.7845×10^6 MNP/ μL . This shift reflects a medium-to-large expansion in tissue burden over time, as indicated by a matched-pairs rank-biserial correlation of $r = 0.4069$. This longitudinal rise suggests that environmental exposure continuously exceeds physiological clearance, and accumulation is non-linear (Figure 2). While macro-environmental drivers (e.g., the COVID-19 surge in single-use plastics) raised baseline exposure population-wide, individual trajectories remain highly variable, likely driven by personal lifestyle, micro-environmental factors, and metabolic clearance differences.

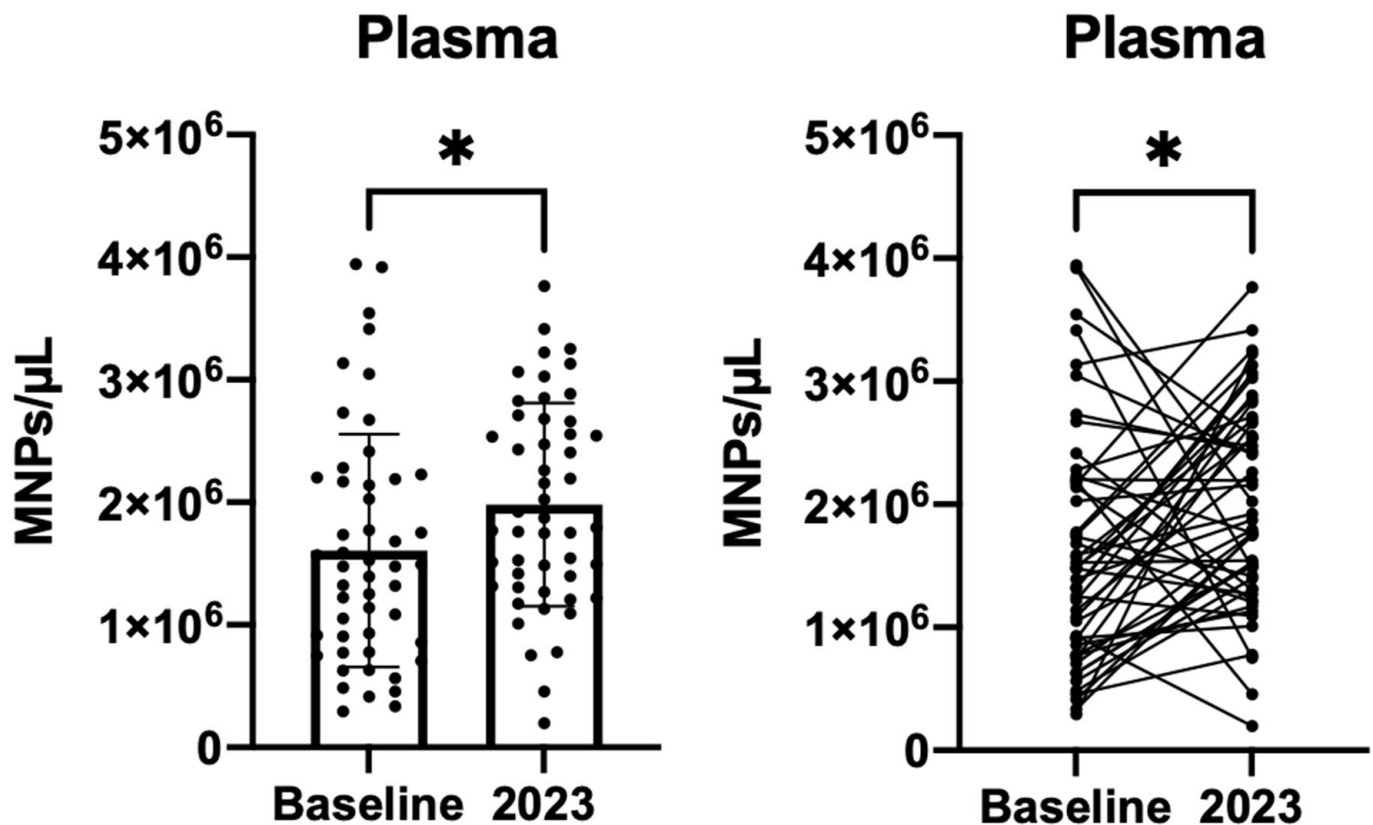


Figure 2. Comparison of MNP Accumulation in Human Plasma Across Time. This figure illustrates the concentration of MNPs detected in human plasma samples, analyzed by longitudinal comparison (Baseline vs. 2023). A paired analysis of subjects reveals a statistically significant increase in median MNP concentration from Baseline to 2023 (Wilcoxon matched-pairs signed rank test, $p = 0.0145$), suggesting progressive bioaccumulation over time. (* $p < 0.05$).

3.2. Effect of Sex and Body Mass Index on Plasma MNP Accumulation

A comparative analysis of plastic body burden by biological sex revealed a statistically significant difference in median MNP accumulation (Mann–Whitney $U = 150.0$, $p = 0.0068$), representing a large effect size ($r = 0.4652$). Males ($n = 17$) exhibited a higher median concentration of 2.09×10^6 NP/ μL , while females ($n = 33$) presented a lower median accumulation of 1.58×10^6 NP/ μL . This resulted in an actual difference in medians of 0.52×10^6 NP/ μL . The non-parametric Hodges–Lehmann median difference between the sexes was 0.4704×10^6 MNP/ μL (95% CI: 0.1287×10^6 to 0.8541×10^6 MNP/ μL), indicating that the male cohort carried a substantially greater internal load within this study population. The influence of body mass on MNP sequestration was assessed by comparing the polar extremities of the BMI distribution (Q1 vs. Q4). When pooling all data points ($n = 100$), the highest BMI quartile (Q4, $n = 19$) demonstrated a significantly greater median plastic accumulation of 2.01×10^6 NP/ μL compared to the lowest quartile (Q1, $n = 31$), which had a median of 1.53×10^6 NP/ μL (Mann–Whitney $U = 138.5$, $p = 0.0173$, Figure 3). The Hodges–Lehmann median difference between these extreme strata was 0.3601×10^6 MNP/ μL (95% CI: 0.0763×10^6 to 0.9421×10^6 MNP/ μL), confirming a robust effect size ($r = 0.4302$). These findings suggest that increased MNP accumulation occurs within extreme phenotypic groups (Figure 3).

Analysis of BMI quartiles at individual time points revealed consistent trends, though they did not reach independent statistical significance when analyzed in isolation. At the Baseline time point, a marginal non-significance was observed ($p = 0.0571$), with median accumulation in Q4 reaching 1.74×10^6 NP/ μL versus 1.22×10^6 NP/ μL in Q1. At the

2023 Follow-up time point, the trend persisted ($p = 0.1150$), with Q4 exhibiting a median of 2.53×10^6 NP/ μ L compared to 1.75×10^6 NP/ μ L in Q1. The relative decrease in statistical strength at the 2023 follow-up may be attributed to the use of baseline BMI measurements for later time points. Because BMI is dynamic, individual fluctuations in body mass over the intervening years may have introduced variability that masks the strength of the association in later cross-sections.

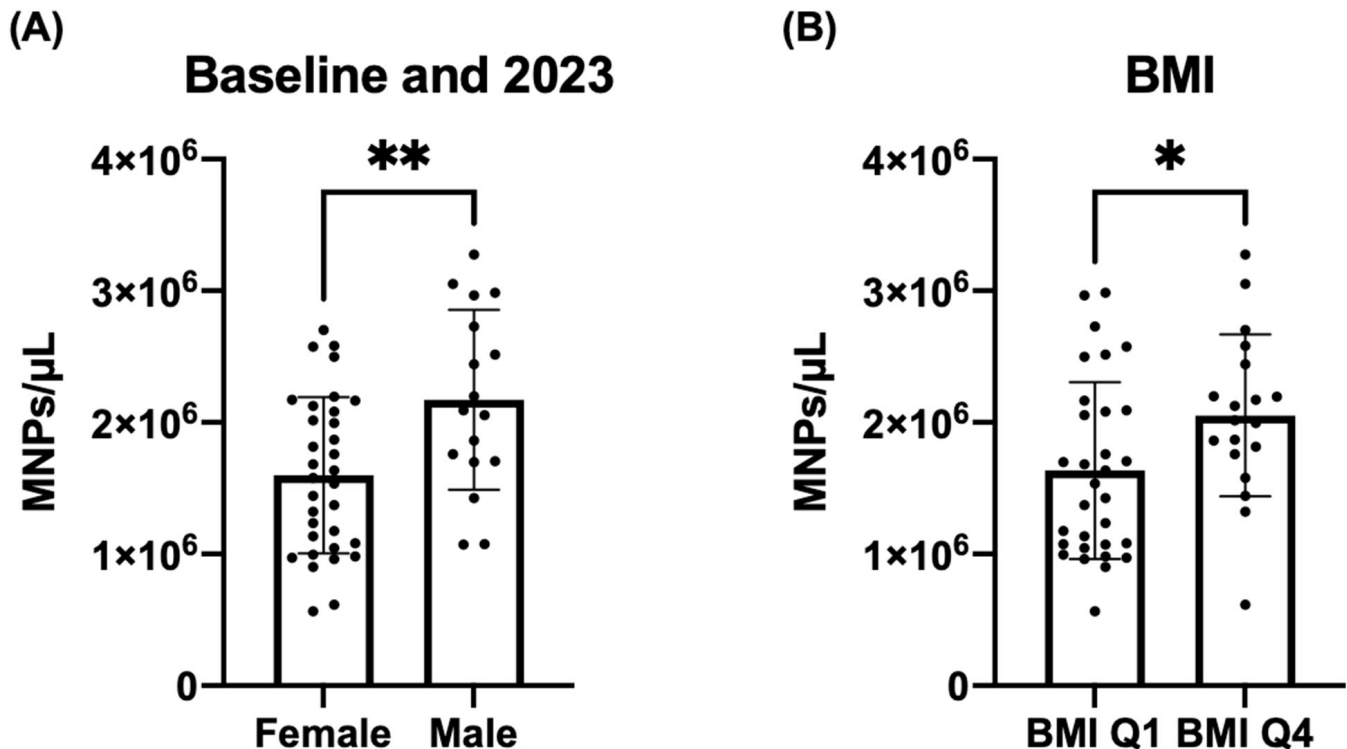


Figure 3. Influence of Sex and Body Mass Index (BMI) on MNP Accumulation in Human Plasma. This figure illustrates MNP concentrations (particles/unit volume) across demographic and anthropometric factors ($n = 100$ total samples). Boxplots represent the median, IQR, and range. (A) Sex-Dependent Accumulation: Males exhibit significantly higher median concentrations (2.09×10^6 NP/ μ L) than females (1.58×10^6 NP/ μ L) (Mann–Whitney U, $p = 0.0068$), suggesting sex-specific toxicokinetics. (B) BMI Influence: The highest BMI quartile (Q4) shows significantly greater median accumulation (2.01×10^6 NP/ μ L) compared to the lowest (Q1; 1.53×10^6 NP/ μ L) (Mann–Whitney U, $p = 0.0173$), supporting the hypothesis that adipose tissue acts as a sequestration reservoir. (* $p < 0.05$, ** $p < 0.01$).

3.3. Extended Analysis of Trends and Demographics

The longitudinal assessment of plastic particle accumulation in human plasma over several years demonstrates a consistent upward trend, suggesting a gradual accumulation of these particles within the body over time. While initial statistical analysis did not show significant differences in concentration across individual years, the observable trajectory indicates a steady increase in internal burden (Figure 4).

When stratifying this accumulation by both sex and Body Mass Index (BMI) quartiles, a clear pattern emerges where male groups consistently exhibit higher median MNP concentrations than female groups across all BMI categories. Furthermore, within both sex subgroups, there is a visible trend showing an increase in median MNP concentration from the lowest (Q1) to the highest (Q4) BMI quartiles. The highest overall concentrations are concentrated within the male Q4 group, likely reflecting the combined influence of greater sequestration capacity in individuals with high BMI and overall sex-specific exposure or metabolic dynamics (Figure 5). This demographic interplay is further supported by cohort-level analysis—comparing Baseline Females, Baseline Males, 2023 Females, and

2023 Males—which highlights how both sex and time point jointly influence circulating MNP levels (Figure 6).

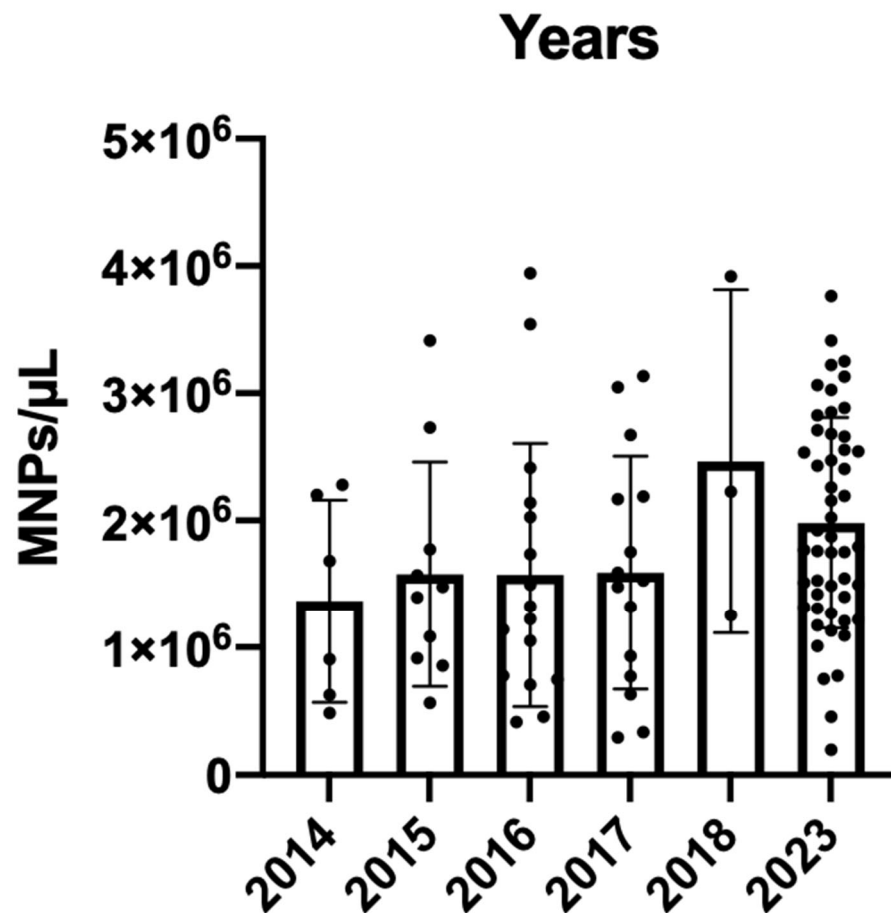


Figure 4. Longitudinal trends of plastic particle accumulation in human plasma. The graph illustrates the concentration of plastic particles detected in plasma samples collected over several years. Although statistical analysis revealed no significant differences in concentration across the years, a consistent upward trend suggesting gradual accumulation over time is observable. Error bars denote the standard error of the mean.

A focused longitudinal assessment of extreme BMI quartiles (Q1 vs. Q4) reveals that while MNP concentrations rose across all cohorts by 2023, individuals in the highest quartile consistently maintained a significantly elevated plastic burden than those in the lowest (Figure 7). This sustained trend points to a positive correlation between higher BMI and MNP sequestration, suggesting that adipose tissue distribution or metabolic traits influence the retention of hydrophobic nanoplastics. Rather than relying on broad averages, these data demonstrate how individual physiological factors dictate long-term particle kinetics, reinforcing the role of adipose tissue as a biological reservoir and sex as a key determinant in internal plastic load.

Comparing the outcomes across Figures 4–7 reveals a coherent, complementary pattern that strongly reinforces our baseline findings (Figures 1–3). While demographic and metabolic stratifications (Figures 4 and 5) establish male sex and elevated BMI as major drivers of MNP burden, the longitudinal trajectories (Figures 6 and 7) demonstrate that individual accumulation progresses alongside changing metabolic profiles over time. Crucially, because our early technical controls (Figures 1–3) confirmed minimal analytical variance, the stability of the global cohort means alongside heterogeneous individual slopes suggests

that systemic MNP levels are governed by a dynamic equilibrium between environmental exposure, metabolic sequestration, and physiological clearance.

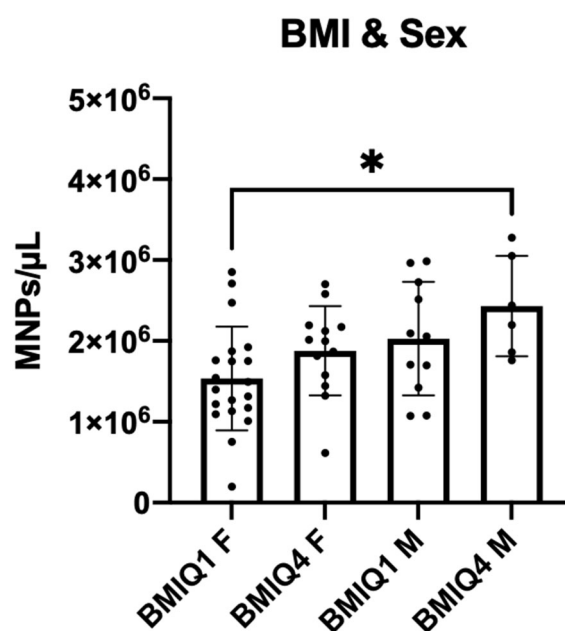


Figure 5. Stratified Analysis of Plasma MNP Accumulation Across BMI Quartiles and Sex. This stratified analysis compares MNP concentrations across BMI quartiles (Q1 vs. Q4) for both sexes. The data reveals a consistent trend: male groups exhibit higher median MNP levels than females, and concentrations increase alongside BMI in both subgroups. The male Q4 group shows the highest overall accumulation, suggesting that higher BMI combined with sex-specific metabolic or exposure factors significantly amplifies MNP sequestration. (* $p < 0.05$).

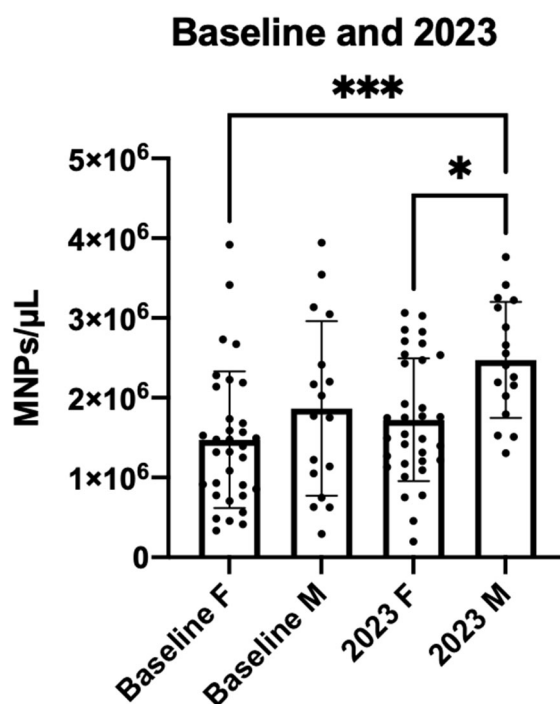


Figure 6. Comparative Analysis of MNP Concentrations Stratified by Biological Sex at Baseline (2014) and Follow-up (2023). Concentration of MNPs across four distinct cohorts, stratified by both sex and time point (Baseline and 2023). The X-axis displays the four groups: Baseline Females, Baseline Males, 2023 Females, and 2023 Males. (* $p < 0.05$, *** $p < 0.001$).

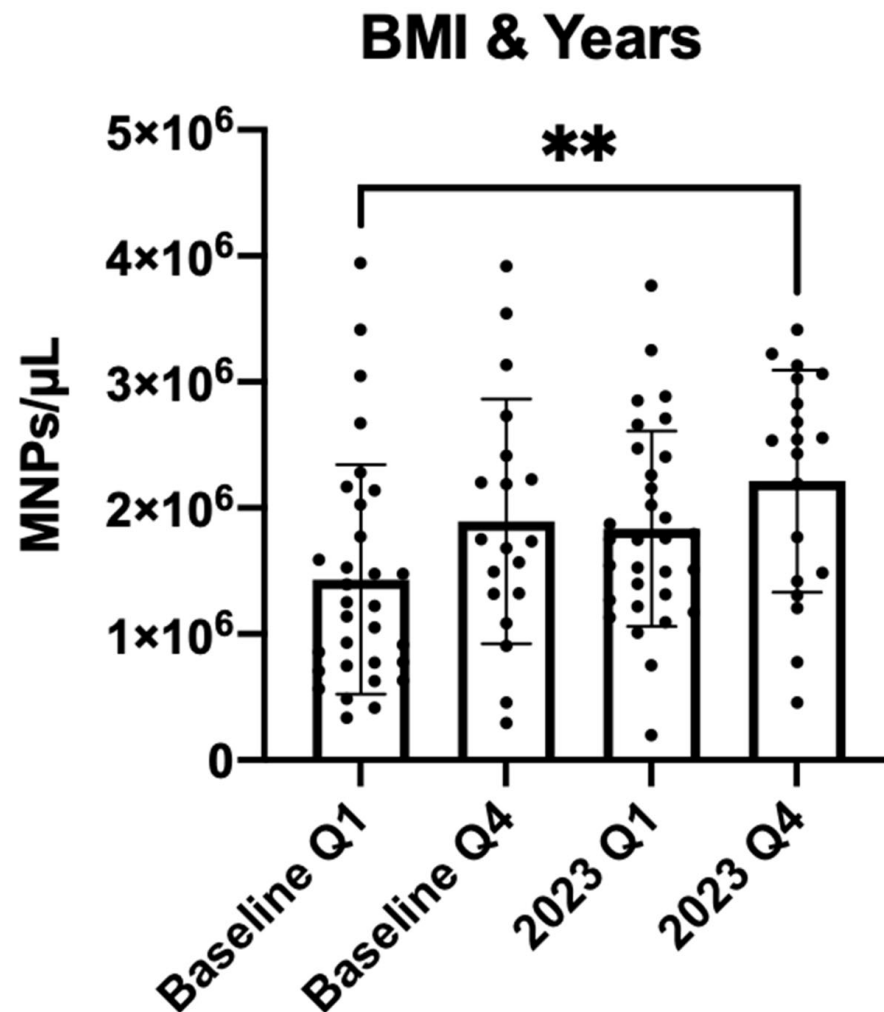


Figure 7. Longitudinal Analysis of Plasma MNP Concentrations Stratified by Body Mass Index (BMI) Quartiles. Comparison of plasma MNP concentrations (MNPs/μL) between the lowest (Q1) and highest (Q4) BMI quartiles across two time points (Baseline vs. 2023). Individual data points, medians, and interquartile ranges (IQR) are shown for each group (Baseline Q1, Baseline Q4, 2023 Q1, and 2023 Q4). A statistically significant increase in plastic accumulation is observed when comparing the lowest baseline group to the highest follow-up group ($p < 0.01$), illustrating the combined effects of temporal bioaccumulation and body mass on internal MNP burden. (** $p < 0.01$).

4. Discussion

4.1. Bioaccumulation and Environmental Loading

Plasma MNP concentrations showed a statistically significant temporal increase ($p = 0.0145$). While this trend aligns with the global surge in plastic production during the COVID-19 pandemic, a direct causal link remains unproven. Nonetheless, these findings suggest that ongoing microplastic exposure may exceed the body's clearance capacity, leading to progressive bioaccumulation throughout adulthood [20,22]. Our longitudinal findings are consistent with recent evidence of systemic MNP distribution in other large mammalian models. For instance, nanocytometry studies on fin whales (*Balaenoptera physalus*) show that MNPs travel beyond the digestive tract into peripheral blood and cross the placenta, highlighting their high mobility and persistence in the mammalian circulatory system [19]. The ubiquity of this exposure is further underscored by recent analyses of common dietary sources. Research shows MNPs are present in beverages and infant foods across all packaging types [20]. This suggests that widespread environmental contamination drives primary human intake. Detection of MNPs in human plasma is also

supported by recent clinical findings. For example, plastic particles have been identified in human peripheral blood [21], thrombi [11], and carotid artery plaques, where their presence has been linked to an increased risk of cardiovascular events [11,15].

The positive median difference (0.41×10^6 NP/ μ L particles) highlights the scale of this accumulating burden. The analysis of the paired data from Baseline to 2023 revealed a statistically significant increase ($p = 0.0145$) in the measured values. The positive sum of signed ranks ($W = 503.0$) and a substantial median difference of 0.41×10^6 indicate that the majority of subjects experienced an upward shift over this period. However, it is important to note that the pairing was not significantly effective ($p = 0.0943$). This lack of correlation suggests that an individual's starting value at Baseline was not a strong predictor of their 2023 outcome. Visually, while the overall trend is upward, there is considerable heterogeneity in the individual trajectories (i.e., some subjects increased sharply while others remained stable or decreased). While the intervention or time period (2023) resulted in a significant population-level improvement compared to Baseline, the high degree of individual variability suggests that other unmeasured factors may be influencing how specific subjects respond over time. These higher absolute particle counts (1.53×10^6 to 2.09×10^6 particles/ μ L) are detected by our analytical platform. While conventional techniques (e.g., mass spectrometry or spectroscopy) report findings in mass units or are restricted to particles larger than 10–20 μ m, our system uses a violet side-scatter filter kit (405 nm) and low flow-rate (12.5 μ L/min) volumetric absolute counting. This configuration optimizes the signal-to-noise ratio for sub-micron and nanometer-scale particles, which increase exponentially in number by weight as diameter decreases.

4.2. Sex as a Determinant of Microplastic Burden

The data from the Normal Q–Q Plot demonstrated a non-normal distribution of the data, revealing individual outliers at the lower extremities while most subjects followed a predictable distribution. Furthermore, the weak and non-significant pairing effectiveness ($r_s = 0.1891$) demonstrates that individual longitudinal trajectories are highly heterogeneous.

A highly significant difference was observed between the sexes ($p = 0.0068$), with males exhibiting substantially higher median circulatory concentrations. While this disparity may be influenced by differential environmental exposure, such as occupational or behavioral differences, it is also linked to established physiological mechanisms of lipid transport, metabolism, and body fat distribution. Because nanoplastics are lipophilic, their vascular transport is mediated by plasma lipoproteins, specifically apolipoprotein B (ApoB)-containing fractions like LDL and VLDL [26]. Endogenous estrogens downregulate hepatic VLDL production and increase LDL receptor expression, resulting in lower circulating ApoB-lipoprotein concentrations in females compared to age-matched males [27]. Consequently, males possess a larger pool of circulating lipid vehicles available to retain lipophilic contaminants within the bloodstream (Wang et al. 2011) [28].

This transport dynamic interacts directly with sex-specific adipose tissue topography and metabolic rates. Females exhibit a predominantly gluteofemoral, subcutaneous adipose distribution with low metabolic turnover, which functions as a stable repository for lipophilic compounds, matching a profile of stable peripheral tissue sequestration and lower systemic concentrations [29]. In contrast, males exhibit a visceral, abdominal fat pattern characterized by higher lipolytic activity. In overweight (25.0 to 29.9 kg/m²) and obese (greater than or equal to 30 kg/m²) profiles, this elevated lipolysis continually mobilizes stored fatty acids and associated MNPs back into the portal vein and systemic circulation, favoring ongoing vascular transport and visceral mobilization [30].

Ultimately, these findings suggest that hormonal regulation of lipid metabolism fundamentally influences the rates at which MNPs are cleared from the blood or se-

questered into tissues. This establishes sex-specific lipid kinetics and physiological variations as critical variables that must be integrated into future risk assessment modeling and toxicokinetic research [31].

4.3. *The Role of Adipose Tissue as a Plastic Reservoir*

The significant association between the highest body mass index quartile and increased MNP concentration ($p = 0.0173$) aligns with the hydrophobic and lipophilic properties of common synthetic polymers and plasticizing additives, which partition preferentially into lipid-rich environments [32]. Providing strong empirical support for this sequestration model, individuals in the highest quartile exhibited a significantly greater median plastic accumulation of 2.01×10^6 particles/ μL compared to the 1.53×10^6 particles/ μL found in the lowest quartile. Because these MNPs possess high surface-area-to-volume ratios, they associate with lipid core matrices and bypass standard hepatic or renal clearance pathways by hitchhiking on circulating apolipoprotein B-containing lipoproteins for systemic distribution [33]. This correlation may indicate that adipose tissue does not merely store inert particles but functions as a dynamic metabolic sink, meaning individuals with higher adiposity carry a larger long-term tissue reservoir.

Vascular concentration is modulated by lipid storage capacity and metabolic mobilization. Adipose tissue undergoes continuous cellular remodeling and lipid exchange; during periods of fasting, stress, weight loss, or metabolic shifts, lipolysis breaks down stored triglycerides into free fatty acids, co-mobilizing sequestered environmental contaminants back into the bloodstream [30]. This fat-storage dynamic acts in tandem with pronounced sex-specific differences in median concentration ($p = 0.0068$). This divergence is driven by physiological and behavioral variations, including distinct anatomical adipose topography; female gluteofemoral subcutaneous fat exhibits lower lipolytic rates and acts as a stable repository, while male visceral fat undergoes rapid lipolysis, promoting continuous vascular transport and particle re-release into the portal circulation [27]. Together, these findings indicate that sex and body mass index are critical determinants of the internal plastic burden.

While cross-sectional comparisons at isolated time points, Baseline ($p = 0.0571$) and 2023 ($p = 0.1150$), did not independently reach significance, the robustness of the pooled dataset ($p = 0.0173$) indicates that the relationship between body mass and plastic burden is persistent and biologically relevant, but requires high statistical power to resolve against background noise and inter-individual variability. Furthermore, a low pairing correlation demonstrates that internal levels are not a linear function of chronological time or cumulative past exposure. Instead, the human plastic load exists in a state of dynamic equilibrium where current environmental interactions and recent, high-intensity lifestyle changes can rapidly supersede an individual's historical baseline. This supports the hypothesis that abrupt changes in external exposure—such as the massive surge in single-use polypropylene personal protective equipment during COVID-19 lockdowns—may potentially influence circulating vascular concentrations [34].

4.4. *Limitations and Future Directions*

While this study provides evidence of an increasing internal MNP burden, several limitations must be acknowledged. First, the observed temporal increase is correlative; without a non-pandemic control cohort, it is difficult to isolate the specific impact of the COVID-19 plastic surge from the general upward trend in global plastic pollution. Second, while plasma is a critical matrix for monitoring systemic trafficking, it represents only a transient transport phase and does not account for the total accumulated burden sequestered in deep tissues. Third, our current methodology relies on NR staining com-

bined with flow cytometry. Despite its value as an established screening tool for MNPs in complex biological matrices, high-throughput nanocytometry faces distinct analytical constraints. Crucially, it cannot provide direct chemical polymer identification (e.g., via μ FTIR, Raman spectroscopy, or Py-GC/MS). Moreover, because NR is a solvatochromic dye that partitions non-specifically into hydrophobic domains rather than distinct polymer species, the method remains susceptible to false-positive counts driven by persistent biogenic debris. Furthermore, because NR can also label residual biogenic lipids and hydrophobic cellular debris, there is an inherent risk of generating false-positive signals [19]. Despite these boundaries, this flow-cytometric approach offers a crucial analytical advantage over traditional spectroscopy. While μ FTIR and Raman spectroscopy are widely regarded as the gold standards for chemical characterization, they feature distinct operational bottlenecks. Micro-FTIR is fundamentally restricted by the diffraction limit of infrared light, which prevents it from resolving or identifying the sub-micron NP fractions (under 1000 nm) that readily traverse biological barriers. Raman spectroscopy offers superior spatial resolution but is severely hindered when processing complex biological matrices due to intense autofluorescence from remaining protein residues and organic matter, which can completely mask the characteristic vibrational signatures of target polymers.

To rigorously address the optical limitations of NR staining and ensure the validity of our particle counts against potential false positives, we implemented a highly controlled thermochemical alkaline digestion protocol. Rather than relying on mild enzymatic or acidic treatments that may leave proteinaceous residues intact, this study utilized an exhaustive 10-day digestion in 1% KOH at 60 °C, operating at a high chemical intensity with a pH exceeding 13.0. This extreme alkaline environment does not merely unfold or clump proteins; instead, it achieves complete alkaline hydrolysis by cleaving peptide bonds, effectively reducing complex plasma proteins and their associated protein coronas into soluble constituent amino acids and small peptides. This deep chemical degradation successfully eliminates the organic matrix, preventing the formation of macro-aggregates that could otherwise cause non-specific dye binding or lead to erratic fluorescence signals.

Our fast-screening platform overcomes these spectroscopic limitations by combining optimized flow-cytometric detection with absolute volumetric particle counting. We validated its nanoscale sensitivity using high-precision fluorescent calibration beads ranging from 100 to 800 nm. This rigorous protocol enabled the instrument to reliably resolve, capture, and quantify sub-100 nm particles. Ultimately, this facilitates the characterization of the highly transportable NP fraction, particles capable of traversing biological barriers while remaining virtually undetectable by conventional spectroscopy.

Acknowledging these analytical boundaries, future workflows must combine ultra-pure, pyrogen-free negative controls with complementary techniques like Py-GC/MS to successfully cross-verify total mass concentrations alongside hyperspectral imaging. This multi-analytical approach is essential to clarify whether internal MNP increases are driven by medical-grade polymers, dietary packaging influences, or broader environmental sources.

Previous research indicates that MNP accumulation in human plasma is both time- and age-dependent, with older demographics exhibiting higher median concentrations. The age range for this longitudinal study was informed by preliminary data [22], which demonstrated a clear correlation between age and internal MNP body burden. While MNPs are detectable at birth, concentrations rise progressively through adulthood, with the most significant accumulation typically occurring after the third decade of life. Consequently, the current cohort was selected to capture the escalation during these high-accumulation years. Nevertheless, investigating physically active 20- to 30-year-olds represents a logical next step. This demographic resides at the threshold of the observed upward trend in

MNP sequestration. Understanding how high-intensity physical activity, and its associated environmental exposures, such as elevated respiratory rates and the use of synthetic polymer hydration containers, influences this early-stage accumulation is essential for developing preventative health strategies. While our paired longitudinal design offers high statistical power by utilizing internal controls, the findings may not be fully generalizable to populations with distinct lifestyles, such as elite athletes or rural cohorts. Future studies should expand this nested design to larger, more diverse populations to validate these trends across broader demographics.

Through high-sensitivity nanocytometry on a paired human cohort, our study provides compelling evidence of a rising internal plastic burden over time. Importantly, our results challenge the long-held assumption that MNP contamination is simply a passive reflection of environmental exposure. Instead, we show that individual physiology plays a dominant role in plastic retention: males consistently carry higher blood concentrations than females, likely due to differences in lipid transport and visceral fat metabolism, while higher body fat acts as a storage reservoir for lipophilic plastic particles. Overall, this research shifts the paradigm toward viewing internal plastic accumulation as a dynamic, bio-individual burden driven by metabolic profiles, sex-specific physiology, and global plastic trends.

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