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Cadmium Exposure Disrupts Gut Microbiota-Derived Short-Chain Fatty Acid Synthesis, Suppresses Peptide YY Secretion, Accumulates Fat, and Increases HBA1C

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Abstract. Cadmium (Cd), as a pervasive environmental toxicant, can enter the human body through food and cigarette smoke [1]. The response triggered by cadmium includes irritation to the digestive tract, build-up of fluids in the lungs, and damage to the kidneys and bones [2]. In addition, cadmium affects the human gut microbiota environment. Therefore, it is hypothesized that chronic cadmium exposure can disrupt short-chain fatty acid production in gut microbiota, suppressing peptide YY secretion, accumulating fat, and elevating hemoglobin A1C. Male C57BL/6 mice (n=12/ group) were exposed to 10, 20, 30, 40, and 50 ppm $CdCl_2$ for 12–36 weeks to test the hypothesis. SCFAs, PYY, fat pad mass, and A1C were analyzed alongside positive controls (high-fat diet, high-protein diet, prebiotic fiber, streptozotocin) and saline-negative controls. Dose-dependent reductions in SCFAs (up to 40% decrease) and PYY (25% suppression) are observed, while increases in weights of the fat pads (15% increases) and elevated HBA1C levels (above 6.5%) are observed [3]. While cadmium's effects on gut microbiota composition are documented, its impact on SCFA bioactivity and downstream metabolic regulation remains unclear. These findings highlight cadmium's role in disrupting gut-metabolic axes and suggest therapeutic strategies targeting SCFA restoration.

Keywords: Cadmium, SCFA, PYY, A1C.

1. Introduction

Cadmium is an element widely distributed in the Earth's crust, air, and water. Often existing in the ecological environment as Cd^{2+}, CdO, $CdCl_2$, or Cd_2SO_4, it can be emitted into water and soil by burning fossil fuels, the use of phosphate fertilizers, and industrial processes, including Cd. Cadmium enters the food chain through bioaccumulation in individual organisms and biomagnification along food chains, particularly in aquatic systems. The detrimental effects of Cd are also of concern to humans. Cadmium may disrupt the sensitive microbial ecosystem, which triggers abnormal physiological responses. Chronic exposure to cadmium can induce dysbiosis of the gut flora, shown by a decline in SCFA-producing bacterial taxa such as Bifidobacterium and Lactobacillus4, irritation of the digestive tract, fluid buildup in the lungs, and damage to the kidneys

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and bones. Despite such responses, prolonged cadmium exposure can cause several types of cancer in the breast, lung, prostate, nasopharynx, pancreas, and kidneys.

Living within the human intestine, trillions of microbes create an intricate microbial community that affects normal human functions through metabolic actions and host-bacterial interactions. Acetate, propionate, and butyrate, collectively known as short-chain fatty acids, are produced by microbial fermentation of indigestible dietary fibers within the host. The gut metabolites function by acting as colonocyte fuel and influencing worldwide appetite regulation, glucose metabolism, and lipid processes [4].

The gastrointestinal tract generates short-chain fatty acids (SCFAs) through bacterial fermentation of dietary fibers. The uptake of SCFAs occurs through substrate transporters such as monocarboxylate transporter 1 and sodium-coupled monocarboxylate transporter 1, which enhance cellular metabolic activity [5]. Scientific investigations have supported that metabolites of microbes function as essential executors of dietary microbiota influence on the host. The intestinal epithelial cells (IECs) use SCFAs as vital energy sources, while the gut microbiota decomposes the major dietary carbon flux through this process. The vital biological processes that SCFAs enable include glucagon-like peptide-1 secretion by IEC for glucose control, G protein-coupled receptors activation, including GPR41 and GPR43, and histone deacetylase inhibition, which controls IEC proliferation, differentiation, and functionality. The production of SCFAs regulates intestinal movements and protective barriers, in addition to host metabolic activities. The metabolic regulatory functions of SCFAs extend across three metabolic levels: local, intermediate, and peripheral. The three main SCFAs include acetate, propionate, and butyrate, which regulate immune responses as well as apoptosis, inflammation, and lipid metabolic pathways. The peptide YY (PYY) secretion from enteroendocrine L-cells increases when SCFAs stimulate PYY release. Disorders, such as obesity and insulin resistance, can result from SCFA production disturbances, which shows that microbiota acts as an essential health mediator. Cd ion causes substantial metabolic functional changes in the gut microbiome, with significant inhibitions on gene pathways associated with the metabolism of amino acids, carbohydrates, and energy, as well as promotions on metabolic pathways such as glutathione metabolism and aminobenzoate degradation.

Research has shown that it disrupts the balance of gut microbiota composition and function, which maintains metabolic stability[6]. This paper extends previous findings about heavy metal exposure, gut dysbiosis, and metabolic problems while focusing on cadmium's effects on short-chain fatty acid production and peptide YY secretion. The majority of existing research has examined direct cadmium toxicity to organs such as the liver and kidneys, yet there is limited study on its effects on SCFA synthesis and subsequent metabolic regulation. Moreover, the mammalian gut microbiome shows high sensitivity to both short-term and subchronic cadmium (Cd) intoxication, yet researchers have not determined the susceptibility patterns after prolonged Cd exposure at levels typical in the environment [5]. Therefore, this research investigates how exposure to 10 and 50 ppm cadmium in drinking water after 12-36 weeks affects gut microbiome composition in mice, which systematically examines the dose and duration effects of cadmium exposure on these processes. Filling the information gaps is significant because SCFAs and PYY function as essential regulators of energy balance, glucose metabolism, and appetite control, and their disruption could worsen metabolic dysregulation.

The goal of this paper is to understand how the duration and dose of cadmium exposure affect the microbiota of the gut and metabolism to better understand its harmful effects. This study will use controlled experimental approaches to expose mouse models to different cadmium doses and exposure times while examining SCFA concentrations, PYY secretion, blood glucose, and fat

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content as metabolic indicators. This research aims to pinpoint the exact pathways through which alterations in the gut microbiota induced by cadmium give rise to metabolic issues. Doing so will furnish crucial information for the development of future therapeutic strategies aimed at minimizing the metabolic impacts stemming from environmental exposure to cadmium. Additionally, the study seeks to bridge the fields of toxicology, microbiology, and endocrinology, thereby enabling a more comprehensive comprehension of the relationship between environmental toxins, gut microbiota, and metabolic well-being [7]. The findings of this study can serve as a foundation for subsequent research, facilitating the formulation of interdisciplinary solutions that incorporate microbial therapies and policy modifications to effectively counteract cadmium toxicity.

2. Hypothesis

Predict that increasing concentrations and treatment durations of cadmium reduce the production of short-chain fatty acids (SCFAs) by gut microbiota, leading to decreased peptide YY (PYY) and therefore increased fat accumulation and elevated HBA1C levels.

3. Materials and methods

3.1. Sample preparation

The male C57BL/6 mice (n=12/group, 8 weeks old) were kept under the following conditions: a twelve-hour light and twelve-hour dark cycle, twenty-five degrees Celsius. There will be 3 replicates in each group exposed to different extents of cadmium exposure (10, 20, 30, 40, and 50 ppm cadmium chloride – CdCl2 in drinking water). These mice will be observed at time intervals for 12, 24, and 36 weeks.

3.2. Method 1 – GC-MS

The short-chain fatty acids (SCFAs) were determined in fecal samples using a GC-MS system having a polar capillary column (DB-WAX, 30 m × 0.25 mm × 0.25 μm). The 100 mg of fresh or frozen fecal samples were homogenized in 1 mL of 0.1% phosphoric acid using a vortex mixer for 5 minutes and then sonicated in an ice-cold ultrasonic bath (4°C, 10 minutes) to extract the SCFAs. The homogenate was centrifuged at 12,000 × g for 15 minutes, and the supernatant was passed through 0.22 μm organic solvent-compatible membranes into 2 ml screw cap tubes. Isotope-labeled internal standards (10 μL of 10 mM $^{2}H_{4}$-butyrate) were added for quantification; acidification was optional using 10 μL concentrated HCl. The chromatographic separations and mass spectrometric detection were performed according to the standardized conditions, and SCFA concentrations were determined using internal standard calibration curves from peak area ratios. The positive control was performed using prebiotic fiber with 10% inulin, while the negative control was a 0.9% NaCl saline solution. Critical equipment included a centrifuge, pipettes (10–100 μL), and organic solvent-compatible filters to ensure precision and reproducibility.

3.3. Method 2 – ELISA

Standard laboratory equipment used in this method included blood collection tools, a centrifuge, a microplate reader, precision pipettes (10–1,000 μL), an incubator, an ultra-low temperature freezer, and a commercial ELISA kit, along with materials such as phosphate-buffered saline (PBS) and protease inhibitor cocktail. First, add 100 μL of the standard samples into each pe-coated 96-well

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plate well. Then, place the plate in the incubator at 37°C for 2 hours. After three washes with PBS containing 0.15% Tween-20 (PBST), 100 μL per well of biotinylated anti-mouse PYY antibody was added, and the plates were placed in the incubator at 37°C for 1 hour. Next, the streptavidin-HRP conjugate (1:1,000 dilution, 100 μL/well) was added and incubated for 30 minutes, then 100 μL/well TMB substrate was added, and the plates were placed in the dark for 15 minutes. The reaction was stopped by using 2M H_2SO_4, and the absorbance at 450 nm was measured using a microplate reader. The PYY concentrations were determined by using a four-parameter logistic standard curve that was derived from the absorbance data. The statistical analysis was done using one-way ANOVA with Tukey's post hoc test ($p < 0.05$) in GraphPad Prism. The positive controls included a high-protein diet (HPD, 40% kcal protein), and the negative control was 0.9% NaCl saline solution.

3.4. Method 3 – dissection and weighing of fat pads

The study makes use of some laboratory equipment which includes an anesthesia system, centrifuge, – 80°C freezer, sterile surgical scissors, forceps, dissection trays, a precision electronic balance, and ice-cold phosphate-buffered saline (PBS) are the material used. The fat pads that were dissected and isolated were washed with ice-cold PBS and dried on filter paper. The wet weight of the fat pads was measured with the electronic balance (in grams), and group averages and standard deviations were recorded. Fat mass was normalized to body weight (expressed as g per 100 g of body weight) to standardize measurements across individuals. Positive controls included a high-fat diet (HFD, 60% kcal fat) to induce obesity and 0.9% NaCl saline solution as the negative control. All steps followed standardized protocols to guarantee reproducibility and accuracy.

3.5. Method 4 – ABC-ELISA

The double antibody sandwich ABC-ELISA method was employed to measure HbA1c levels. The assay involves monoclonal anti-rat HbA1c antibody-coated microplates where 100 μL standards (200, 100, 50, 25, 12.5, 6.25, 3.12, 0%) or samples are added and incubated at 37°C for 120 minutes. After 4–6 washes with washing buffer, biotinylated anti-rat HbA1c antibody (100 μL) is added and incubated at 37°C for 60 minutes, followed by streptavidin-HRP conjugate (100 μL) incubation for 30 minutes. Following additional washes, 100 μL TMB substrate is added, and the reaction is developed at 37°C in the dark for 15 minutes before stopping with 100 μL sulfuric acid. Absorbance is measured at 450 nm within 30 minutes. HbA1c concentrations are calculated by subtracting blank values and interpolating sample OD values from a standard curve. The standard curve is generated by plotting OD values against HbA1c percentages. Positive controls included streptozotocin (STZ, 50 mg/kg intraperitoneal injection) to induce hyperglycemia, while the negative control was 0.9% NaCl saline solution. All steps were conducted to ensure precise measurement and comparison of A1C levels across experimental groups. When HbA1C levels were greater than 6.5%, the mice were diagnosed with hyperglycemia, which supports our hypothesis.

4. Statistical analysis

4.1. Method 1 – Principal Component Analysis (PCA)

For the GC-MS analysis of short-chain fatty acids (SCFAs) in fecal samples, Principal Component Analysis (PCA) can be a powerful statistical tool. PCA is a dimensionality-reduction technique that transforms a set of correlated variables into a set of uncorrelated variables called principal components. First, the raw data from the GC-MS analysis, which consists of peak areas for different

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SCFAs in each fecal sample, need to be pre-processed. This includes normalizing the data to account for differences in sample volume or extraction efficiency. We can divide the peak area of each SCFA by the total peak area of all SCFAs in the sample. Using statistical software like R or Python (with libraries such as `scikit - learn` in Python), we can perform PCA on the pre-processed data. The goal is to identify the principal components that explain the majority of the variance in the data. The first principal component (PC1) accounts for the largest amount of variance, followed by the second principal component (PC2), and so on.

The scores of each sample on the principal components can be plotted on a scatter plot. Samples from different experimental groups (e.g., control vs. cadmium-exposed) can be colored differently. If there is a clear separation between the groups on the PCA plot, it indicates that there are significant differences in the SCFA profiles between the groups. Additionally, the loadings of each SCFA on the principal components can be examined. A high loading of a particular SCFA on a principal component means that this SCFA contributes significantly to the variance explained by that component. This can help us identify which SCFAs are most affected by the experimental treatment.

4.2. Method 2 – Post-hoc tests

In the ELISA method for measuring PYY concentrations, since multiple measurements are taken over time (for example, if samples are measured at different time points during the experiment), a Repeated Measures ANOVA is appropriate. The data for each subject (mouse) includes the PYY concentrations measured at different time points. The factors in the analysis are the experimental group (e.g., high-protein diet group, control group) and the time factor. Before performing the Repeated Measures ANOVA, we need to check the assumptions of sphericity. If the assumption of sphericity is violated, we can use corrections such as the Greenhouse-Geisser corrections. The Repeated Measures ANOVA($p<0.05$) will test for the main effects of the experimental group and the time factor, as well as their interaction. A significant main effect of the experimental group indicates that there are differences in PYY concentrations between the groups. A significant main effect of the time factor is that PYY concentrations change over time. A significant interaction effect implies that the change in PYY concentrations over time is different between the experimental groups. Post hoc tests may be performed to determine which specific groups (10, 20, 30, 40, or 50 ppm) or time points (12, 24, or 36 weeks) are significantly different if ANOVA results are significant.

4.3. Method 3 – Kruskal-Wallis test

The Kruskal–Wallis test is a nonparametric version of the one-way ANOVA. It is applicable for the data obtained from the dissection and weighing of fat pads, especially when the data does not fulfill the assumption of normality. The data includes wet weights of fat pads (normalized to body weight) from different experimental groups. The Kruskal–Wallis test combines all the data points from all the groups and then compares the sum of ranks for each group. The null hypothesis of the Kruskal–Wallis test is that the medians of all the groups are the same. If the t-p-value of the Kruskal–Wallis test is less than the significance level (e.g., $p < 0.05$), we reject the null hypothesis and conclude that there is a significant difference in the fat pad weights between at least two of the groups. To find out which groups are different, we can use post-hoc Dunn’s tests.

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4.4. Method 4 – Receiver Operating Characteristic (ROC) curve analysis

For the ABC-ELISA method used for the measurement of HbA1c levels and diagnosis of hyperglycemia, ROC curve analysis can be quite useful. There are two types of data that are usable: HbA1c concentrations obtained using the ELISA method and the actual disease status of the mice (hyperglycemic or nonhyperglycemic). The HbA1c concentrations are used as the test variable, and the disease status is the binary outcome. The ROC curve is generated by plotting the true positive rate (sensitivity) against the false positive rate (1 – specificity) at different cut-off values of the HbA1c concentration. With the aid of statistical software, we can calculate the area under the ROC curve (AUC). The AUC is the measure of the diagnostic accuracy of the test. A value of 0.5 for the AUC indicates that the test is not statistically supported, while a value of 1.0 indicates perfect accuracy. Therefore, if the AUC is close to 1, the measurement of HbA1c by the ABC–ELISA method is a good predictor of hyperglycemia. Besides, the appropriate cut-off value for diagnosing hyperglycemia can be obtained from the ROC curve, the point on the curve that gives the maximum sensitivity and specificity.

5. Results table

Table 1. Hypothetical results

Combination of possible results (CR)	Cadmium reduces the production of SCFAs by measure SCFA in fecal samples through GC-MS	Cadmium decreases peptide YY secretion by ELISA	Cadmium increases fat accumulation by dissecting out fat pad and weighing it	Cadmium increases glycated hemoglobin by A1C test	Support hypothesis
CR1	+	+	+	+	Full
CR2	+	+	+	-	Partial
CR3	+	+	-	+	Partial
CR4	+	-	+	+	Partial
CR5	-	+	+	+	Partial
CR6	+	+	-	-	Partial
CR7	+	-	+	-	Partial
CR8	+	-	-	+	Partial
CR9	-	+	+	-	Partial
CR10	-	+	-	+	Partial
CR11	-	-	+	+	Partial
CR12	+	-	-	-	Partial
CR13	-	+	-	-	Partial
CR14	-	-	+	-	Partial
CR15	-	-	-	+	Partial
CR16	-	-	-	-	Fully contradicts

In Table 1, "+" indicates a reduction of SCFAs, a decrease in peptide YY secretion, an increase in fat content, and a rise in A1C levels, which are in line with the proposed hypothesis. "-" indicates an

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increase of SCFAs, an increase in peptide YY secretion, a decrease in fat content, and a decrease in A1C levels, which are not in line with the proposed hypothesis.

CR1: If I got this result(+ + + +), I would observe cadmium decreasing SCFAs, decreasing PYY, increasing fat deposition, and A1C levels. Higher cadmium concentrations and prolonged exposure would probably enhance all four effects, i.e., SCFA reduction by disrupting the gut microbiota, PYY suppression, fat accumulation, and elevated A1C. Lower doses or shorter durations, like 2-8 weeks, may produce weaker or delayed responses. For instance, temporary SCFA loss may occur without A1C changes. Extremely high doses could paradoxically reduce fat mass due to cytotoxicity, thereby hiding adipogenic effects.

CR2: If I obtain this result(+ + + –), I would see cadmium lowers SCFAs, decreases PYY, and increases fat deposition, but A1C is not affected. The SCFA/PYY/fat effects at moderate concentrations and intermediate durations may not be accompanied by A1C elevation, since pancreatic β-cells may still secrete insulin to compensate. Higher doses or longer exposure may lead to hyperglycemia (A1C+). The shorter durations (<3 weeks) may cause isolated gut/adipose effects before glucose dysregulation occurs.

CR3: If I obtain this result (+ + – +), I would see cadmium decreases SCFAs and PYY and increases A1C, but does not affect fat mass. High doses may reduce fat accumulation through cytotoxicity such as adipocyte apoptosis, whereas shorter durations may favor gut/endocrine dysfunction and hyperglycemia (A1C+). Lower doses with longer exposure periods may lead to an increase in fat mass when compensatory mechanisms fail.

CR4: If I obtain this result (+ – + +), I would see cadmium decrease SCF and As, and increase fat mass and A1C, but PYY is not affected. Low to moderate doses may preserve PYY pathways while still causing SCFA depletion, fat deposition, and A1C elevation. Longer durations could suppress the compensatory PYY secretion, thus converting CR4 to CR1. High doses may disrupt PYY earlier, thereby masking this result.

CR5: If I obtain this result (– + + +), I would see cadmium decrease PYY, and increase fat mass and A1C, but SCFAs do not change. Particular cadmium thresholds may affect the endocrine and glucose systems (PYY↓, A1C↑, fat↑) while leaving the SCFA-producing microbiota intact. Short-term exposure may isolate these effects; prolonged exposure may later reduce SCFAs as dysbiosis develops.

CR6: If I obtain this result (+ + – –), I would see cadmium decreases SCFAs and PYY but does not affect fat mass or A1C. Low doses or short exposure may not have significant effects on gut endpoints (SCFA/PYY↓) without any effects on systemic metabolic changes. Compensatory mechanisms can be overcome with higher concentrations or longer durations to trigger fat/A1C effects.

CR7: If I obtain this result (+ – + –), I would see cadmium decreases SCFAs and increases fat mass, and there is no effect on PYY and A1C. The mid-range doses may specifically impair the gut microbiota (SCFA↓) and adipogenesis (fat↑) without any effects on endocrine and glucose metabolism. Short-term exposure, such as 2-8 weeks, may reveal this pattern of responses, while longer exposure may support the relationship between SCFA depletion and PYY suppression or A1C increase.

CR8: If I obtain this result (+ – – +), I would see cadmium decreases SCFAs and increases A1C, and there is no change in PYY and fat mass. High doses may cause direct damage to β-cells (A1C↑) and the gut microbiota (SCFA↓) without effects on PYY/fat. These effects may be isolated for shorter durations, but chronic exposure may eventually lead to fat accumulation through insulin resistance or insulin resistance with delayed fat mass changes.

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CR9: If I obtain this result (– + + –), I would see cadmium decreases PYY and increases fat mass, but does not affect SCFAs or A1C. Some threshold doses may block PYY secretion and adipogenesis but not gut/glucose metabolism. Short-term exposure could focus on these changes; longer durations could later reduce SCFAs or elevate A1C as toxicity spreads.

CR10: If I get this result (– + – +), then cadmium lowers PYY and raises A1C, but SCFAs and fat mass are unchanged. High doses may be more toxic to the pancreas (A1C↑) and endocrine system (PYY↓) without the involvement of the gut or adipose. Acute exposure might show this pattern, whereas chronic exposure could trigger fat accumulation or SCFA loss.

CR11: If I obtain this result (– – + +), I would see increased fat mass and A1C, but SCFAs and PYY are unchanged. High doses may directly induce fat accumulation, pancreas dysfunction (A1C↑), and adipogenesis (fat↑) without a gut effect. Short-term exposure could isolate these outcomes; prolonged durations might later reduce SCFAs or PYY.

CR12: If I obtain this result (+ – – –), I would see cadmium reduces SCFAs, but other parameters are unchanged. Small doses or short duration may disrupt microbiota (SCFA↓) but not the whole body. Higher concentrations or longer exposure could extend the spectrum of the damage to other systems.

CR13: If I obtain this result (– + – –), I would see Cadmium lowers PYY, but SCFAs, fat mass, and A1C are unchanged. Particular doses may be harmful to L-cells (PYY↓) with no other metabolic impact. Short-term exposure could produce this result; longer durations might link PYY loss to SCFA/A1C changes. In addition, early PYY suppression could precede later metabolic effects.

CR14: If I obtain this result (– – + –), I would see cadmium increases fat mass only. Moderate doses could promote fat cell formation (fat↑) by activating PPARγ without affecting glucose, or endocrine system, or the gut. Exposure for a longer period might cause fat mass to normalize through lipolysis or lead to secondary effects (A1C↑).

CR15: If I obtain this result (– – – +), I would see cadmium elevates A1C only. High doses of cadmium may be more toxic to the β-cells of the pancreas (A1C↑) without the involvement of the gut. Acute exposure (1–3 weeks) might isolate hyperglycemia; chronic exposure could later reduce SCFAs or PYY. Meanwhile, chronic hyperglycemia could eventually trigger fat/SCFA changes.

CR16: If I obtain this result (– – – –), I would see no observed effects align with the hypothesis. Subtoxic doses or insufficient durations fail to induce measurable changes. Experimental design flaws (e.g., insensitive assays and poor cadmium bioavailability) could also explain this result. Higher doses or longer exposure would likely shift outcomes toward partial/full support.

6. Discussion

CR1 (+ + + +): I believe that this result, in which cadmium decreases SCFAs, decreases PYY, increases fat mass, and elevates A1C, supports the hypothesis. High concentrations and long exposure periods are likely to cause metabolic disturbances throughout the body, indicating cadmium affects gut microbiota, endocrine function, adipogenesis, and glucose metabolism. However, this raises questions about specificity—whether effects are direct or due to general toxicity. Future experiments should include dose-response curves to determine the minimal effective doses, time-course studies to establish the critical exposure periods, and specificity checks by administering cadmium chelators to reverse the effects and to examine off-target toxicity. These steps will help to establish whether cadmium's effects are specific to certain metabolic pathways or if they are part of a broader toxic response.

CR2 (+ + + –): Here, cadmium decreases SCFAs, decreases PYY, and increases fat mass, and A1C is not changed. This suggests that the glucose metabolism is either very stable or requires a

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higher dose of cadmium. Lower doses or shorter exposures might protect pancreatic beta cells. To support the assumption, future experiments should include higher dose levels that cause hyperglycemia. Then, measure insulin secretion and β-cell viability and examine compensatory mechanisms such as insulin sensitivity and gluconeogenesis. These steps will help in understanding if the glucose metabolism dysregulation is dose-dependent or a delayed response.

CR3 (+ + – +): In this case, cadmium decreases SCFAs and PYY and increases A1C but does not change fat mass. High doses may cause the death of adipocytes (apoptosis), which would prevent fat formation. This shows that adipogenesis is not necessarily a universal effect and may be influenced by the type and duration of the exposure. Future experiments should include an adipocyte viability assay for apoptosis/necrosis, longer exposure times to watch for delayed fat accumulation, and leptin and adiponectin as lipolysis markers. These studies will define the relationship between cadmium exposure and fat deposition.

CR4 (+ – + +): This result indicates loss of SCFA, fat accumulation, and A1C elevation but no changes in PYY. Low to moderate doses might leave the L-cells intact or stimulate compensatory PYY release. Future experiments should include measurement of other gut hormones such as GLP-1 and ghrelin, use of PYY knockout models to check if fat/A1C changes remain, and microbiota transfers to study the effect of the gut. This will help to reveal PYY-independent pathways and to explain the endocrine effects of cadmium.

CR5 (– + + +): PYY suppression, fat accumulation, and A1C elevation occur, but SCFAs are stable. Some specific thresholds might affect endocrine and glucose systems without inducing changes in gut dysbiosis. Future experiments should include microbiota sequencing to examine the taxonomic alterations, SCFA supplementation to determine the reversibility, and fecal transplantations to determine the stability of the microbiota. This will help to define the involvement of gut microbiota in cadmium-induced metabolic disturbances.

CR6 (+ + – –): I believe that this result shows the gut dysfunction (SCFA/PYY↓) without any metabolic changes. Low concentrations and brief exposure may prevent systemic effects. Future experiments should prolong exposure times to follow delayed fat and A1C changes, measure intestinal permeability, and measure inflammatory cytokines to determine if there is any immune activation. These experiments will help to determine if gut effects occur before metabolic effects on the rest of the body.

CR7 (+ – + –): In this instance, cadmium reduces SCFAs and increases fat mass but does not change PYY or A1C. Mid-range doses could be more selective for the gut and adipose tissue. Future experiments should include analysis of adipogenesis pathways such as PPARγ and C/EBPα, and perform microbiota metabolomics to look for non-SCFA metabolites and combine cadmium with a high-fat diet to look at synergies. These studies will help to explain the gut-adipose axis in cadmium-induced metabolic dysfunction.

CR8 (+ – – +): I believe that this result shows the loss of SCFAs and A1C increase, but no changes in PYY and fat mass, as higher doses may directly damage the beta cells and the microbiota. Future experiments should include an assessment of insulin signaling pathways, quantification of β cell mass by immunofluorescence, and assessment of vagal nerve activity in glucose regulation. This will help to clarify the independent pathways for gut and glucose effects.

CR9 (– + + –): In this study, PYY suppression and fat accumulation are observed, but SCFAs and A1C remain unchanged. Threshold doses might affect endocrine/adipose systems. Future experiments should include the use of PYY receptor antagonists to examine their role in fat accumulation, lipid species profiling in adipose tissue, and metabolic rate assessment via indirect calorimetry. These studies will define the role of PYY in adipogenesis.

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CR10 (– + – +): I believe that this outcome shows that PYY is decreased and A1C is elevated, but SCFAs and fat mass are stable. High doses might preferentially cause pancreatic/endocrine toxicity. Future experiments should include glucose clamps to determine insulin secretion, use PYY agonists to determine reversibility, and assess autonomic nervous system input to the pancreas. This will help to explain the relationship between PYY and glucose dysregulation.

CR11 (– – + +): Fat accumulation and A1C elevation are observed, but SCFAs and PYY are stable. High doses might activate adipogenesis and β-cell toxicity directly. Future experiments should include cadmium exposure of adipocyte cultures in vitro, assessment of mitochondrial function in β cells, and genetic models to test fat-glucose crosstalk. These studies will help to understand the mechanisms independent of the gut.

CR12 (+ – – –): This result shows only isolated SCFA reduction. This result partially supports the hypothesis by showing that cadmium has effects on gut microbiota while questioning the role of the gut in systemic regulation. This result shows that the gut is sensitive to cadmium but may also be resilient. This result stresses the need to determine whether SCFA reduction is a biomarker of cadmium toxicity or an effect that is not related to the rest of the body. In addition, it suggests that SCFA reduction alone may not be sufficient to cause endocrine or metabolic dysfunction. Future experiments should include the identification of compensatory metabolites and antibiotics to test microbiota depletion effects and track SCFA recovery post-exposure. These steps will help to understand gut sensitivity and resilience.

CR13 (– + – –): PYY suppression occurs without other effects. The doses of cadmium could target specifically the enteroendocrine L-cells that produce PYY. Cadmium may disrupt the functioning of these cells by interfering with their signaling pathways or ion channels. Since other parameters are not changed, it indicates that exposure to cadmium at this dose does not have any major effect on the ability of gut microbiota to produce SCFAs, nor on adipose tissue metabolism or glucose regulation at this stage. The reduction in PYY may be compensated for by the body's other mechanisms for SCFAs, fat, and glucose without showing any changes.

CR14 (– – + –): The result shows that there is only fat accumulation. Moderate doses of cadmium may stimulate adipogenesis through the activation of peroxisome proliferator-activated receptor gamma (PPARγ) pathways. In addition, Cd may directly act on adipocytes, promoting their differentiation and lipid synthesis. The lack of effects on SCFAs, PYY, and A1C suggests that at this dose, cadmium is not significantly affecting the gut microbiota - SCFA - PYY axis or glucose metabolism. The body's feedback mechanisms regarding PYY and glucose regulation may still act normally despite an increase in fat mass.

CR15 (– – – +): A1C elevation occurs without other effects. High doses (>6 mg/kg) may rapidly cause β-cell toxicity. Future experiments should isolate islets to test cadmium's direct effects, use antioxidants to reduce oxidative stress, and perform glucose tolerance tests to assess insulin response. These studies will elucidate pancreatic vulnerability.

CR16 (– – – –): This result shows no effects, likely due to subtoxic doses or insufficient durations (<2 weeks). Future experiments should measure cadmium bioavailability, escalate exposure parameters incrementally, and use genetically susceptible strains or aged animals. These steps will correct experimental limitations and validate the hypothesis.

7. Overall analysis of concentration and treatment duration in the experiment

In this study, the effects of cadmium exposure with different doses and durations are important variables that have a considerable effect on the results. Cadmium is administered to male C57BL/6 mice at concentrations of 10, 20, 30, 40, and 50 ppm in drinking water for periods of 12, 24, and 36

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weeks. This allows for the investigation of dose and time effects on several metabolic parameters, including SCFA, PYY, fat, and A1C.

Effects of Concentration

SCFA Production

In fecal samples, as determined by GC-MS, SCFA production tends to decrease with increasing cadmium concentration. Higher concentrations of cadmium are more likely to interfere with the bacteria that produce SCFAs. For example, at 50 ppm, the gut microbiota may be disrupted to such an extent that SCFA production is greatly inhibited. This could be because cadmium is toxic to bacteria that produce SCFAs, including Bifidobacterium and Lactobacillus. However, at 10 ppm, the effect on SCFA production might be less severe, and the gut microbiota may still be able to produce SCFAs at near-normal levels, or a small decrease could be detected. It is identified that the microbiome in the human gut works collaboratively to enhance butyrate production and maintain this synergism at Cd concentrations up to 5 ppm [8].

PYY Secretion

Concentration also influences PYY secretion. Higher cadmium concentrations are more likely to suppress PYY secretion, as measured by ELISA [9]. Cadmium may act on the enteroendocrine L-cells that produce PYY. It is reported that a high level of cadmium (Cd) boosts phosphodiesterase activity and reduces leptin secretion in isolated rat adipocytes. Additionally, treating 3T3-L1 cells with a low concentration of Cd leads to an enhancement in glucose uptake mediated by glucose transporter (GLUT)1 in 3T3-L1 adipocytes.

Fat accumulation

Fat accumulation shows a complex relationship with cadmium concentration. Fat pad mass tends to increase following moderate cadmium concentrations through dissection and weight measurement, in our assumption. PPARγ pathways may become activated, which leads to fat accumulation. When cadmium reaches high concentrations (100 ppm), it has the potential to destroy adipocytes through toxicity, which might decrease fat mass.

A1C Levels

The measurement of HPLC shows that A1C levels increase when cadmium concentration rises. High cadmium levels reaching 100 ppm lead to β-cell damage or insulin resistance that results in elevated A1C levels. Previous studies proposed that hexose uptake in Chinese hamster ovary fibroblasts could be regulated by changing glucose transporter activity, not the number. Using 3T3-L1 fibroblasts and adipocytes, this study found low – low-micromolar cadmium (18 h) increased sugar uptake 5 – 6-fold. In fibroblasts lacking GLUT4, cadmium lowered the amount of 3 - O – 3-O-methylglucose, with little change in GLUT1 amount or cell-surface level. Insulin didn't further boost cadmium-activated transport. In adipocytes (with GLUT1 and GLUT4), insulin and cadmium had additive effects on hexose transport. Cadmium minimally affected adipocyte GLUT1/GLUT4, while insulin recruited GLUT4 to the membrane [10]. The effect of 50 ppm cadmium concentration on A1C measurements remains either undetectable or minimal because the human body maintains glucose homeostasis through its regulatory systems.

Effects of Treatment Duration

SCFA Production

Extended treatment periods tend to worsen the decrease in SCFA production. The extended 36-week exposure to cadmium produces cumulative toxic effects that destroy SCFA-producing bacteria which resulting in reduced SCFA production levels. A 12-week exposure duration would create only minor disturbances to gut microbiota, which leads to minor reductions in SCFAs. The gut microbiota

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shows limited adaptability to cadmium exposure because longer exposure results in more serious disruptions.

PYY Secretion

The duration of treatment administration determines how much PYY gets secreted. The exposure duration directly determines the level of PYY suppression. The damage to enteroendocrine L – L-L-cells becomes more pronounced after 36 weeks of cadmium exposure, thus reducing PYY secretion. During initial cadmium exposure of 12 weeks, the effects on PYY secretion appear minimal. One possibility is that the body may start to repair initial cadmium-related damage.

Fat Accumulation

The duration of exposure to cadmium influences how much fat accumulates based on the cadmium concentration levels. A moderate cadmium concentration combined with long exposure times (36 weeks) tends to result in continuous fat mass increase due to prolonged adipogenesis. The cytotoxic effect on adipocytes at high concentrations might intensify with longer exposure times which resulting in fat mass reduction even though the initial adipogenic effect occurs. Fat accumulation depends on how long cadmium exposure occurs because adipogenesis and adipocyte death rates create an equilibrium.

A1C Levels

The duration of treatment directly affects the chances of detecting elevated A1C levels. The combination of β-cell damage and insulin resistance occurring during 36 weeks of cadmium exposure leads to hyperglycemia, which elevates A1C levels. A 12-week exposure period may not result in substantial glucose dysregulation, so A1C levels would probably remain within the normal range.

The combined effect of cadmium concentration levels and exposure time determines the overall metabolic consequences. The combination of low concentration (10 ppm) and short treatment duration (12 weeks) may lead to no significant changes across metabolic parameters. A 36-week exposure duration may reveal more pronounced effects on SCFA production as well as PYY secretion and potentially fat accumulation and A1C levels. A short exposure of 12 weeks at 100 ppm concentration may cause major disruptions to SCFA production and PYY secretion, while extended exposure leads to intensified cytotoxic effects on adipocytes and β – β-cells, resulting in complicated changes to fat mass and A1C levels.

Both cadmium concentration levels together with treatment duration produce substantial and interconnected effects on the metabolic parameters studied in this research. Assumption and hence confirmations about these associations proves essential for both identifying how cadmium exposure disrupts the gut biome and creating methods to protect human health from cadmium's adverse effects. Research should investigate how molecular mechanisms produce dose and time-dependent effects so more effective interventions can be developed.

8. Conclusions

The research conducted in this study examined multiple cadmium exposure impacts on metabolic health through four specific measurements: SCFA production in fecal samples (GC-MS), peptide YY (PYY) secretion (ELISA), fat accumulation through fat pad dissection with weight measurements, and glycated hemoglobin (A1C) determination via HPLC. The research investigated sixteen potential outcome results (CR1–CR16) to understand how cadmium toxicity affects gut microbiota and endocrine function, together with adipogenesis and glucose regulation.

Cadmium exposure through the GC-MS SCFA Production experiment consistently decreased SCFA amounts in fecal samples throughout different scenarios (CR1, CR2, CR6, CR12), which

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confirmed the proposed effect of cadmium on gut microbiota composition and function. Isolated SCFA loss in CR12 scenarios did not always result in systemic metabolic dysfunction, indicating that compensatory mechanisms such as microbial redundancy or alternative energy sources might provide protective effects against downstream consequences.

PYY suppression occurred frequently in the following results according to ELISA: CR1, CR2, CR9, and CR13, indicating cadmium toxicity to enteroendocrine L-cells. The reduction in PYY did not lead to metabolic problems in all cases (e.g., CR13), possibly due to redundant gut hormones such as GLP-1 or ghrelin or adaptive hormonal responses.

Fat Pad Dissection results showed that increased fat mass occurred in the scenarios where cadmium promoted adipogenesis (e.g., CR1, CR4, CR9, CR14). High doses of cadmium led to unexpected fat mass reductions (CR3, CR8) because the high doses caused adipocyte death. Cadmium demonstrates dual actions on adipose tissue through dose-dependent mechanisms that lead to lipid storage at moderate doses yet cause cell death at excessive concentrations.

The ABC-ELISA experiment demonstrated that A1C levels rose in scenarios CR1, CR3, CR8, and CR15, which supported cadmium's link to glucose dysregulation through β-cell toxicity or insulin resistance. The elevation of A1C sometimes occurred without gut or adipose involvement (CR15), which shows pancreatic cells are sensitive to cadmium regardless of other metabolic disturbances.

The 16 possible result combinations revealed that cadmium's metabolic effects are context-dependent, and influenced by dose, duration, and compensatory mechanisms. The full validation of cadmium-induced metabolic dysfunction appeared in CR1 results, while partial validation occurred in CR2 through CR15, which demonstrated the nonlinear system-specific nature of toxicity. CR16 lacked any observable effects, which demonstrated how critical it is to optimize dose-time parameters during experimental design.

Previous research concentrated on individual endpoints between cadmium and diabetes or gut dysbiosis but failed to reveal the concurrent effects of cadmium on interconnected systems. This study closes the gaps in existing research through a demonstration of cadmium's impact on four areas: First, the GC-MS results indicate that cadmium decreases SCFA production by changing microbial diversity and function, which creates problems with colonic health and metabolite signaling. Second, PYY suppression (via ELISA) indicates that L-cell toxicity leads to disrupted satiety signaling and energy homeostasis. Third, fat accumulation (via dissection) shows that cadmium has adipogenic effects, but the cytotoxicity at high doses makes the dose-response interpretation more complicated. Fourth, A1C elevation (via HPLC) demonstrates cadmium's ability to cause hyperglycemia independently of or synergistically with gut and adipose effects. The research creates a significant advancement in cadmium toxicity mapping by demonstrating its effects across multiple systems and showing that holistic models must integrate gut-endocrine-adipose-pancreas interactions. The research demonstrates important cadmium toxicity thresholds along with its temporal characteristics. The isolated effects demonstrated through CR12 and CR13 show that early biomarkers like SCFA loss and PYY suppression appear before systemic dysfunction develops. The partial support scenarios for CR2 and CR7 show how cadmium targets specific systems, including the gut and adipose tissues, while it doesn't attack other human systems. CR1 demonstrates full support, and CR3 and CR8 show paradoxical results because compensatory mechanisms reach saturation or because of cytotoxicity.

The experimental approaches present both advantages and disadvantages in their research design. The SCFA Measurement via GC-MS presents two main advantages, which include its ability to detect minimal microbial metabolic variations and precise SCFA quantification, yet it cannot

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determine which microbial taxa generate SCFAs or distinguish between host and microbiota SCFAs. Second, the PYY Quantification via ELISA. The approach demonstrates both excellent specificity to PYY and an affordable cost structure and large sample scalability, but cannot assess bioactive PYY and does not detect PYY secretion patterns. Fat Pad Dissection and Weighing represent the third method. This approach allows researchers to measure adipose tissue mass and produces results that are both easy to repeat and consistent. This method fails to distinguish between hypertrophic (cell enlargement) and hyperplastic (cell proliferation) fat growth patterns while ignoring liver cell ectopic fat accumulation. A1C Measurement via HPLC represents the final method. A1C stands as the definitive measure for long-term blood sugar control, yet it shows no response to short-term glucose changes while providing averaged blood sugar readings for a year, which limits its ability to detect early-stage β-cell problems.

The systematic evaluation of 16 outcomes presented in this paper improves interpretation, but the reliance on animal subjects hinders direct human applications. The results' extrapolation to human subjects faces challenges because rodent biology, together with cadmium processing, differs from human systems. The application of a fixed dosage does not align with actual human exposure conditions. The use of A1C as a snapshot measurement fails to detect temporal variations. The study faces a limitation because it focuses on phenotypic results (fat mass, A1C) without exploring the underlying molecular processes. The use of GC-MS for SCFA quantification does not allow researchers to analyze microbial communities, which restricts their ability to study dysbiosis.

The research provides new insights into cadmium toxicity effects by demonstrating its ability to disrupt gut microbiota and endocrine signaling, adipose homeostasis, and glucose regulation through dose- and duration-dependent mechanisms, integrating the study results, followed by an analysis of knowledge advancement potential together with an evaluation of experimental design benefits and challenges. Mapping 16 possible outcomes, the theoretical approaches generated strong qualitative data, yet the limitations in mechanistic understanding and translational application require future work using integrated multidisciplinary approaches. The study findings may help to promote public health strategies to reduce cadmium exposure and promote therapeutic developments focused on gut-endocrine-metabolic connections in metabolic disorders.

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