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Original Article

Cobalt protoporphyrin modulates antioxidant enzyme activity in the hypothalamus and motor cortex of female rats

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Abstract

Cobalt protoporphyrin (CoPP) is a synthetic heme analog that has been observed to reduce food intake and promote sustained weight loss. While the precise mechanisms responsible for these effects remain elusive, earlier research has hinted at the potential involvement of nitric oxide synthase in the hypothalamus. This study aimed to delve into CoPP's impact on the activities of crucial antioxidant enzymes: superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferase (GST) across seven distinct brain regions (hippocampus, hypothalamus, prefrontal cortex, motor cortex, striatum, midbrain, and cerebellum), as well as in the liver and kidneys. Female Wistar rats weighing 180 to 200 grams received a single subcutaneous dose of 25 µmol/kg CoPP. After six days, brain tissue was extracted to assess the activities of antioxidant enzymes and quantify malondialdehyde levels. Our findings confirm that CoPP administration triggers the characteristic effects of decreased food intake and reduced body weight. Moreover, it led to an increase in SOD activity in the hypothalamus, a pivotal brain region associated with food intake regulation. Notably, CoPP-treated rats exhibited elevated enzymatic activity of catalase, GR, and GST in the motor cortex without concurrent signs of heightened oxidative stress. These results underscore a strong connection between the antioxidant system and food intake regulation. They also emphasize the need for further investigation into the roles of antioxidant enzymes in modulating food intake and the ensuing weight loss, using CoPP as a valuable research tool.

Keywords: Antioxidant enzymes, Brain, Decreased body weight, CoPP, Food intake.

1. Introduction

Cobalt protoporphyrin (CoPP), a synthetic metalloporphyrin, has gained attention for its ability to induce heme oxygenase-1 (HO-1), an enzyme responsible for breaking down heme into biliverdin, carbon monoxide, and free iron [1]. This intriguing compound has demonstrated the capacity to induce weight loss and reduce food intake in various species [2-7], including transgenic mice used as obesity models [8]. However, the precise mechanisms underlying CoPP's anorexigenic effects and its impact on the central nervous system's antioxidant system remain in mystery.

Previous studies have unveiled the selective accumulation of CoPP in the hypothalamus following intracerebroventricular administration [2], hinting at the potential involvement of this brain region in mediating its influence on food intake; notably, this effect is not directly linked to HO-1 induction [5]. Also, CoPP-induced weight loss cannot be attributed to increased energy demand [4, 5, 7]. Research probing the mechanisms behind CoPP-induced hypophagia and weight loss has unveiled alterations in creatine concentrations [3] and reduced nitric oxide synthase activity in the hypothalamus [6].

The regulation of food intake is a very complex process involving the integration of sensory, hormonal, and metabolic signals, with the hypothalamus serving as a pivotal hub. Moreover, the interaction of other brain regions, such as the midbrain and striatum, known for their role in the reward system, can significantly influence food intake.

Emerging evidence suggests that reactive oxygen species (ROS) regulate food intake. Mitochondria-generated ROS are implicated in the hypothalamus´s nutrient detection and glucose sensing [9]. Furthermore, ROS production during amphetamine treatment has been associated

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with appetite control [10], and exogenous ROS administration has been shown to induce satiety in mice [11].

Conversely, elevated oxidative stress levels can disrupt hypothalamic neuropeptides linked to appetite control, potentially leading to inflammation and disruptions in energy balance and food intake patterns [12]. The brain possesses inherent protective mechanisms against oxidative stress, involving activating antioxidant enzymes and maintaining mitochondrial function. ROS refers to a large family of oxidants derived from molecular oxygen, such as superoxide anion radical ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($HO^{\bullet}$); the antioxidant system includes several enzymes and molecules that neutralize ROS and maintain cellular redox balance, such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferase (GST).

Despite CoPP not being considered an inducer of oxidative stress, its impact on the central nervous system antioxidant system remains largely unexplored. Consequently, understanding how CoPP affects antioxidant enzyme activities in different brain regions could provide valuable insights into its role in regulating appetite and body weight.

Given these considerations, the present study assessed CoPP's capacity to influence antioxidant enzyme activities and oxidative stress in specific brain regions, including the midbrain, striatum, and motor cortex. This research holds promise for unraveling the complex interplay between CoPP and the antioxidant system and its potential implications for food intake and metabolic health. Such insights may pave the way for identifying novel targets to address metabolic disorders like obesity.

2. Materials and methods

2.1. Chemical reagents

CoPP (Co654-9) was purchased from Frontier Scientific (Logan, UT, USA). Most chemicals, including nitroblue tetrazolium (NBT), xanthine, xanthine oxidase, ammonium molybdate, reduced glutathione (GSH), oxidized glutathione, GR, beta-Nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium salt (NADPH), 1-chloro-2,4-dinitrobenzene (CDNB), tetramethoxypropane, and 1-methyl-2-phenylindole (1M2F) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade and commercially available.

2.2. Animals and bioethics

We used 24 female Wistar rats of 180-200 g (7-9 weeks old); they were kept in individual boxes under controlled temperature and humidity conditions, under 12-h light/dark cycles with free access to water and standard food (5001 Laboratory Rodent Diet). The experimental procedures followed the Norma Oficial Mexicana NOM-062-ZOO-1999. The local Committee on Research Ethics approved the experimental design (Approval number 32/18, date: June 18th, 2018).

2.3. Experimental design and sample obtention

Rats were divided into a control group and a CoPP-treated group. Rats received a single subcutaneous (s.c.) injection of 2 ml/kg of saline as the vehicle for the control group and 25 µmol/kg of CoPP for the experimental group. After six days, rats were anesthetized with 50 mg/kg sodium pentobarbital and euthanized by cardiac perfusion with saline solution. Once the perfusion was finished, the livers and kidneys were extracted, and the brains were dissected on ice to obtain the hypothalamus, hippocampus, prefrontal cortex, motor cortex, striatum, midbrain, and cerebellum. All the tissues were immediately frozen in liquid nitrogen and stored at -80°C until used. Blood was extracted from the right atrium of the animal's heart before perfusion and rested for 30 min. Later, it was centrifuged at 2,000 x g for 10 min for serum collection.

2.4. Body weight and food intake

On the day of CoPP or vehicle administration, each rat's initial body weight was recorded, and 300-400 g of food was placed in the rats' cages. The remaining food and ending body weight of rats were measured on days 5 and 6, respectively. Both the difference in the means of food consumption and the body weight of the rats when administering the respective treatments (initial body weight, first day) and at the end of the experiment (final body weight, sixth day) were calculated.

2.5. Serum parameters

Blood serum was used to determine biochemical, renal, and hepatic markers through commercial kits. We measured blood urea (Spinreact, 1001325), triglycerides (Spinreact, 41032), alkaline phosphatase (Spinreact, 1001131), and alanine aminotransferase (Spinreact, 41282), following the manufacturer's instructions. According to the manufacturer's instructions, blood urea nitrogen (BUN) was calculated by correcting the blood urea value by a 2.14 factor.

2.6. Tissue homogenization

Brain tissues were homogenized in a 1:3 (w/v) ratio, and the liver and kidney were homogenized in a 1:10 (w/v) ratio with a cold 50 mM phosphate buffer pH 7 containing 0.05% Triton X-100. Brain cell disruption was performed on ice using an ultrasonic homogenizer, and hepatic and renal tissues were homogenized with a Kinematica-Polytron homogenizer. The homogenates were centrifuged at 10,000 x g at 4°C for 30 minutes. The supernatants were stored at -70°C until enzymatic activity and oxidative stress were measured.

2.7. Total SOD activity

This assay was made based on the enzymatic ability to prevent the reduction of NBT by $O_2^{\bullet-}$ generated by the xanthine-xanthine oxidase reaction. The reaction mixture consisted of 0.3 mM xanthine, 150 µM NBT, 0.6 mM ethylenediaminetetraacetic acid (EDTA), and 0.4 M sodium carbonate. The reaction was initiated by adding 0.1 U/ml xanthine oxidase and monitored spectrometrically at 560 nm. One unit of SOD was defined as the amount of enzyme that inhibited NBT reduction by 50%. Data were expressed as units per milligram of protein.

2.8. Catalase activity

The enzymatic decomposition of 20 mM H_2O_2 by catalase was evaluated at 37 ºC for three minutes. The undecomposed H_2O_2 reacts with ammonium molybdate to produce a yellowish color with a maximum optical density (OD) at 374 nm. The rate constant of a first-order reaction (k) equation was used to determine catalase activity:

$$\text{Catalase Activity (kU)} = (2.303 / t) \times \log (So / [S - M]) \times (Vt / Vs)$$

Where t=time; So=20 mM H_2O_2 OD; S=Sample OD; M=sample blank OD; Vt=Total volume; Vs=Sample volume

Data were expressed as units (μmoles H_2O_2 decomposed per minute)/mg of protein.

2.9. GPx activity

The activity of this peroxidase was measured using a reaction mixture consisting of 1 mM EDTA, 1 mM GSH, 1 mM sodium azide, 0.2 mM NADPH, and 1 U/ml GR in 50 mM phosphate buffer (pH 7.4). Hepatic and renal samples (0.036 ml) were mixed with 0.3 ml of the reaction mixture and 0.036 ml of 2.5 mM H_2O_2 solution (1.25 mM H_2O_2 solution was used for brain samples). The absorbance at 340 nm was recorded for three minutes, and the activity was calculated from the slope of these lines. One unit of enzyme was considered as the amount of enzyme that oxidizes 1 μmol of NADPH/min. Blank reactions with the enzyme source substituted for distilled water were subtracted from each assay. Data were expressed as units per milligram of protein.

2.10. GR activity

The activity of this enzyme was measured using oxidized glutathione as a substrate, and the consumption of NADPH was measured. The reaction mixture consisted of 0.5 mM EDTA, 1.25 mM oxidized glutathione, and 0.1 mM NADPH in 100 mM phosphate buffer (pH 7.6). Supernatants (0.015 ml) were added to the 0.3 ml reaction mixture, and the absorbance at 340 nm was recorded for 3 minutes. The activity was calculated from the slope of these lines, and one GR unit was defined as the amount of enzyme that oxidizes 1 μmol of NADPH/min. Data were expressed as units per milligram of protein.

2.11. GST activity

It was measured with a reaction mixture consisting of 1 mM GSH and 1 mM CDNB in 50 mM phosphate buffer (pH 6.5). Supernatants (0.008 ml) were added to the 0.35 ml reaction mixture, and the OD was measured at 340 nm for three minutes. One unit of enzyme conjugates 1 μmol CDNB with GSH/min. Data were expressed as units per milligram of protein.

2.12. Malondialdehyde (MDA) content

This oxidative stress marker was measured as follows: Supernatants and tetramethoxypropane standards (0.2 ml) were added to 0.65 ml of 10 mM 1M2F in an acetonitrile/methanol (3:1 v/v) mixture. The reaction was started by adding 0.15 ml of 37% hydrochloric acid and incubating for 40 minutes at 45°C. The samples were then centrifuged at 3,000 x g for 5 minutes. The OD of the supernatant was measured at 586 nm, and data were expressed as nmol of MDA per milligram of protein.

2.13. Data analysis

The results were expressed as mean ± SEM. Graphs from data and unpaired t-tests were performed using GraphPad Prism version 10.0.0 for Windows (GraphPad Software, Boston, MA, USA, www.graphpad.com). Differences between groups were considered significant at $p<0.05$.

3. Results

3.1. Body weight and food intake

A comprehensive analysis of body weight and food intake was undertaken to validate the influence of CoPP administration on these parameters. As depicted in Figure 1A, the initial weights recorded on the first day of saline or CoPP administration exhibited a comparable baseline. However, a noteworthy divergence became apparent six days following CoPP treatment (Figure 1B), characterized by a significant 10% reduction in body weight compared to the control group ($t=2.476$, $df=17$, $p=0.0241$). This effect was further evident in food intake (Figure 1C), as CoPP administration resulted in a substantial 27% decrease relative to the control group ($t=6.15$, $df=10$, $p = 0.0001$).

3.2. Serum parameters

To assess the potential adverse effects of CoPP administration on liver and kidney function, we examined BUN levels, triglycerides, alkaline phosphatase activity, and alanine aminotransferase (Figure 2). While no statistically significant differences were observed in triglycerides and alkaline phosphatase, there was a discernible tendency towards increased alkaline phosphatase in the CoPP-administered group (Figure 2C). Conversely, BUN levels displayed a substantial 40% increase relative to the saline-

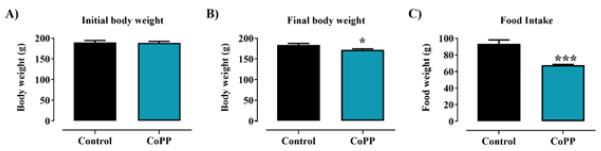

Fig. 1. Effect of CoPP administration on body weight and food intake. Female rats were treated with vehicle (Control) or CoPP (a single dose of 25 μmol/kg s.c.). A) The average body weight of the rats is shown at the beginning of the experiment (initial body weight), and B) six days after (final body weight). C) The total food intake (average of the differences between the first and fifth days of treatment). Unpaired t-test, mean ± SEM, n=9-10, *p<0.05 and ***p<0.001 vs. control. CoPP: cobalt protoporphyrin.

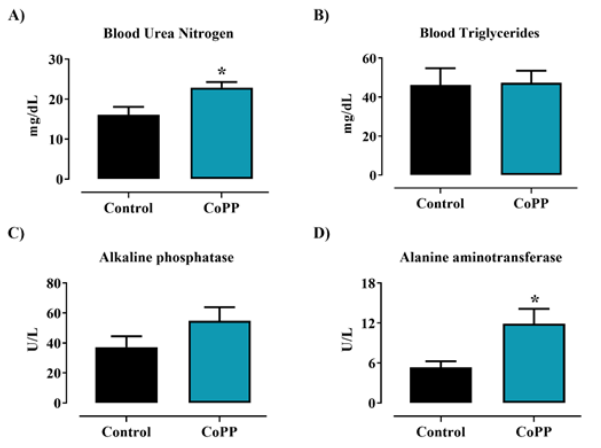

Fig. 2. Systemic effects of CoPP. Serum parameters to assess systemic toxicity of CoPP. A) blood urea nitrogen, B) triglycerides, C) alkaline phosphatase, and D) alanine aminotransferase. Unpaired t-test, mean ± SEM, n=10-12, *p<0.05 vs. control. CoPP: cobalt protoporphyrin.

administered group (t=2.805, df=10, p = 0.0186), and alanine aminotransferase (Figure 2D) exhibited a remarkable 100% elevation in the CoPP group compared to the control (t=2.682, df=10, p = 0.023).

3.3. Antioxidant Enzymes Activity and Malondialdehyde Production

To investigate the impact of CoPP on oxidative stress and antioxidant enzyme activity, we assessed levels of MDA and enzymes, including SOD, catalase, GPx, GR, and GST in various brain regions. The hypothalamus (Figure 3), a pivotal brain region governing food intake, exhibited a notable 15% increase in SOD activity upon CoPP administration compared to the control group (t=4.312, df=10, p=0.0015). However, no significant alterations were observed in the activity of other enzymes or MDA levels. In the motor cortex (Figure 4), a region that receives signals from the thalamus and, in turn, from the ventral tegmental area, CoPP administration substantially elevates the activity of several antioxidant enzymes. Specifically, catalase activity increased by 130% (t=7.445, df=11, p=0.0001), GR activity showed a 25% rise (t=3.011, df=11, p=0.0118), and GST activity demonstrated a 35% increase (t=2.307, df=11, p=0.0415) compared to the group administered with saline.

Other notable changes in antioxidant enzyme activities included a 10% decrease in GST activity in the hippocampus (t=2.502, df=10, p=0.0314) and a similar 10% reduction in catalase activity in the cerebellum (t=2.493, df=10, p=0.0318). No statistically significant differences were observed in the activities of antioxidant enzymes in the striatum, midbrain, cerebellum, liver, or kidney (Table 1). Furthermore, malondialdehyde levels remained unaffected across all examined tissues.

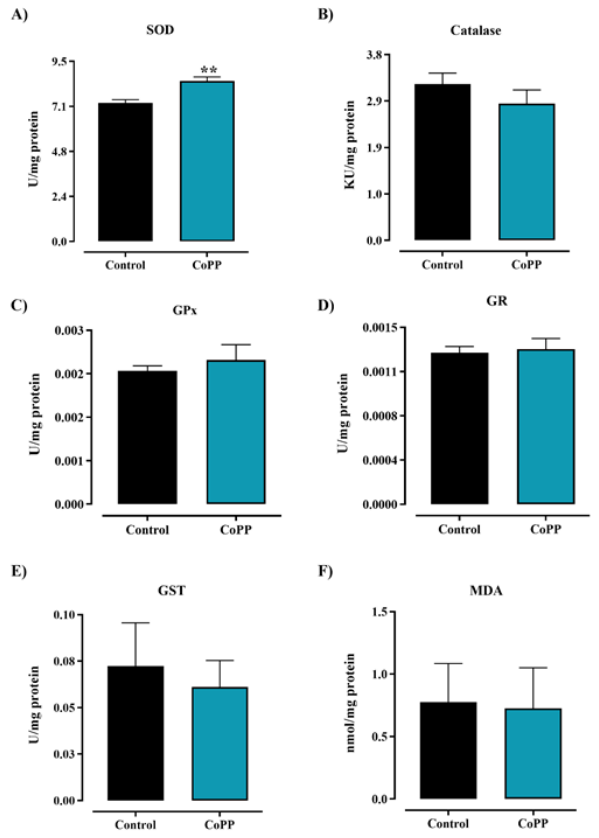

Fig. 3. Effect of CoPP on the activity of antioxidant enzymes and oxidative stress in the hypothalamus. A) superoxide dismutase (SOD), B) catalase, C) glutathione peroxidase (GPx), D) glutathione reductase (GR), E) glutathione-S-transferase (GST) and F) malondialdehyde (MDA). Unpaired t-test, mean ± SEM, n=10-12, **p<0.01 vs. control. CoPP: cobalt protoporphyrin.

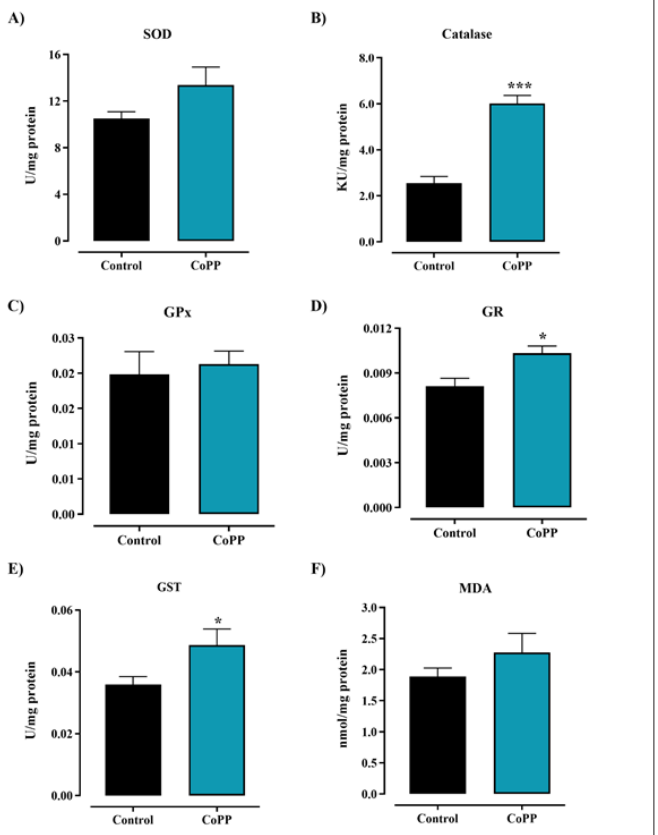

Fig. 4. Effect of CoPP on the activity of antioxidant enzymes and oxidative stress in the motor cortex. A) superoxide dismutase (SOD), B) catalase, C) glutathione peroxidase (GPx), D) glutathione reductase (GR), E) glutathione-s-transferase (GST) and F) malondialdehyde (MDA). Unpaired t-test, mean ± SEM, n=10-12, *p<0.05 and ***p<0.001 vs. control. CoPP: cobalt protoporphyrin.

4. Discussion

This study sought to explore the impact of CoPP on the activity of antioxidant enzymes and malondialdehyde (MDA) content in various brain regions, particularly those implicated in food intake regulation, including the hypothalamus, midbrain, striatum, and cortex. Our findings align with previous research, confirming that a single dose of CoPP significantly reduces food intake and prevents weight gain in rats [4, 5, 7]. Additionally, we observed increased enzymatic activity of SOD in the hypothalamus, and elevated catalase, GR, and GST activities in the motor cortex, all without evidence of heightened oxidative stress. The above suggests a compelling link between the antioxidant system and food intake regulation.

CoPP is widely recognized as an inducer of HO-1, often associated with cytoprotection, even though it has been reported that some stimuli do not trigger HO-1 induction [13]. While CoPP is generally considered non-toxic, we assessed potential systemic toxicity by monitoring BUN, triglycerides, alkaline phosphatase, and alanine aminotransferase levels, reflecting kidney and liver function. CoPP had no significant effect on triglyceride levels, in line with previous findings [14]. Unexpectedly, BUN and alanine

Table 1. Antioxidant enzyme activities and malondialdehyde content

	SOD (U/mg prot)		Catalase (kU/mg prot)		GPx (U/mg prot)		GR (U/mg prot)		GST (U/mg prot)		MDA (nmoles/mg prot)	
	Control	CoPP	Control	CoPP	Control	CoPP	Control	CoPP	Control	CoPP	Control	CoPP
Hippocampus	7.1 ± 0.1	7.0 ± 0.2	3.5 ± 0.2	3.7 ± 0.3	0.010 ± 0.0002	0.009 ± 0.0001	0.011 ± 0.0003	0.011 ± 0.0001	1.3 ± 0.02	1.2 ± 0.03*	2.0 ± 0.4	2.3 ± 0.2
Prefrontal Cortex	14.9 ± 0.1	14.5 ± 0.6	4.7 ± 0.1	4.7 ± 0.3	0.019 ± 0.001	0.016 ± 0.001	0.016 ± 0.0004	0.015 ± 0.0013	2.1 ± 0.1	3.5 ± 0.1***	0.6 ± 0.02	0.5 ± 0.07
Striatum	18.0 ± 0.9	19.7 ± 0.3	3.7 ± 0.4	4.3 ± 0.3	0.03 ± 0.002	0.03 ± 0.001	0.0076 ± 0.0003	0.0075 ± 0.0004	0.045 ± 0.003	0.049 ± 0.003	2.3 ± 0.1	1.8 ± 0.3
Midbrain	17.3 ± 0.8	18.9 ± 1.3	7.2 ± 0.7	8.1 ± 0.5	0.029 ± 0.001	0.028 ± 0.002	0.006 ± 0.0002	0.007 ± 0.0005	0.05 ± 0.003	0.05 ± 0.004	2.3 ± 0.6	1.2 ± 0.3
Cerebellum	21.0 ± 0.9	20.3 ± 0.5	25.9 ± 0.9	22.8 ± 0.8	0.027 ± 0.002	0.027 ± 0.001	0.023 ± 0.0013	0.022 ± 0.0002	0.38 ± 0.02	0.39 ± 0.01	3.2 ± 0.2	3.2 ± 0.2
Liver	22 ± 2.4	20 ± 1.4	405 ± 42	331 ± 43	1.2 ± 0.1	1.1 ± 0.1	0.055 ± 0.007	0.060 ± 0.003	18.9 ± 3.4	17.0 ± 1.3	1.9 ± 0.6	2.1 ± 0.6
Kidney	15.0 ± 0.7	13.3 ± 1.0	465 ± 42	455 ± 22	0.24 ± 0.02	0.24 ± 0.02	0.019 ± 0.0009	0.017 ± 0.0020	0.25 ± 0.03	0.24 ± 0.03	2.9 ± 0.9	2.5 ± 0.6

Data are shown as mean ± SEM. *p<0.05 and ***p<0.001 vs. Control group. SOD: Superoxide dismutase; GPx: glutathione peroxidase; GR: glutathione reductase; GST: glutathione-S-transferase; MDA: malondialdehyde. CoPP: cobalt protoporphyrin.

aminotransferase levels exhibited an increase following CoPP administration. However, it is essential to note that these changes did not signify renal or liver damage, as the recorded values remained within the physiological range. The assessment of renal function using BUN is not exclusive to intrinsic renal activity, as the blood urea level is significantly influenced by factors such as protein intake, catabolism, and hydration status [15].

Traditionally, research into food intake regulation has primarily focused on hormonal input to the hypothalamus. However, other factors, such as mitochondrial ROS, have gained attention recently [16]. In this context, our study examined the activity of various antioxidant enzymes across different brain regions. We detected significant variations in the hypothalamus and motor cortex, which govern autonomic and executive processes. The increased SOD activity in the hypothalamus and catalase, GR, and GST activities in the motor cortex may suggest elevated ROS levels. The SOD change in the hypothalamus could be in response to increased O_2•- levels, with SOD dismutating O_2•- into H_2O_2. However, the absence of changes in H_2O_2-related enzymes like catalase and GPx suggests that the increase in H_2O_2 levels was moderate and insufficient to induce oxidative stress. Future research could investigate the impact of CoPP on protein or nucleic acid oxidation. Our data was collected six days post-CoPP administration, yet it is essential to note that ROS production has been reported to occur rapidly following a stimulus [9].

Lipid peroxidation, a process with the potential to cause cell damage and various pathological conditions, has been suggested to play a significant role in food addiction, serving as both a causative and mediating factor in obesity [17]. ROS have been implicated in cell signaling, food intake regulation [9, 16], and adipogenesis control [18]. Changes in antioxidant enzyme activities have been observed under conditions affecting food intake, such as high-sugar diets [19], lactobacilli administration [20], or hormonal interventions [21], pointing to the adaptability of the hypothalamus to nutraceutical compounds and gender-specific responses [21]. Additionally, antioxidant enzymes like SOD and catalase have been associated with protection against neurodegenerative disorders [22], and SOD activity has been reported to be rarely affected by dietary restriction [23].

Antioxidant enzyme activities respond to the level of ROS produced in cells. We propose that the increase in SOD activity in the hypothalamus is due to elevated O_2•- levels. This increase in SOD activity could also be associated with heightened H_2O_2 production. Both O_2•- and H_2O_2 are key redox signaling agents. ROS are essential in regulating food intake in the hypothalamus by affecting nerve cells, including anorexigenic and orexigenic neurons. ROS also function as physiological sensors, detecting glucose and stimulating insulin release [16, 17]. Our findings align with previous studies indicating that intracerebroventricular administration of ROS scavengers or H_2O_2 can increase or decrease food intake. This effect is linked to hypothalamic proopiomelanocortin (POMC) neurons [11].

Recent research highlights the balance between catalase activity and H_2O_2 levels as a regulator of lipid levels. Excessive H_2O_2 production can reduce lipid levels and disrupt metabolism, suggesting increased H_2O_2 inhibits adipogenesis. Catalase is critical in maintaining appropriate adipocyte lipid levels [24]. Thus, future studies should determine if H_2O_2 concentration increases after CoPP administration, shedding light on the role of increased SOD and peroxidase activity.

Moreover, the motor cortex, which controls muscle motor functions, including tongue [25] and jaw movements during chewing [26], appears to be an intriguing brain region involved in the feeding process. In humans, the motor cortex is activated in the motor and imaginary execution of self-feeding [27] and response to high-calorie food pictures [28]. Our results are consistent with previous findings illustrating that GR is responsive to changes in food intake and body weight [29]. Notably, catalase activity increases with improved nutritional status, as evidenced in patients with anorexia nervosa [30]. GST refers to a family of enzymes known for their dual role in detoxifying xenobiotic toxic molecules and maintaining cellular redox homeostasis; it might be responsible for detoxifying the organism from CoPP. Previous reports support this by associating high GST levels with drug resistance, even when the drug is not a substrate [31].

While this study provides valuable insights, it has limitations. For example, the research was conducted exclusively in female rats and focused on a single time point. As our prior research [7] outlined, our selection of female rats over male rats was deliberate. This decision was motivated by the substantial and enduring alterations in androgen metabolism observed in male rats following a single injection of cobalt-protoporphyrin (50 μmol/kg), as reported by Galbraith and Jellinck [32]. This distinction is pivotal, given that changes in testosterone levels have been linked to notable effects on locomotor activity and anxiety behavior in male rats, as demonstrated in studies by Jeong et al. [33] and Domonkos et al. [34]. Furthermore, the impact of CoPP administration on body weight and its underlying mechanisms have been more extensively documented in male rats than in their female counterparts. Previous studies by Csongradi et al. [8], Galbraith and Kappas [4, 5], and Galbraith et al. [2, 3] have contributed substantially to the understanding of these effects in male rat models. However, examining the effects of CoPP in both male and female rats at various time points could significantly augment our comprehension of how CoPP influences food intake. Additionally, the study could benefit from examining inflammation-related molecules following CoPP administration, as inflammation and oxidative stress in the hypothalamus have been identified as contributing factors to obesity [35].

5. Conclusion

In conclusion, our study demonstrated that CoPP treatment leads to decreased body weight, reduced food intake, and altered antioxidant enzyme activities in the hypothalamus and motor cortex. Our findings raise several intriguing research avenues, including the role of O_2•-, H_2O_2, and SOD in the hypothalamus and motor cortex, as well as the potential involvement of the motor cortex in feeding behavior. These results underscore the urgent need for further research to elucidate the roles of antioxidant enzymes in modulating food intake and the resultant weight loss, making CoPP a valuable investigative tool.

Abbreviation

CoPP: Cobalt protoporphyrin; SOD: superoxide dismutase; GPx: glutathione peroxidase; GR: glutathione reduc-

tase; GR: glutathione-S-transferase; HO-1: heme oxygenase-1; ROS: reactive oxygen species; H_2O_2: hydrogen peroxide; NBT: nitroblue tetrazolium; GSH: educed glutathione; NADPH: beta-Nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium salt; CDNB: 1-chloro-2,4-dinitrobenzene; 1M2F: tetramethoxypropane, and 1-methyl-2-phenylindole; EDTA: ethylenediaminetetraacetic acid; OD: optical density; s.c.: subcutaneous; MDA: Malondialdehyde; BUN: blood urea nitrogen.

Conflict of Interests
The authors have no conflicts with any step of the article preparation.

Consent for publications
The authors read and approved the final manuscript for publication.

Ethics approval and consent to participate
The experimental procedures followed the Norma Oficial Mexicana NOM-062-ZOO-1999. The local Committee on Research Ethics approved the experimental design (Approval number 32/18, date: June 18th, 2018).

Informed Consent
The authors declare that no patients were used in this study.

Availability of data and material
The data supporting this study's findings are available from the corresponding author upon reasonable request.

Authors' contributions
Raúl Pinete-Sánchez: Investigation, Writing - original draft, Formal analysis; Norma Serrano-García: Investigation, Writing - original draft, Formal analysis; Omar Noel Medina-Campos: Investigation, Writing - review & editing, Formal analysis; Minerva Adriana Ramos-Santander: Investigation; José Pedraza-Chaverri: Writing - review & editing, Resources; Marisol Orozco-Ibarra: Conceptualization, Funding acquisition, Writing – review & editing, Formal analysis, Supervision.

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