Nanotechnology, Science and Applications

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Consequences of Dietary Manganese-Based Nanoparticles Supplementation or Deficiency on Systemic Health and Gut Metabolic Dynamics in Rats

Przemysław Sołek[1], Karolina Różaniecka[1], Jerzy Juśkiewicz[2], Bartosz Fotschki[2], Anna Stępniowska[1], Katarzyna Ognik[1]

[1]Department of Biochemistry and Toxicology, University of Life Sciences, Lublin, Poland; [2]Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland

Correspondence: Przemysław Sołek, Department of Biochemistry and Toxicology, University of Life Sciences, Akademicka 13, Lublin, 20-950, Poland, Tel +48814456839, Email pp.solek@gmail.com; przemyslaw.solek@up.lublin.pl

Introduction: Trace elements such as manganese (Mn) are essential for various biological processes, including enzyme activation, metabolic pathways, and antioxidant defences. Given its involvement in these critical processes, maintaining adequate Mn levels is crucial for overall health.

Methods: The experimental design involved 24 male Wistar rats divided into three groups (n=8 per group): a control group receiving standard Mn supplementation (65 mg/kg), an Mn-deficient group, and a group supplemented with Mn_2O_3 nanoparticles (65 mg/kg). The 12-week feeding trial assessed selected physiological parameters, tissue composition, caecal health, and biochemical markers.

Results: Body and major organ weights were not significantly affected across groups (p=0.083 to p=0.579). However, significant differences were observed in fat tissue percentage (p=0.016) and lean tissue percentage (p<0.001). Caecal parameters showed higher ammonia levels (p=0.030) and increased pH (p=0.031) in the nano-Mn group. In turn, total SCFA concentrations were highest in the control group, followed by the Mn-deficient and nano-Mn groups (p<0.001). Enzymatic activities of caecal bacteria differed significantly between the groups, with reduced activity in the nano-Mn group (p<0.001). Blood plasma analysis revealed significantly lower insulin (p<0.001) and neurotransmitter levels, including dopamine and serotonin, in the Mn-deficient and nano-Mn groups compared to controls.

Discussion: Our findings suggest that both Mn supplementation and deficiency can lead to physiological and biochemical alterations, affecting fat metabolism, gut health and microbial enzymatic activity or neurotransmitter levels highlighting the critical role of Mn in maintaining metabolic homeostasis or its potential implications for nutritional and pharmaceutical interventions.

Keywords: manganese, trace elements, nanoparticles, gut health, metabolic homeostasis, enzymatic activity

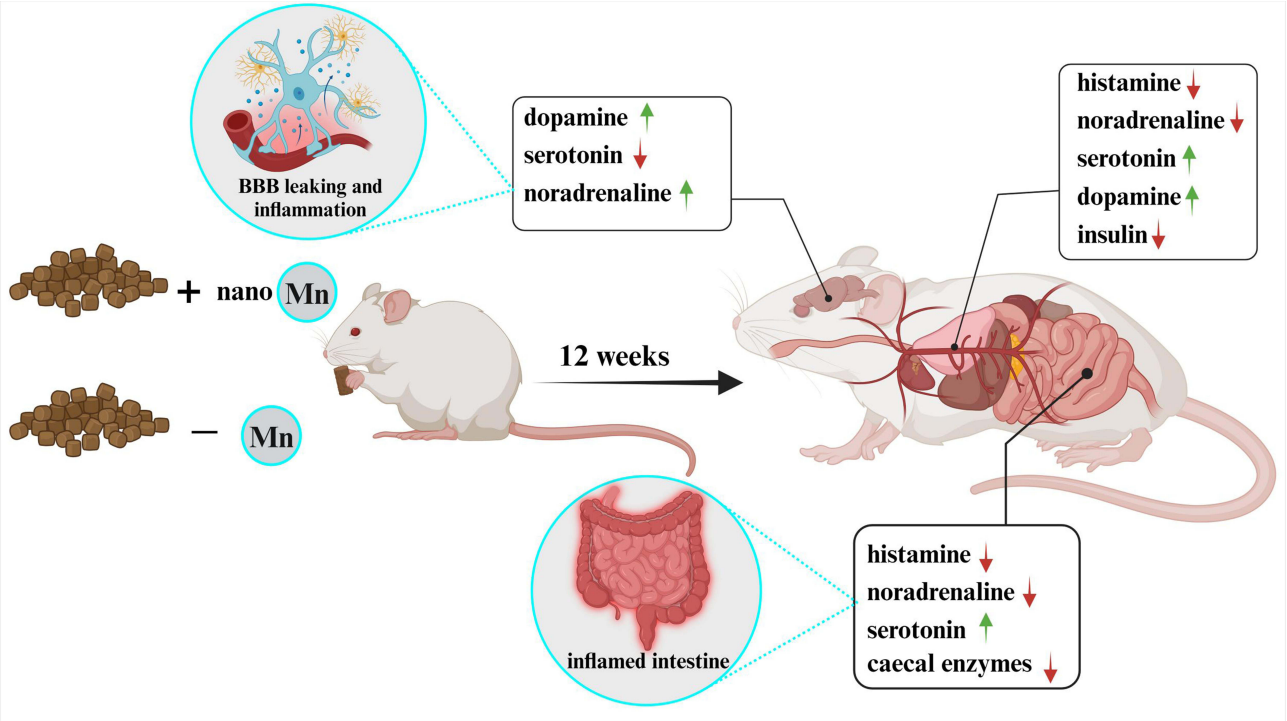
Introduction

Trace elements perform unique and essential functions within the body, and their appropriate intake is crucial for health and well-being. Although required in minimal amounts, they play significant roles in numerous biological processes. Thus, deficiencies in trace elements can lead to various disorders and diseases, depending on the specific functions they fulfil in the body.[1]

Manganese (Mn) is one such trace element that plays a versatile role in the metabolism and energy production of the body, acting as a cofactor for various enzymes involved in key biochemical pathways.[2] Its presence is essential for the proper progression of numerous processes that ensure the maintenance of the body homeostasis. Primarily, manganese is an integral component of enzymes involved in glycolysis, the Krebs cycle, and the pentose phosphate pathway, all fundamental for ATP production, the basic energy carrier in cells.[3,4] Beyond carbohydrates, manganese is involved in

Received: 3 September 2024
Accepted: 17 January 2025
Published: 15 February 2025

Graphical Abstract



amino acids and protein metabolism. It is a constituent of enzymes catalyzing transamination, decarboxylation, and other amino acid transformations[5] necessary for the new protein synthesis and the production of bioactive compounds such as neurotransmitters, important for proper nervous system function. Manganese also participates in lipid metabolism by being a cofactor for enzymes responsible for cholesterol and fatty acid biosynthesis.[6] Cholesterol is a key component of cell membranes and a precursor for the synthesis of steroid hormones such as cortisol, estrogen, and testosterone.[7] Furthermore, manganese impacts the immune system and inflammatory processes, modulating the inflammatory response by influencing immune cell functions and cytokine production.[8]

At the cellular level, manganese is involved in mechanisms protecting cells from oxidative stress, which is associated with the development of many chronic conditions like cardiovascular diseases, cancers, and neurodegenerative diseases, including Alzheimer's and Parkinson's diseases.[9,10] Manganese is an essential component of superoxide dismutase (MnSOD), an antioxidant enzyme that catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide, a process crucial for free radical scavenging.[11] It also affects the function of other antioxidant and detoxifying enzymes, such as glutathione S-transferases (GST), that participate in neutralizing toxic substances by conjugating with glutathione (GSH), thus protecting cells from damage caused by reactive metabolic products and xenobiotics.[12] Protection against oxidative stress is vital for maintaining cellular integrity and biological functions, particularly in tissues with high metabolic activity, such as the brain, muscles, and liver.[13] Thus, manganese indirectly enhances cellular energy efficiency and their ability to regenerate or adapt to environmental conditions changes.

Data suggests that dietary, pharmaceutical, and nanomedicine excipients may exert significant effects on gut microbiota and metabolic health. Studies have demonstrated that these compounds alter gut microbial populations, leading to downstream impacts on systemic physiological processes.[14] Furthermore, recent reviews have highlighted the role of trace elements and excipients in key metabolic and immune function modulation, underscoring their extensive physiological influence.[15,16] Together, these insights underscore the critical need for comprehensive investigations into

the safety profiles and metabolic implications of these substances, especially given their prevalent application in pharmaceutical formulations and dietary products.[17]

The research aimed to investigate the effects of manganese in different forms (traditional supplementation vs nano-emulsion) under conditions of deficiency on various physiological and metabolic parameters. We hypothesize that manganese-based nanoparticles, through their unique physicochemical properties, influence metabolic health by altering gut microbiota composition and enzymatic activity, which in turn affects systemic metabolic processes. Notice that in this study, the control group refers to the diet supplemented with manganese in its standard form. Our approach was intended to a direct comparison between the effects of manganese deficiency and standard supplementation on selected physiological parameters in rats.

We specifically evaluated body composition and examined organ metrics. Additionally, we analyzed caecal health by measuring ammonia levels, pH, and short-chain fatty acid concentrations. We also focused on caecal enzymatic activities to understand how different levels of manganese impact digestive enzyme function and microbial interactions. Furthermore, we examined the influence of manganese on neurotransmitter levels in the blood plasma, intestine, and brain to gain insights into its broader effects on metabolic and neurological health. Our findings aim to elucidate the impact of manganese on metabolic processes and gut health, providing valuable insights into manganeses role in maintaining physiological homeostasis and preventing related disorders.

Materials and Methods

All animal care and experimental protocols complied with the current laws governing animal experimentation in Poland and by an ethical committee according to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, Directive 2010/63/EU for animal experiments (OJEU, 2010), and were approved by the respective Local Institutional Animal Care and Use Committee (No. 13/2022; Olsztyn, Poland, 16.03.2022).

Thirty-eight healthy, male, albino Wistar rats (Cmdb: WI) aged 4 weeks were divided into 3 groups (n=8, each). Rats were housed randomly and individually in stainless steel cages under a stable temperature (21–22°C), relative humidity of 60 ± 10%, a 12-h light-dark cycle, and a ventilation rate of 15 air changes per hour. For 12 weeks, the rats had free access to tap water and semipurified diets, which were prepared and then stored at 4°C in hermetic containers until the end of the experiment (details in Table 1). The diets were modified to a casein diet for laboratory rodents as recommended by the American Institute of Nutrition. In the study, three experimental sets were used to evaluate the effects of different treatments with supplemental Mn in the diet. The control (K) diet contained 65 mg/kg of additional Mn originating from the mineral mixture), group B (negative control) was fed a diet deprived of Mn from the mineral mixture, and group N was fed a diet containing 65 mg/kg Mn from the Mn_2O_3 nanoparticles. The Mn_2O_3 NPs were sourced from Sky Spring Nanomaterials Inc. (Houston, TX, USA). These nanoparticles were selected due to their stable and well-characterized physical and chemical properties, which include a melting point of 1519 K, a boiling point of 2334 K, purity of 99.9%, a size of 40–60 nm and a density of 7.3 g/cm³. The nanoparticles were prepared following standard protocols provided by the supplier to ensure consistency and reproducibility in the experimental setup. To keep the operator safe while preparing the experimental diets, the Mn_2O_3 NPs preparation was added to a diet not in the mineral mixture but as an emulsion along with dietary rapeseed oil. The detailed composition of mineral mixtures used in all experimental groups has been provided in Tables 1 and 2.

Experimental Design

The study aimed to evaluate the effects of different manganese (Mn) sources on various physiological parameters in rats over a 12-week feeding period. The experimental design included three groups with specific dietary manganese conditions, as detailed below (Figure 1).

Sample Collection

Blood and tissue fragments were collected and immediately processed to preserve their integrity for ELISA analysis. Blood samples were transferred into anticoagulant-coated tubes to prevent clotting and gently mixed. Tissue fragments were placed into sterile, pre-labeled containers for further processing. For blood samples, the collected tubes were centrifuged at 4°C for 10–15 minutes at 1,500–2,000 g and the supernatant was then carefully transferred into new, sterile

Table 1 Composition of Basal Experimental Diet Fed to Rats

Ingredient	Content (%)
Unchangeable Ingredients	
Casein[1]	**14.8**
DL-methionine	**0.2**
Cellulose[2]	**8.0**
Choline chloride	**0.2**
Rapeseed oil	**8.0**
Cholesterol	**0.3**
Vitamin mix[3]	**1.0**
Maize starch[4]	**64.0**
Changeable ingredient:	
Mineral mix (MX)[5]	**3.5**
Calculated content	
Crude protein	**13.5**

Notes: [1]Casein preparation: crude protein 89.7%, crude fat 0.3%, ash 2.0%, and water 8.0%. [2]α-Cellulose (SIGMA, Poznan, Poland), the main source of dietary fibre. [3]AIN-93G-VM (Reeves, 1997), g/kg mix: 3.0 nicotinic acid, 1.6 Ca pantothenate, 0.7 pyridoxine-HCl, 0.6 thiamin-HCl, 0.6 riboflavin, 0.2 folic acid, 0.02 biotin, 2.5 vitamin B-12 (cyanocobalamin, 0.1% in mannitol), 15.0 vitamin E (all-rac-α-tocopheryl acetate, 500 IU/g), 0.8 vitamin A (all-trans-retinyl palmitate, 500000 IU/g), 0.25 vitamin D-3 (cholecalciferol, 400000 IU/g), 0.075 vitamin K-1 (phylloquinone), 974.655 powdered sucrose. [4]Maize starch preparation: crude protein 0.6%, crude fat 0.9%, ash 0.2%, total dietary fibre 0%, and water 8.8%. [5]Changeable dietary ingredient in relation to manganese level; mineral mixture (the base according to NRC, 1995) with standard Mn level and deprived of Mn.

Table 2 Composition of Mineral Mixtures (MX) Used in Experimental Diets

	MX with Standard Mn Dosage[1]	MX Deprived of Mn[2]
Calcium carbonate anhydrous $CaCO_3$	**357**	**357**
Potassium phosphate monobasic K_2HPO_4	**196**	**196**
Potassium citrate $C_6H_5K_3O_7$	**70.78**	**70.78**
Sodium chloride NaCl	**74**	**74**
Potassium sulphate K_2SO_4	**46.6**	**46.6**
Magnesium oxide MgO	**24**	**24**
Microelements mixture[#]	**18**	**18**
Starch	**To 1000 g = 213.62**	**To 1000 g = 213.62**

(*Continued*)

Table 2 (Continued).

	MX with Standard Mn Dosage[1]	MX Deprived of Mn[2]
Microelements mixture		
Ferric citrate [16.7% Fe]	**31**	**31**
Zinc carbonate $ZnCO_3$ [56% Zn]	**4.5**	**4.5**
Manganous carbonate $MnCO_3$ [44.4% Mn]	**23.4**	**0**
Copper carbonate $CuCO_3$ [55.5% Cu]	**1.85**	**1.85**
Potassium iodate KJ	**0.04**	**0.04**
Citric acid $C_6H_8O_7$	**To 100 g = 39.21 g**	**To 100 g = 62.61**

Notes: [1]given to K group (12 weeks of feeding), [2]given to B and N groups (12 weeks of feeding), but the N group was provided with the appropriate amount of Mn from Mn_2O_3 nanoparticles preparation as an emulsion along with dietary rapeseed oil.

tubes for ELISA analysis. For tissue samples, the fragments were immediately homogenized in an appropriate lysis buffer (PBS with protease inhibitors) using a tissue homogenizer. The homogenized tissue was centrifuged and the supernatant was collected for ELISA analysis. Both plasma and tissue supernatant samples were stored at −80°C until required for ELISA assays. For the ELISA procedure, the samples were thawed on ice, mixed thoroughly, and then diluted as necessary before being added to the microplate wells according to the assay protocol.

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA assay was performed in accordance with.[18] In detail, each standard and sample were added to the appropriate wells of the microplate and incubated for 1 hour at room temperature with gentle shaking to ensure adequate binding of the target antigen to the immobilized antibodies. Following incubation, the plates were washed four times with wash buffer to remove any unbound substances. After washing, 100 µL of the enzyme-conjugated detection antibody solution was added to each well. The plates were then incubated for an additional 1 hour at room temperature with gentle shaking.

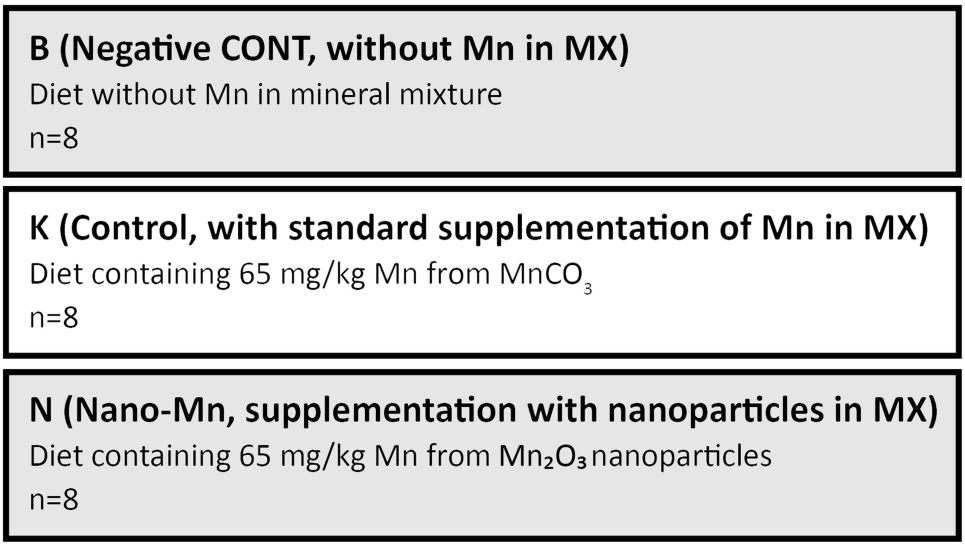


Figure 1 Experimental groups and feeding protocols. Experimental design showing the allocation of rats into three groups based on dietary manganese (Mn) supplementation over a 12-week feeding period.

Abbreviations: 5-HT, Serotonin; ANOVA, Analysis of Variance; ATP, Adenosine Triphosphate; B: Mn-Deficient Group (Negative Control); DA, Dopamine; ELISA, Enzyme-Linked Immunosorbent Assay; EU, European Union; GSH, Glutathione; GST, Glutathione S-Transferase; HIST, Histamine; K: Control Group; miRNAs, MicroRNAs; Mn, Manganese; MnO, Manganese(II) oxide; MnO_2, Manganese dioxide; Mn_2O_3 NPs, Manganese(III) oxide nanoparticles; MnSOD, Manganese Superoxide Dismutase; N: Nano-Mn Group; NA, Noradrenaline (Norepinephrine); NP, Nanoparticle; PBS, Phosphate-buffered saline; PPARγ, Peroxisome proliferator-activated receptor gamma; PSCFA, Putrefactive Short-Chain Fatty Acids; SCFA, Short-Chain Fatty Acids; SEM, Standard Error of the Mean; T3, Triiodothyronine; T4, Thyroxine.

Subsequently, the plates were washed four times with wash buffer to remove any unbound detection antibody. To develop the signal, 100 µL of the substrate solution was added to each well. The plates were incubated at room temperature in the dark for 30 minutes. To terminate the enzymatic reaction, stop solution was added to each well and absorbance was measured using a microplate reader at 450 nm wavelength. The absorbance values were analyzed and compared to the standard curve to determine the concentration of the target antigen in each sample.

Power Analysis and Statistical Significance

The sample size for this study (n=8 per group) was determined based on prior studies investigating the physiological and metabolic effects of manganese in rodent models, as well as practical considerations related to ethical guidelines and resource availability. While a formal power analysis was not conducted, this sample size aligns with similar experimental designs reported in the literature and was deemed sufficient to detect differences between groups tested. Moreover, the study design was developed in accordance with the ARRIVE guidelines to ensure transparency and reproducibility. Results were expressed as mean values with standard error of the mean (SEM). The statistical significance of differences between the experimental groups [Control (K), Nano-Mn (N), and Without Mn (B)] was assessed using a one-way analysis of variance (ANOVA) followed by a post-hoc test.

Results

At the beginning, manganese supplementation or deficiency did not significantly affect body weight among the experimental groups (p=0.083). Furthermore, no significant differences were observed in the weights of testes, pancreas, lungs, liver, heart, and spleen per 100 g of body weight, with p-values ranging from 0.122 to 0.579. In contrast, a significant difference was detected in fat tissue percentage among the groups (p=0.016). Specifically, the nano-Mn group had a higher fat tissue percentage (8.77%) compared to the without Mn group (6.99%), while the control group (7.92%) fell between these two values. A highly significant difference was also found in lean tissue percentage (p<0.001). The without Mn group demonstrated a significantly higher lean tissue percentage (66.8%) compared to both the control group (64.6%) and the nano-Mn group (65.4%). Additionally, a significant difference in kidney weight per 100 g body weight was observed (p=0.006). The without Mn group exhibited a higher kidney weight (0.609 g) compared to both the control (0.577 g) and nano-Mn (0.584 g) groups (Table 3).

Table 3 Comparative Analysis of Body Weight and Organ Metrics in Rats Exposed to Different Manganese Treatments

	Control (K)	Nano-Mn (N)	Without Mn (B)	SEM	p-value
Body weight, g	387	386	368	4.242	0.083
Fat tissue, %	7.92^{ab}	8.77^{a}	6.99^{b}	0.295	0.016
Lean tissue, %	64.6^{b}	65.4^{b}	66.8^{a}	0.257	<0.001
Testes, g/100 g BW	0.810	0.817	0.836	0.006	0.122
Pancreas, g/100 g BW	0.233	0.231	0.277	0.018	0.338
Lungs, g/100 g BW	0.319	0.314	0.318	0.003	0.579
Liver, g/100 g BW	4.21	4.25	4.34	0.030	0.093
Heart, g/100 g BW	0.370	0.375	0.361	0.003	0.123
Spleen, g/100 g BW	0.204	0.197	0.197	0.003	0.330
Kidneys, g/100 g BW	0.577^{b}	0.584^{b}	0.609^{a}	0.005	0.006

Notes: SEM, pooled standard error of mean (standard deviation for all rats divided by the square root of rat number, n=24); ^{a,b} Mean values within a row with unlike superscript letters are shown to be significantly different (P<0.05); Fat and Lean tissue measured by NMR procedure (nuclear magnetic resonance).

The assessment of manganese effects on caecal parameters revealed several significant findings. Ammonia levels were notably higher in the nano-Mn group (0.300 mg/g) compared to both the control (0.258 mg/g) and Mn-deficient groups (0.298 mg/g), with significant differences observed (p=0.030). The pH of the digesta was also significantly affected, being higher in the nano-Mn group (6.93) compared to the control (6.71) and marginally higher than the Mn-deficient group (6.79), with a significant p-value of 0.031.

In terms of short-chain fatty acids (SCFAs), significant variations were noted. Acetic acid levels were highest in the control group (38.1 µmol/g), followed by the Mn-deficient group (33.7 µmol/g), and lowest in the nano-Mn group (28.6 µmol/g), with substantial differences (p<0.001). For propionic acid, we observed a similar trend, being highest in the control group (9.58 µmol/g) and lowest in the nano-Mn group (6.87 µmol/g) (p<0.001). Iso-butyric acid levels were significantly higher in the nano-Mn group (1.26 µmol/g) compared to the control (0.978 µmol/g) and Mn-deficient groups (1.20 µmol/g), with p=0.004. We also noted that butyric acid levels were significantly affected, with the highest concentration in the control group (5.29 µmol/g), lower in the Mn-deficient group (4.54 µmol/g), and lowest in the nano-Mn group (4.07 µmol/g; p=0.002). In turn, iso-valeric acid and valeric acid concentrations did not show significant differences among the groups (p=0.083 and p=0.185, respectively). However, the sum of putrefactive SCFAs (PSCFA) was significantly higher in the nano-Mn group (3.03 µmol/g) compared to the control (2.56 µmol/g) and marginally lower than the Mn-deficient group (3.08 µmol/g; p = 0.022). We observed that total SCFA concentrations were highest in the control group (55.5 µmol/g), followed by the Mn-deficient group (49.8 µmol/g), and lowest in the nano-Mn group (42.5 µmol/g), with the differences being statistically significant (p<0.001). Moreover, the relative proportions of specific SCFAs, such as acetic acid, propionic acid, and butyric acid, did not show significant variation among the groups, with p-values of 0.222, 0.319, and 0.543, respectively (Table 4).

Next, analysis of caecal bacterial enzymatic activity revealed significant variations among the control, nano-Mn, and Mn-deficient groups. For α-glucosidase activity, extracellular enzyme levels were markedly higher in the control group (14.6 µmol/h/g digesta) compared to the nano-Mn group (7.87 µmol/h/g digesta), with the Mn-deficient group at

Table 4 Impact of Nano-Manganese and Manganese Deficiency on Caecal Parameters in Experimental Groups

	Control (K)	Nano-Mn (N)	Without Mn (B)	SEM	p-value
Ammonia, mg/g	0.258^{b}	0.300^{a}	0.298^{a}	0.008	0.030
pH of digesta	6.71^{b}	6.93^{a}	6.79^{ab}	0.041	0.031
SCFA:					
Acetic acid, µmol/g	38.1^{a}	28.6^{c}	33.7^{b}	1.021	<0.001
Propionic acid, µmol/g	9.58^{a}	6.87^{c}	8.48^{b}	0.303	<0.001
Iso-butyric acid, µmol/g	0.978^{b}	1.26^{a}	1.20^{a}	0.042	0.004
Butyruicacid, µmol/g	5.29^{a}	4.07^{b}	4.54^{b}	0.170	0.002
Ico-valeric acid, µmol/g	0.826	0.958	0.970	0.033	0.083
Valericacid, µmol/g	0.761	0.811	0.908	0.042	0.185
PSCFA, µmol/g	2.56^{b}	3.03^{a}	3.08^{a}	0.092	0.022
Total SCFA, µmol/g	55.5^{a}	42.5^{c}	49.8^{b}	1.335	<0.001
Acetic acid profile, % of SCFA	68.5	67.1	67.6	0.442	0.222
Propionic acid, % of SCFA	17.3	16.2	17.1	0.395	0.319
Butyric acid profile, % of SCFA	9.55	9.56	9.12	0.271	0.543

Notes: C2, acetic acid; C3, propionic acid; C4i, iso-butyric acid; C4, butyric acid; C5i, iso-valeric acid; C5, valeric acid; PSCFA, putrefactive SCFA (sum of C4i, C5i and C5); SCFA, short-chain fatty acid; SEM, pooled standard error of mean (standard deviation for all rats divided by the square root of rat number, n=24); ^{a,b} Mean values within a row with unlike superscript letters are shown to be significantly different (P<0.05).

13.9 µmol/h/g digesta ($p<0.001$). Intracellular α-glucosidase activity was also noted significantly higher in the control group (11.1 µmol/h/g digesta) than in the nano-Mn group (7.36 µmol/h/g digesta) and the Mn-deficient group (4.44 µmol/h/g digesta; $p=0.002$). Total α-glucosidase activity followed a similar trend, being highest in the control group (25.7 µmol/h/g digesta), and lowest in the nano-Mn group (15.2 µmol/h/g digesta) and Mn-deficient group (18.4 µmol/h/g digesta; $p<0.001$). The release rate of α-glucosidase, expressed as a percentage of its total activity, was significantly higher in the Mn-deficient group (75.6%) compared to the control (58.3%) and nano-Mn groups (52.7%; $p<0.001$).

Regarding β-glucosidase activity, extracellular levels were significantly higher in the control group (4.86 µmol/h/g digesta) than in the nano-Mn group (2.23 µmol/h/g digesta), with similar values in the Mn-deficient group (4.30 µmol/h/g digesta; $p<0.001$). Intracellular β-glucosidase activity was observed also significantly elevated in the control group (22.1 µmol/h/g digesta) compared to the nano-Mn group (9.37 µmol/h/g digesta) and the Mn-deficient group (11.6 µmol/h/g digesta; $p<0.001$). Total β-glucosidase activity was highest in the control group (27.0 µmol/h/g digesta), lower in the Mn-deficient group (15.9 µmol/h/g digesta), and lowest in the nano-Mn group (11.6 µmol/h/g digesta; $p<0.001$). The release rate of β-glucosidase, as a percentage of its total activity, was significantly higher in the Mn-deficient group (27.7%) compared to the control (18.6%) and nano-Mn groups (20.3%; $p=0.005$).

For α-galactosidase, extracellular enzyme activity was highest in the control group (18.8 µmol/h/g digesta), lower in the Mn-deficient group (16.2 µmol/h/g digesta), and lowest in the nano-Mn group (8.77 µmol/h/g digesta; $p<0.001$). Intracellular α-galactosidase activity was significantly higher in the control group (40.6 µmol/h/g digesta) compared to both the nano-Mn group (18.0 µmol/h/g digesta) and the Mn-deficient group (18.2 µmol/h/g digesta; $p=0.002$). Total α-galactosidase activity in the control group was highest (59.4 µmol/h/g digesta), reduced in the nano-Mn group (26.8 µmol/h/g digesta), and intermediate in the Mn-deficient group (34.3 µmol/h/g digesta; $p<0.001$). The release rate of α-galactosidase was significantly higher in the Mn-deficient group (48.6%) compared to the control (31.8%) and nano-Mn groups (41.6%; $p=0.012$) (Table 5).

Table 5 Caecal Enzymatic Activity and Release Rates of α-Glucosidase, β-Glucosidase, or α-Galactosidase in Different Treatment Groups

	Control (K)	Nano-Mn (N)	Without Mn (B)	SEM	p-value
α-Glucosidase					
Extracellular, µmol/h/g digesta	14.6[a]	7.87[b]	13.9[a]	0.765	<0.001
Intracellular, µmol/h/g digesta	11.1[a]	7.36[b]	4.44[b]	0.901	0.002
Total, µmol/h/g digesta	25.7[a]	15.2[b]	18.4[b]	1.286	<0.001
Release rate, %	58.3[b]	52.7[b]	75.6[a]	2.834	<0.001
β-Glucosidase					
Extracellular, µmol/h/g digesta	4.86[a]	2.23[b]	4.30[a]	0.291	<0.001
Intracellular, µmol/h/g digesta	22.1[a]	9.37[b]	11.6[b]	1.436	<0.001
Total, µmol/h/g digesta	27.0[a]	11.6[b]	15.9[b]	1.598	<0.001
Release rate, %	18.6[b]	20.3[b]	27.7[a]	1.363	0.005
α-Galactosidase					
Extracellular, µmol/h/g digesta	18.8[a]	8.77[b]	16.2[a]	1.053	<0.001
Intracellular, µmol/h/g digesta	40.6[a]	18.0[b]	18.2[b]	3.368	0.002
Total, µmol/h/g digesta	59.4[a]	26.8[b]	34.3[b]	3.873	<0.001
Release rate, %	31.8[b]	41.6[ab]	48.6[a]	2.688	0.012

Notes: Release rate, extracellular enzyme activity expressed as the percentage of its total activity. SEM, pooled standard error of mean (standard deviation for all rats divided by the square root of rat number, n=24); [a,b] Mean values within a row with unlike superscript letters are shown to be significantly different ($P<0.05$).

The enzymatic activity of β-galactosidase exhibited significant differences among the groups. Extracellular activity was highest in the control group (22.8 µmol/h/g digesta), followed by the Mn-deficient group (20.1 µmol/h/g digesta), and lowest in the nano-Mn group (14.2 µmol/h/g digesta; $p<0.001$). Intracellular activity mirrored this trend, with the control group demonstrating the highest levels (76.1 µmol/h/g digesta), while the nano-Mn (26.7 µmol/h/g digesta) and Mn-deficient (43.3 µmol/h/g digesta) groups showed significantly reduced activity ($p<0.001$). Consequently, total β-galactosidase activity was substantially higher in the control group (98.9 µmol/h/g digesta) compared to the markedly lower levels in the nano-Mn (40.9 µmol/h/g digesta) and Mn-deficient groups (63.4 µmol/h/g digesta; $p<0.001$). The release rate, expressed as a percentage of total activity, was significantly elevated in the nano-Mn (35.7%) and Mn-deficient groups (32.5%) relative to the control group (23.3%; $p=0.009$).

We observed a similar pattern for β-glucuronidase activity. Extracellular enzyme levels were significantly reduced in the nano-Mn group (12.4 µmol/h/g digesta) compared to the control (17.9 µmol/h/g digesta) and Mn-deficient groups (21.5 µmol/h/g digesta; $p<0.001$). The intracellular activity peaked in the Mn-deficient group (80.1 µmol/h/g digesta), followed by the control group (58.2 µmol/h/g digesta), and was lowest in the nano-Mn group (33.0 µmol/h/g digesta; $p<0.001$). Total β-glucuronidase activity was significantly higher in the Mn-deficient group (102 µmol/h/g digesta) compared to the control (76.1 µmol/h/g digesta) and nano-Mn groups (45.5 µmol/h/g digesta; $p<0.001$). The release rate was highest in the nano-Mn group (27.9%), intermediate in the control group (23.6%), and lowest in the Mn-deficient group (21.5%; $p=0.014$).

For β-xylosidase activity, extracellular levels were notably lower in the nano-Mn group (4.83 µmol/h/g digesta) than in the control (7.16 µmol/h/g digesta) and Mn-deficient groups (6.88 µmol/h/g digesta; $p=0.011$). The intracellular activity was highest in the control group (28.5 µmol/h/g digesta), followed by the Mn-deficient (18.7 µmol/h/g digesta) and nano-Mn groups (11.5 µmol/h/g digesta; $p<0.001$). Total β-xylosidase activity was significantly elevated in the control group (35.6 µmol/h/g digesta) compared to the lower levels in the Mn-deficient (25.6 µmol/h/g digesta) and nano-Mn groups (16.3 µmol/h/g digesta; $p<0.001$). The release rate was highest in the nano-Mn group (30.3%), compared to the control (20.5%) and Mn-deficient groups (27.8%; $p=0.022$) (Table 6).

Next, the analysis of α-arabinopyranosidase activity revealed significant variations across the different treatment groups. Extracellular enzyme activity was highest in the control group, registering at 3.96 µmol/h/g digesta, which was significantly greater than the 2.06 µmol/h/g observed in the nano-Mn group. The Mn-deficient group exhibited extracellular activity levels of 3.79 µmol/h/g digesta ($p<0.001$). Intracellular activity also showed the highest values in the

Table 6 Caecal Enzymatic Activity and Release Rates of β-Galactosidase, β-Glucuronidase, or β-Xylosidase in Different Treatment Groups

	Control (K)	Nano-Mn (N)	Without Mn (B)	SEM	p-value
β-Galactosidase					
Extracellular, µmol/h/g digesta	22.8[a]	14.2[b]	20.1[a]	1.042	<0.001
Intracellular, µmol/h/g digesta	76.1[a]	26.7[c]	43.3[b]	4.770	<0.001
Total, µmol/h/g digesta	98.9[a]	40.9[c]	63.4[b]	5.371	<0.001
Release rate, %	23.3[b]	35.7[a]	32.5[a]	1.936	0.009
β-Glucuronidase					
Extracellular, µmol/h/g digesta	17.9[a]	12.4[b]	21.5[a]	1.072	<0.001
Intracellular, µmol/h/g digesta	58.2[b]	33.0[c]	80.1[a]	4.570	<0.001
Total, µmol/h/g digesta	76.1[b]	45.5[c]	102[a]	5.332	<0.001
Release rate, %	23.6[ab]	27.9[a]	21.5[b]	1.057	0.014

(*Continued*)

 https://doi.org/10.2147/NSA.S494533

Table 6 (Continued).

	Control (K)	Nano-Mn (N)	Without Mn (B)	SEM	p-value
β-Xylosidase					
Extracellular, μmol/h/g digesta	7.16[a]	4.83[b]	6.88[a]	0.378	0.011
Intracellular, μmol/h/g digesta	28.5[a]	11.5[c]	18.7[b]	1.815	<0.001
Total, μmol/h/g digesta	35.6[a]	16.3[c]	25.6[b]	1.994	<0.001
Release rate, %	20.5[b]	30.3[a]	27.8[ab]	1.723	0.022

Notes: rate, extracellular enzyme activity expressed as the percentage of its total activity. SEM, pooled standard error of mean (standard deviation for all rats divided by the square root of rat number, n=24); [a,b] Mean values within a row with unlike superscript letters are shown to be significantly different (P<0.05).

control group (2.90 μmol/h/g digesta), while both the nano-Mn (1.25 μmol/h/g digesta) and Mn-deficient groups (1.34 μmol/h/g digesta) had significantly lower levels (p=0.001). When considering total α-arabinopyranosidase activity, the control group demonstrated the highest activity (6.86 μmol/h/g digesta), followed by the Mn-deficient group (5.13 μmol/h/g digesta), and the lowest activity was found in the nano-Mn group (3.31 μmol/h/g digesta; p<0.001). The release rate of α-arabinopyranosidase, indicating the proportion of extracellular activity to total activity, was highest in the Mn-deficient group (74.4%), intermediate in the nano-Mn group (61.7%), and lowest in the control group (58.3%; p=0.038).

In the assessment of β-cellobiosidase activity, extracellular levels were significantly elevated in the control group at 1.24 μmol/h/g digesta, compared to 0.783 μmol/h/g in the nano-Mn group and 1.21 μmol/h/g in the Mn-deficient group (p<0.001). Also, intracellular activity was similarly highest in the control group (0.811 μmol/h/g digesta) and markedly reduced in the nano-Mn (0.195 μmol/h/g digesta) and Mn-deficient groups (0.264 μmol/h/g digesta; p<0.001). Total β-cellobiosidase activity was found high in the control group (2.05 μmol/h/g digesta), significantly lower in the Mn-deficient group (1.47 μmol/h/g digesta), and lowest in the nano-Mn group (0.978 μmol/h/g digesta; p<0.001). The release rate was significantly higher in the nano-Mn (79.9%) and Mn-deficient (82.7%) groups compared to the control group (61.1%; p<0.001).

For β-mannosidase activity, the control group showed the highest extracellular levels at 1.25 μmol/h/g digesta, with the nano-Mn group significantly lower at 0.869 μmol/h/g digesta, and the Mn-deficient group similar to the control at 1.18 μmol/h/g digesta (p=0.015). Intracellular activity peaked in the control group (1.63 μmol/h/g digesta) and was significantly diminished in both the nano-Mn (0.152 μmol/h/g digesta) and Mn-deficient groups (0.445 μmol/h/g digesta; p<0.001). Total β-mannosidase activity followed this trend, being highest in the control group (2.88 μmol/h/g digesta), lower in the Mn-deficient group (1.62 μmol/h/g digesta), and lowest in the nano-Mn group (1.02 μmol/h/g digesta; p<0.001). The release rate of β-mannosidase was significantly higher in the nano-Mn group (85.2%) and the Mn-deficient group (72.0%) compared to the control (44.4%; p<0.001) (Table 7).

We further investigated the role of manganese in various physiological processes management. Our analysis revealed distinct patterns in the levels of different biomarkers across the control, nano-Mn, and Mn-deficient groups. In blood plasma, insulin concentrations were highest in the control group (8.68 μIU/mL), with significantly lower levels observed in both the nano-Mn (6.28 μIU/mL) and Mn-deficient (6.22 μIU/mL) groups (p<0.001). Similarly, histamine (HIST) concentrations were significantly elevated in the control (8.20 ng/mL) and nano-Mn (6.81 ng/mL) groups compared to the Mn-deficient group (4.06 ng/mL) (p<0.001). Dopamine (DA) levels were also higher in the control (26.4 ng/mL) and nano-Mn (29.6 ng/mL) groups relative to the Mn-deficient group (16.1 ng/mL) (p=0.002). A comparable pattern was observed for noradrenaline (NA), with concentrations significantly greater in the control group (3.27 ng/mL) than in both the nano-Mn (2.57 ng/mL) and Mn-deficient (2.68 ng/mL) groups (p=0.009). Additionally, serotonin (5-HT) levels were significantly elevated in the control (26.08 ng/mL) and nano-Mn (28.70 ng/mL) groups compared to the Mn-deficient group (16.02 ng/mL) (p<0.001). In contrast, thyroxine (T4), triiodothyronine (T3), and cortisol levels did not differ

Table 7 Caecal Enzymatic Activity and Release Rates of α-Arabinopyranosidase, β-Cellobiosidase, or β-Mannosidase in Different Treatment Groups

	Control (K)	Nano-Mn (N)	Without Mn (B)	SEM	p-value
α-Arabinopyranosidase					
Extracellular, μmol/h/g digesta	3.96[a]	2.06[b]	3.79[a]	0.237	<0.001
Intracellular, μmol/h/g digesta	2.90[a]	1.25[b]	1.34[b]	0.227	0.001
Total, μmol/h/g digesta	6.86[a]	3.31[c]	5.13[b]	0.344	<0.001
Release rate, %	58.3[b]	61.7[ab]	74.4[a]	3.067	0.038
β-Cellobiosidase					
Extracellular, μmol/h/g digesta	1.24[a]	0.783[b]	1.21[a]	0.062	<0.001
Intracellular, μmol/h/g digesta	0.811[a]	0.195[b]	0.264[b]	0.072	<0.001
Total, μmol/h/g digesta	2.05[a]	0.978[c]	1.47[b]	0.113	<0.001
Release rate, %	61.1[b]	79.9[a]	82.7[a]	2.637	<0.001
β-Mannosidase					
Extracellular, μmol/h/g digesta	1.25[a]	0.869[b]	1.18[a]	0.064	0.015
Intracellular, μmol/h/g digesta	1.63[a]	0.152[b]	0.445[b]	0.146	<0.001
Total, μmol/h/g digesta	2.88[a]	1.02[c]	1.62[b]	0.183	<0.001
Release rate, %	44.4[c]	85.2[a]	72.0[b]	3.837	<0.001

Notes: Release rate, extracellular enzyme activity expressed as the percentage of its total activity. SEM, pooled standard error of mean (standard deviation for all rats divided by the square root of rat number, n=24); [a,b] Mean values within a row with unlike superscript letters are shown to be significantly different (P<0.05).

significantly among the groups. Specifically, T4 concentrations were 85.28 ng/mL in the control group, 81.47 ng/mL in the nano-Mn group, and 82.61 ng/mL in the Mn-deficient group, with a p-value of 0.634. T3 levels were 50.62 pg/mL in the control group, 46.20 pg/mL in the nano-Mn group, and 45.35 pg/mL in the Mn-deficient group, with a p-value of 0.067, indicating a non-significant trend. Cortisol levels were 10.03 ng/mL in the control group, 11.07 ng/mL in the nano-Mn group, and 9.99 ng/mL in the Mn-deficient group, with a p-value of 0.088, further indicating no significant differences across the groups (Table 8).

Subsequently, we analyzed the concentrations of key neurotransmitters in the intestine. Histamine concentrations varied significantly among the groups, with the control group (21.95 ng/g) and the without Mn group (22.01 ng/g) exhibiting significantly higher levels than the nano-Mn group (15.08 ng/g) (p=0.001). In contrast, dopamine levels did not differ significantly across the groups, with concentrations recorded at 185 ng/g in the control group, 172 ng/g in the nano-Mn group, and 168 ng/g in the without Mn group (p=0.225). Noradrenaline levels were notably higher in the without Mn group (36.95 ng/g) compared to the control (20.14 ng/g) and nano-Mn (25.13 ng/g) groups (p<0.001). Similarly, serotonin concentrations were highest in the without Mn group (228 ng/g), significantly exceeding those in the control (196 ng/g) and nano-Mn (192 ng/g) groups (p=0.021) (Table 8).

Finally, the same neurotransmitters were analyzed in the brain. Histamine levels showed a trend towards significance, with the control group (14.96 ng/g) having slightly lower levels compared to the nano-Mn (17.77 ng/g) and without Mn (18.87 ng/g) groups (p=0.052). Dopamine levels demonstrated significant differences, with the nano-Mn group showing the highest concentration (187 ng/g), followed by the control group (138 ng/g), and the lowest levels in the without Mn group (120 ng/g) (p=0.002). Similarly, noradrenaline concentrations were significantly higher in the nano-Mn group (33.08 ng/g) compared to both the without Mn (26.56 ng/g) and control (22.34 ng/g) groups (p=0.002). Serotonin levels

were markedly elevated in the control group (229 ng/g), significantly surpassing those in the nano-Mn (183 ng/g) and without Mn (166 ng/g) groups ($p<0.001$) (Table 8).

Discussion

Manganese is a critical trace element that plays essential roles in various metabolic processes. As a cofactor for numerous enzymes, manganese is indispensable for proper physiological function and protection of the organism. Despite its significance, the impact of manganese on body composition, metabolic and gut health remains largely underexplored. Our study aimed to evaluate the effects of manganese supplementation and deficiency on body composition, organ function, hormonal balance, neurotransmitter levels, and gut health in laboratory animals.

First, our result indicates that manganese, whether supplemented or deficient, does not have a pronounced effect on the overall mass of major organs, such as testes, pancreas, lungs, liver, heart or spleen. This suggests that manganese may not serve as a critical regulator of organ mass under the experimental conditions employed. Other studies support that inadequate manganese intake can lead to adverse health outcomes, such as impaired growth and poor bone formation.[19] While manganese has been reported to influence the growth of reproductive organs, it does not appear to affect body growth or the weights of the liver or kidneys,[20] which is reflected in our results. This lack of pronounced effect on organ

Table 8 Comparison of Neurotransmitter and Hormonal Markers in Blood Plasma, Intestine, and Brain Across Control, Nano-Mn, and Mn Deficient Groups

	Control (K)	Nano-Mn (N)	Without Mn (B)	SEM	p-value
Blood plasma					
Insulin, µIU/mL	8.68[a]	6.28[b]	6.22[b]	0.320	<0.001
HIST, ng/mL	8.20[a]	6.81[a]	4.06[b]	0.455	<0.001
DA, ng/mL	26.4[a]	29.6[a]	16.1[b]	1.839	0.002
NA, ng/mL	3.27[a]	2.57[b]	2.68[b]	0.111	0.009
5-HT, ng/mL	26.08[a]	28.70[a]	16.02[b]	1.334	<0.001
T4, ng/mL	85.28	81.47	82.61	2.915	0.634
T3, pg/mL	50.62	46.20	45.35	1.123	0.067
Cortisol, ng/mL	10.03	11.07	9.99	0.249	0.088
Intestine					
HIST, ng/g	21.95[a]	15.08[b]	22.01[a]	0.973	0.001
DA, ng/g	185	172	168	5.035	0.225
NA, ng/g	20.14[b]	25.13[b]	36.95[a]	1.848	<0.001
5-HT, ng/g	196[b]	192[b]	228[a]	6.316	0.021
Brain					
HIST, ng/g	14.96	17.77	18.87	0.783	0.052
DA, ng/g	138[b]	187[a]	120[b]	9.186	0.002
NA, ng/g	22.34[c]	33.08[a]	26.56[b]	1.155	0.002
5-HT, ng/g	229[a]	183[b]	166[b]	8.191	<0.001

Notes: SEM, pooled standard error of mean (standard deviation for all rats divided by the square root of rat number, n=24); [a,b] Mean values within a row with unlike superscript letters are shown to be significantly different ($P<0.05$).

mass might imply that manganeses primary roles are more related to cellular and molecular processes rather than macroscopic structural changes in organ size.

Conversely, our study revealed significant alterations in tissue composition with nano-Mn supplementation. Specifically, nano-Mn treatment resulted in an increased percentage of fat tissue and a decreased percentage of lean tissue compared to both the control and manganese-deficient groups. These findings suggest that nano-Mn supplementation may disrupt normal lipid metabolism, promoting adipogenesis and inhibiting myogenesis. Such a shift in cellular differentiation could be mediated by alterations in the PPARγ signaling pathway, a key regulator of adipocyte differentiation.[21] Elevated fat tissue associated with nano-Mn supplementation could have broader implications for metabolic health[22] and body composition[23] by contributing to insulin resistance, chronic inflammation, and an elevated risk of metabolic disorders such as type 2 diabetes and cardiovascular diseases.[24–26] Additionally, the reduction in lean tissue, primarily muscle mass, further exacerbates these risks, as skeletal muscles are a major site for glucose uptake and metabolism.[27] Other studies indicate that excessive Mn_2O_3 NPs intake promotes hepatic lipotoxicity and lipogenesis while inhibiting hepatic lipolysis and fatty acid β-oxidation. Mn_2O_3 NPs also induced hepatic mitochondrial oxidative stress, damaged mitochondrial function, disrupted mitochondrial dynamics, and activated mitophagy.[28] Furthermore, dietary Mn_2O_3 nanoparticles may upregulate de novo lipogenic genes while downregulating specific miRNAs, contributing to lipotoxicity.[29] However, the higher kidney weight in the manganese-deficient group also points to potential compensatory mechanisms in response to low manganese levels.[30]

Furthermore, nano-Mn supplementation significantly impacted caecal parameters. Ammonia levels and pH in the digesta were elevated in the nano-Mn group, suggesting potential alterations in gut microbiome composition or metabolic processes.[30] It is well-established that dietary nanoparticles can alter both the composition and function of the gut microbiota at human-relevant concentrations.[31] Also, the reduced levels of short-chain fatty acids (SCFAs) such as acetic acid, propionic acid, and butyric acid in the nano-Mn group suggest that nano-Mn might adversely affect the gut microbiota functions and activity, particularly related to neurotoxicity.[32] SCFAs play a crucial role in gut-brain axis communication, influencing neurodevelopment, neurotransmitter synthesis, and blood-brain barrier integrity.[33] Thus, a decrease in SCFA production may therefore have significant implications for neurological health, potentially exacerbating conditions such as anxiety, depression, and neurodegenerative diseases.[34,35] In addition, SCFAs have anti-inflammatory properties and play a role in modulating the immune system.[36] A reduction in SCFA levels may impair immune responses and promote inflammatory conditions both locally in the gut and systemically.[37] SCFAs can also influence the expression of genes involved in lipid metabolism supporting our previous findings.[38] Taking together, the observed reduction in SCFAs due to nano-Mn supplementation not only suggests potential adverse effects on gut microbiota function but also highlights broader implications for metabolic, immune, and neurological health.

Moreover, the direct link between manganese supplementation, particularly in nanoparticle form, and disruptions in metabolic and neurological homeostasis is reflected in the elevated levels of insulin and neurotransmitters. The reduced insulin levels detected in both the nano-Mn and Mn-deficient groups suggest potential impairments in glucose metabolism linked to manganese status.[39] This finding is consistent with our previous observations of body composition changes, where nano-Mn supplementation was correlated with increased adipogenesis. Insulin resistance is frequently associated with increased fat accumulation and decreased lean mass,[40] further supporting our hypothesis of altered lipid metabolism induced by nano-Mn. The differences in neurotransmitter levels observed in this study underscore the potential influence of manganese on neurological health.[41] Specifically, the elevated serotonin levels in the control group, contrasted with their significant reduction in both the nano-Mn and Mn-deficient groups, suggest a role for manganese in mood and cognitive functions modulation[42] very likely via the gut-brain axis. This was particularly relevant given the reduced SCFA levels observed in the nano-Mn group, which are known to regulate serotonin synthesis in the gut.[43] The decreased serotonin levels in the nano-Mn group may thus reflect not only altered gut microbiota function[44] but also broader implications for mental health, particularly for anxiety and depression.[45]

Similarly, the significant differences in histamine, dopamine, and noradrenaline levels support findings that manganese, particularly in nanoparticle form, may disrupt neurotransmitter release regulation. The notably elevated dopamine levels in the brain of the nano-Mn group may indicate a compensatory response to manganese-induced oxidative stress,[46] as dopamine is a precursor to noradrenaline. This disruption in neurotransmitter balance may contribute to the neurotoxic

effects of manganese,[47] as suggested by other studies linking excessive manganese exposure to neurodegenerative diseases.[48,49]

Finally, enzyme activities in the caecum were notably higher in the control group compared to both the nano-Mn and manganese-deficient groups. Reduced enzyme activity in these groups could indicate decreased digestive and metabolic efficiency, potentially related to impaired intestinal tight junctions affecting nutrient absorption and gut health.[50] The additional insight may be provided by the potential mechanisms that connect manganese-induced changes in gut integrity and intestinal microbiota to manganese neurotoxicity. Excessive manganese exposure has been associated with alterations in gut microbiota composition and disrupted intestinal metabolic processes, likely damaging enterocytes and compromising gut integrity by disrupting tight junctions.[51] However, interestingly, manganese deficiency led to increased enzyme activity in some cases, potentially reflecting an adaptive response. Conversely, nano-Mn supplementation seemed to impair enzyme activity, possibly linked to the observed changes in tissue composition and caecal parameters. The increased release rates of certain enzymes in the nano-Mn group might suggest a compensatory or altered regulation mechanism in response to supplementation.[52]

Our findings indicate that manganese, particularly in the form of nano-Mn, influences body composition and gut health through molecular mechanisms that warrant further investigation. These insights could enhance our understanding of manganese effects on metabolic health and inform strategies for optimizing manganese supplementation.

Limitations

Our results provide valuable insights into the effects of manganese supplementation and deficiency, however, several limitations must be considered. A notable constraint of this study is the lack of a comparison group with a regular diet, which neither supplements nor deprives manganese. This absence limits our ability to directly compare the effects of manganese supplementation and deficiency to a baseline diet, which could have provided a more comprehensive understanding of manganese's role in health. Here, the control group represents standard manganese intake levels, which allows for the comparison of physiological and metabolic parameters against deficient and nano-Mn-supplemented diets only. Furthermore, the study sample size was relatively small, which may affect the generalizability of the results. The intervention period was also relatively short, which restricts the evaluation of the long-term effects of manganese supplementation and deficiency in terms of health outcomes. Lastly, although significant effects were observed, this investigation did not investigate the underlying molecular mechanisms in depth. Further research are highly requested to explore the molecular pathways to provide a more detailed understanding of manganese physiological roles and potential clinical implications.

Conclusion

A balanced diet is vital for maintaining optimal levels of manganese and other trace elements, which are essential for enzyme function, metabolic regulation, oxidative stress protection, nervous system support, and overall homeostasis. Our study provides insights into how manganese supplementation and deficiency affect body composition, organ metrics, metabolic, and gut health, highlighting manganeses crucial role in these physiological processes. In addition, the insights from this study enhance our understanding of manganese exposure consequences and emphasize the need for targeted nutritional interventions in modern preventive medicine.

Acknowledgments

The animal study protocol was approved by the National Ethics Committee for Animal Experiments (permission No. 73/2021, Warsaw, Poland). This work was supported by the National Science Centre in Poland, Grant No. 2020/39/B/NZ9/00674.

Disclosure

The authors declare that they have no competing interests in this work.

References

1. Mehri A. trace elements in human nutrition (II) - an update. *Int J Prev Med*. 2020;11(1):2. doi:10.4103/ijpvm.IJPVM_48_19
2. Li L, Yang X. The essential element manganese, oxidative stress, and metabolic diseases: links and interactions. *Oxid Med Cell Longev*. 2018;2018 (1):7580707. doi:10.1155/2018/7580707
3. Bonke E, Siebels I, Zwicker K, Dröse S. Manganese ions enhance mitochondrial H2O2 emission from Krebs cycle oxidoreductases by inducing permeability transition. *Free Radic Biol Med*. 2016;99:43–53. doi:10.1016/j.freeradbiomed.2016.07.026
4. Sun Z, Shao Y, Yan K, et al. The Link between trace metal elements and glucose metabolism: evidence from zinc, copper, iron, and manganese-mediated metabolic regulation. *Metabolites*. 2023;13(10):1048. doi:10.3390/metabo13101048
5. Schober L, Dobiašová H, Jurkaš V, Parmeggiani F, Rudroff F, Winkler M. Enzymatic reactions towards aldehydes: an overview. *Flavour and Fragrance Journal*. 2023;38(4):221–242. doi:10.1002/ffj.3739
6. Luo X, Liu Z, Ge X, et al. High manganese exposure decreased the risk of high triglycerides in workers: a cross-sectional study. *BMC Public Health*. 2020;20(1):874. doi:10.1186/s12889-020-09011-x
7. Moon JY, Choi MH, Kim J. Metabolic profiling of cholesterol and sex steroid hormones to monitor urological diseases. *Endocr Relat Cancer*. 2016;23(10):R455–67. doi:10.1530/erc-16-0285
8. Ashique S, Kumar S, Hussain A, et al. A narrative review on the role of magnesium in immune regulation, inflammation, infectious diseases, and cancer. *J Health Popul Nutr*. 2023;42(1):74. doi:10.1186/s41043-023-00423-0
9. De Leo ME, Borrello S, Passantino M, et al. Oxidative stress and overexpression of manganese superoxide dismutase in patients with Alzheimer's disease. *Neurosci Lett*. 1998;250(3):173–176. doi:10.1016/S0304-3940(98)00469-8
10. Chen X, Guo C, Kong J. Oxidative stress in neurodegenerative diseases. *Neural Regen Res*. 2012;7(5):376–385. doi:10.3969/j.issn.1673-5374.2012.05.009
11. Holley AK, Bakthavatchalu V, Velez-Roman JM, St Clair DK. Manganese superoxide dismutase: guardian of the powerhouse. *Int J mol Sci*. 2011;12(10):7114–7162. doi:10.3390/ijms12107114
12. Lauritano C, Carotenuto Y, Roncalli V. Glutathione S-Transferases in Marine Copepods J Mar Sci Eng. 2021;9(9):1025 doi:10.3390/jmse9091025.
13. Olufunmilayo EO, Gerke-Duncan MB, Holsinger RMD. Oxidative stress and antioxidants in neurodegenerative disorders. *Antioxidants*. 2023;12 (2):517. doi:10.3390/antiox12020517
14. Naimi S, Viennois E, Gewirtz AT, Chassaing B. Direct impact of commonly used dietary emulsifiers on human gut microbiota. *Microbiome*. 2021;9 (1):66. doi:10.1186/s40168-020-00996-6
15. Subramaniam S, Kamath S, Ariaee A, Prestidge C, Joyce P. The impact of common pharmaceutical excipients on the gut microbiota. *Expert Opin Drug Delivery*. 2023;20(10):1297–1314. doi:10.1080/17425247.2023.2223937
16. Panyod S, Wu WK, Chang CT, et al. Common dietary emulsifiers promote metabolic disorders and intestinal microbiota dysbiosis in mice. *Commun Biol*. 2024;7(1):749. doi:10.1038/s42003-024-06224-3
17. Subramaniam S, Elz A, Wignall A, et al. Self-emulsifying drug delivery systems (SEDDS) disrupt the gut microbiota and trigger an intestinal inflammatory response in rats. *Int J Pharm*. 2023;648:123614. doi:10.1016/j.ijpharm.2023.123614
18. Cholewińska E, Sołek P, Juśkiewicz J, Fotschki B, Dworzański W, Ognik K. Chromium nanoparticles improve bone turnover regulation in rats fed a high-fat, low-fibre diet. *PLoS One*. 2024;19(5):e0300292. doi:10.1371/journal.pone.0300292
19. Chen X, Yang G, Zhang B, Li F, Liu L, Li F. Effects of manganese-supplemented diets on growth performance, blood biochemistry, nitrogen metabolism and skeletal development of rex rabbits. *J Trace Elem Med Biol*. 2020;61:126543. doi:10.1016/j.jtemb.2020.126543
20. Williams M, Todd GD, Roney N. Toxicological Profile for Manganese. *Atlanta: Agency Toxic Subs Dis Reg*. 2012.
21. Wen X, Zhang B, Wu B, et al. Signaling pathways in obesity: mechanisms and therapeutic interventions. *Signal Trans Targ Thera*. 2022;7(1):298. doi:10.1038/s41392-022-01149-x
22. Sibuyi NRS, Moabelo KL, Meyer M, Onani MO, Dube A, Madiehe AM. Nanotechnology advances towards development of targeted-treatment for obesity. *J Nanobiotechnol*. 2019;17(1):122. doi:10.1186/s12951-019-0554-3
23. Xu B, Chen ZX, Zhou WJ, Su J, Zhou Q. Associations between blood manganese levels and sarcopenia in adults: insights from the National Health and Nutrition Examination Survey. *Front Public Health*. 2024;12:1351479. doi:10.3389/fpubh.2024.1351479
24. Ruan S, Guo X, Ren Y, Cao G, Xing H, Zhang X. Nanomedicines based on trace elements for intervention of diabetes mellitus. *Biomed Pharmacother*. 2023;168:115684. doi:10.1016/j.biopha.2023.115684
25. Hotamisligil GS. Inflammation and metabolic disorders. *Nature*. 2006;444(7121):860–867. doi:10.1038/nature05485
26. Adetunji CO, Michael OS, Rathee S, et al. Potentialities of nanomaterials for the management and treatment of metabolic syndrome: a new insight. *Mater Today Adv*. 2022;13:100198. doi:10.1016/j.mtadv.2021.100198
27. Merz KE, Thurmond DC. Role of skeletal muscle in insulin resistance and glucose uptake. *Compr Physiol*. 2020;10(3):785–809. doi:10.1002/cphy.c190029
28. Zhao T, Zheng H, Xu JJ, et al. MnO(2) nanoparticles trigger hepatic lipotoxicity and mitophagy via mtROS-dependent Hsf1(Ser326) phosphorylation. *Free Radic Biol Med*. 2024;210:390–405. doi:10.1016/j.freeradbiomed.2023.11.037
29. Zhao T, Zheng H, Xu -J-J, Xu Y-C, Liu -L-L, Luo Z. MnO2 nanoparticles and MnSO4 differentially affected hepatic lipid metabolism through miR-92a/acsl3-dependent de novo lipogenesis in yellow catfish Pelteobagrus fulvidraco. *Environ Pollut*. 2023;336:122416. doi:10.1016/j.envpol.2023.122416
30. Baj J, Flieger W, Barbachowska A, et al. Consequences of disturbing manganese homeostasis. *Int J mol Sci*. 2023;24(19). doi:10.3390/ijms241914959
31. Perez L, Scarcello E, Ibouraadaten S, et al. Dietary nanoparticles alter the composition and function of the gut microbiota in mice at dose levels relevant for human exposure. *Food and Cheml Toxicol*. 2021;154:112352. doi:10.1016/j.fct.2021.112352
32. Chi L, Gao B, Bian X, Tu P, Ru H, Lu K. Manganese-induced sex-specific gut microbiome perturbations in C57BL/6 mice. *Toxicol Appl Pharmacol*. 2017;331:142–153. doi:10.1016/j.taap.2017.06.008
33. O'Riordan KJ, Collins MK, Moloney GM, et al. Short chain fatty acids: microbial metabolites for gut-brain axis signalling. *Mole Cell Endocrinol*. 2022;546:111572. doi:10.1016/j.mce.2022.111572

34. Silva YP, Bernardi A, Frozza RL. The role of short-chain fatty acids from gut microbiota in gut-brain communication. *Front Endocrinol*. 2020;11:25. doi:10.3389/fendo.2020.00025
35. Mirzaei R, Bouzari B, Hosseini-Fard SR, et al. Role of microbiota-derived short-chain fatty acids in nervous system disorders. *Biomed Pharmacother*. 2021;139:111661. doi:10.1016/j.biopha.2021.111661
36. Kim CH. Complex regulatory effects of gut microbial short-chain fatty acids on immune tolerance and autoimmunity. *Cell mol Immunol*. 2023;20 (4):341–350. doi:10.1038/s41423-023-00987-1
37. Liu XF, Shao JH, Liao YT, et al. Regulation of short-chain fatty acids in the immune system. *Front Immunol*. 2023;14:1186892. doi:10.3389/fimmu.2023.1186892
38. He J, Zhang P, Shen L, et al. Short-chain fatty acids and their association with signalling pathways in inflammation, glucose and lipid metabolism. *Int J mol Sci*. 2020;21(17):6356. doi:10.3390/ijms21176356
39. Baly DL, Curry DL, Keen CL, Hurley LS. Effect of manganese deficiency on insulin secretion and carbohydrate homeostasis in rats. *J Nutr*. 1984;114(8):1438–1446. doi:10.1093/jn/114.8.1438
40. Fukushima Y, Kurose S, Shinno H, et al. Importance of lean muscle maintenance to improve insulin resistance by body weight reduction in female patients with obesity. *Diabetes Metab J*. 2016;40(2):147–153. doi:10.4093/dmj.2016.40.2.147
41. Harischandra DS, Ghaisas S, Zenitsky G, et al. Manganese-induced neurotoxicity: new insights into the triad of protein misfolding, mitochondrial impairment, and neuroinflammation. *Front Neurosci*. 2019;13:654. doi:10.3389/fnins.2019.00654
42. Sharma A, Feng L, Muresanu DF, et al. Chapter 9 - Manganese nanoparticles induce blood-brain barrier disruption, cerebral blood flow reduction, edema formation and brain pathology associated with cognitive and motor dysfunctions. In: Sharma HS, Sharma A, editors. *Progress in Brain Research*. Elsevier; 2021. 385–406.
43. Buey B, Forcén A, Grasa L, Layunta E, Mesonero JE, Latorre E. Gut microbiota-derived short-chain fatty acids: novel regulators of intestinal serotonin transporter. *Life*. 2023;13(5). doi:10.3390/life13051085
44. Xia Y, Wang C, Zhang X, et al. Combined effects of lead and manganese on locomotor activity and microbiota in zebrafish. *Ecotoxicol Environ Saf*. 2023;263:115260. doi:10.1016/j.ecoenv.2023.115260
45. Racette BA, Nelson G, Dlamini WW, et al. Depression and anxiety in a manganese-exposed community. *Neuro Toxicol*. 2021;85:222–233. doi:10.1016/j.neuro.2021.05.017
46. Pajarillo E, Nyarko-Danquah I, Digman A, et al. Mechanisms of manganese-induced neurotoxicity and the pursuit of neurotherapeutic strategies. *Front Pharmacol*. 2022;13:1011947. doi:10.3389/fphar.2022.1011947
47. Soares ATG, Silva AC, Tinkov AA, et al. The impact of manganese on neurotransmitter systems. *J Trace Elem Med Biol*. 2020;61:126554. doi:10.1016/j.jtemb.2020.126554
48. Martins AC Jr, Gubert P, Villas Boas GR, et al. Manganese-induced neurodegenerative diseases and possible therapeutic approaches. *Expert Rev Neurother*. 2020;20(11):1109–1121. doi:10.1080/14737175.2020.1807330
49. Chib S, Singh S. Manganese and related neurotoxic pathways: a potential therapeutic target in neurodegenerative diseases. *Neurotoxicol Teratol*. 2022;94:107124. doi:10.1016/j.ntt.2022.107124
50. Zhang H, Pan S, Zhang K, et al. Impact of dietary manganese on intestinal barrier and inflammatory response in broilers challenged with salmonella typhimurium. *Microorganisms*. 2020;8(5):757. doi:10.3390/microorganisms8050757
51. Tinkov AA, Martins AC, Avila DS, et al. Gut microbiota as a potential player in mn-induced neurotoxicity. *Biomolecules*. 2021;11(9):1292. doi:10.3390/biom11091292
52. Tian H, Ghorbanpour M, Kariman K. Manganese oxide nanoparticle-induced changes in growth, redox reactions and elicitation of antioxidant metabolites in deadly nightshade (Atropa belladonna L.). *Ind Crops Prod*. 2018;126:403–414. doi:10.1016/j.indcrop.2018.10.042