

# The Effect of Microgravity on Parathyroid Hormone Secretion: A Meta-Analysis

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## Abstract

**Background:** The relationship between microgravity and parathyroid hormone (PTH), a keystone of bone mineral density, remains controversial. Bone loss is a prominent, ongoing issue in spaceflight, and PTH has been suggested as a treatment for microgravity-induced osteopenia, indicating the importance of this hormone. This systematic review and meta-analysis aimed to evaluate the association between exposure to microgravity and the production of PTH.

**Methods:** PubMed, Embase, Scopus, and Google Scholar were searched for studies reporting PTH levels during and after exposure to microgravity. Non-peer-reviewed articles, studies lacking control groups, and articles published earlier than 2002 were excluded. Twelve articles from 2002 to present, with a total of 145 subjects, were identified and the standardized mean differences from baseline PTH levels were combined in a random effects model. Two-way analysis of variance (ANOVA) with Tukey honestly significant difference (HSD) testing on weighted mean differences was conducted to obtain 95% confidence intervals.

**Results:** Compared to baseline measurements, significant changes in PTH levels are found during and after microgravity exposure. In-flight levels significantly decrease ( $P < 0.01$ ), and post-flight levels show increases. Furthermore, there is evidence of an interaction between experimental condition (real or simulated microgravity) and time after removal from microgravity on PTH.

**Conclusions:** The findings of this systematic review and meta-analysis suggest that microgravity affects parathyroid gland function during and after spaceflight, with decreases in function in-flight and an increase at 7 days post-flight. Experimental condition also appears to play a role in the recovery timeline of PTH.

**Keywords:** Microgravity; Endocrine; Parathyroid; Bone metabolism

## Introduction

Spaceflight grows ever ambitious. As astronauts spend longer

periods in space with a future goal of a manned flight to Mars, it is critical to understand the physiological changes that occur in weightless conditions. Loss of bone density during spaceflight is a preeminent issue in aerospace medicine; upon return to the ground, astronauts are predisposed to fracture under the heavier load of Earth's gravity and bone mineral density is recovered slowly if fully recovered at all [1, 2]. Parathyroid hormone (PTH) has gained attention in microgravity research for its role in bone density, having been used clinically to treat osteoporosis [3] and to slow bone loss in rats under simulated weightlessness [4].

Secretions of many hormones have been observed to decrease during spaceflight and in simulations of microgravity, suggesting that the human endocrine system responds to changes in ambient gravity. However, studies can be sparse and report heterogeneous results. Furthermore, several confounding variables in the spacecraft environment may alter parathyroid function separately from microgravity, such as components in the spacecraft itself. For example, iodine is no longer used as a water disinfectant in American spacecraft after observations that iodine contributed to increased thyrotropin (TSH) levels, a marker of hypothyroidism [5].

This indicates a need for a synthesis of microgravity studies to reduce the effect of confounds in single studies, emphasizing recent literature to reflect updates in gravity research. Furthermore, the effect of real versus simulated microgravity conditions on experimental results has not been thoroughly explored. This systematic review and meta-analysis gathers studies from January 1, 2002 to present concerning the effect of microgravity on PTH production to elucidate the relationship between microgravity exposure, experimental condition, and PTH levels.

## Physiology in Microgravity

Decades of spaceflight research identify several physiological changes that occur in weightless conditions, many with foundations in the endocrine system. Osteopenia, primarily from load-bearing bones such as the pelvis and femur [6], is one of the most pressing and well-documented issues in spaceflight due to the potential for post-spaceflight complications, incurring similar depressed hormone profiles and high risks of bone fracture as age-related osteoporosis [7, 8]. Loss of skeletal muscle during flight compounds this risk of fracture, predisposing astronauts to unsteadiness and weakness on the ground [6].

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On top of musculoskeletal changes, an assortment of other adaptations also occurs in weightless conditions. Without gravity, body fluids shift from the feet to the head, incurring reductions in blood volume. This acquired hypovolemia potentiates orthostatic hypotension when astronauts return to normal gravity [9]. Cephalad fluid shifts also increase intraocular pressure, contributing to long-term vision changes seen in astronauts on extended spaceflights [10]. Virtually every body system is affected by microgravity; for example, immune, cardiovascular, and nervous systems all show cellular changes during spaceflight, altering protein expression and the cell cycle, and incurring functional changes in cell adhesion and morphology [11, 12].

As hormones play key regulatory roles in virtually all physiological systems, it is critical to understand how glandular function changes in weightless conditions. Importantly for spacefarers, bone density is principally regulated through the hormones PTH and calcitonin [4, 8], and skeletal muscle mass is maintained in part by the thyroid hormone thyroxine [13]. Understanding how endocrine function is affected by microgravity is thus vitally important to understand, with its roles in preeminent issues such as bone and muscle loss as well as the broad scope of physiological effects seen in weightlessness. This swath of changes makes gravitational physiology research incredibly important to better understand a novel physical environment and to support the human body during long-term spaceflight.

## Parathyroid Gland Function in Microgravity

PTH levels have been observed to drop during exposure to microgravity coupled with an increase above pre-flight baseline after return to Earth. This reduced hormone secretion during spaceflight is paralleled in several other endocrine organs, such as in T3, T4, and computed tomography (CT) from the thyroid [14-16]. Production of insulin and corticosteroids [8] as well as renin [14] from the pancreas, adrenal glands, and kidneys, respectively, are also all negatively impacted by exposure to microgravity.

However, multiple factors obfuscate definite conclusions about the endocrine system in weightless conditions. Complex interactions between several confounding variables in the spaceflight environment obfuscate the effect of microgravity alone, such as background radiation or psychological factors like isolation. Not only does this complicate literature analysis, but also adds technical obstacles to microgravity research by making accurate simulations more difficult to achieve. Low sample sizes and poor inter-study agreement further obfuscate clear trends in study results. These challenges perplex the formation of concrete conclusions regarding endocrine function in microgravity.

Recent discourse on thyroid function during spaceflight exemplifies these difficulties. For example, a study on American spacecraft found that iodine present in the water contributed to decreases in thyroid hormone levels [17], leading to preliminary suggestions that microgravity does not directly influence thyroid function. However, studies conducted on non-American spacecraft show a continued prevalence of de-

creased thyroid levels, such as on Kosmos missions [18-20], Spacelab [21-23], Euromir [24], or Mir [25] where silver was used as a water disinfectant. Furthermore, studies conducted on American spacecraft after the replacement of iodine continue to show decreased in-flight thyroid hormone levels [26-28]. Astronauts also show increased thyroid hormone levels immediately post-flight, suggesting a compensatory response after an in-flight decrease in thyroid function [24, 25, 29]. Hidden in this discourse is also the effect of technological advancement, where changes in both spaceflight research techniques and engineering subtly change the results of studies over time, especially in such a young, highly innovative field as aerospace medicine. This discussion illustrates the effect of confounding variables, high inter-study variation, and limited sample sizes in microgravity research, especially in endocrinological studies, and how these issues obfuscate clear conclusions. These challenges across all microgravity research naturally extend to PTH in microgravity, spawning ongoing knowledge gaps regarding the parathyroid gland in space. In turn, there is a need for a synthesis of modern literature to quantify the effect of weightlessness on parathyroid function.

Changes in endocrine function are paralleled by alterations to tissue and gene expression. Recent histological studies on endocrine cells cultured in microgravity show changes in gene expression, cell adhesion, and tissue morphology in a wide variety of endocrine tissues [11, 12, 26]. Again using the thyroid as an example, cellular changes to the thyroid have been suggested as a potential mechanism for microgravity-associated hypothyroidism. Both carcinoma and benign thyroid cells show increased apoptosis and decreased hormone production as well as altered protein and gene expression profiles and three-dimensional (3D) conglomeration when placed in microgravity [30-32]. In turn, there is substantial evidence to suggest that microgravity influences thyroid tissue at the cellular level. Similar histological changes have been noted in other endocrine glands such as the pancreas, including increases in vacuolation as well as islet size and number, reflecting reductions in insulin production during spaceflight [33]. However, while these structural changes to endocrine tissue have been studied fairly extensively in microgravity, little information is available regarding changes to parathyroid tissue. The parathyroid gland may undergo similar structural changes in microgravity, but further research is required to clarify the specific effect of microgravity on parathyroid tissue.

With the prevalence and strong potential for complications with microgravity-induced osteopenia, studying CT and PTH in microgravity is critical due to their roles as keystones of calcium balance. Isolated observations in the literature show a similar pattern in CT levels with exposure to microgravity, with a drop in CT levels in weightless conditions coupled with an increase after exposure. However, there are not enough data on CT levels to produce substantive results from a systematic review and quantitative analysis. Enough data exist for PTH, however, for meaningful meta-analysis. As loss of bone density continues to impact astronauts returning from space, synthesizing data on PTH levels during and after flight will elucidate the recovery timeline and establish the magnitude of change for the parathyroid gland.

**Table 1.** Keywords Used to Search Individual Databases Until July 19, 2022 With Records Identified From Each Source

Database	Terms	No. of records identified
Embase, Scopus	parathyroid* AND (microgravity OR hypogravity OR “low gravity” OR weightless*)	Embase: 18; Scopus: 16
PubMed	parathyroid* AND (microgravity OR hypogravity OR “low gravity” OR weightless*) (all fields)	9
Google Scholar	parathyroid AND (microgravity OR hypogravity OR “low gravity” OR weightlessness OR weightless)	979
JAMA Network	parathyroid AND (microgravity OR hypogravity OR “low gravity” OR weightlessness)	0

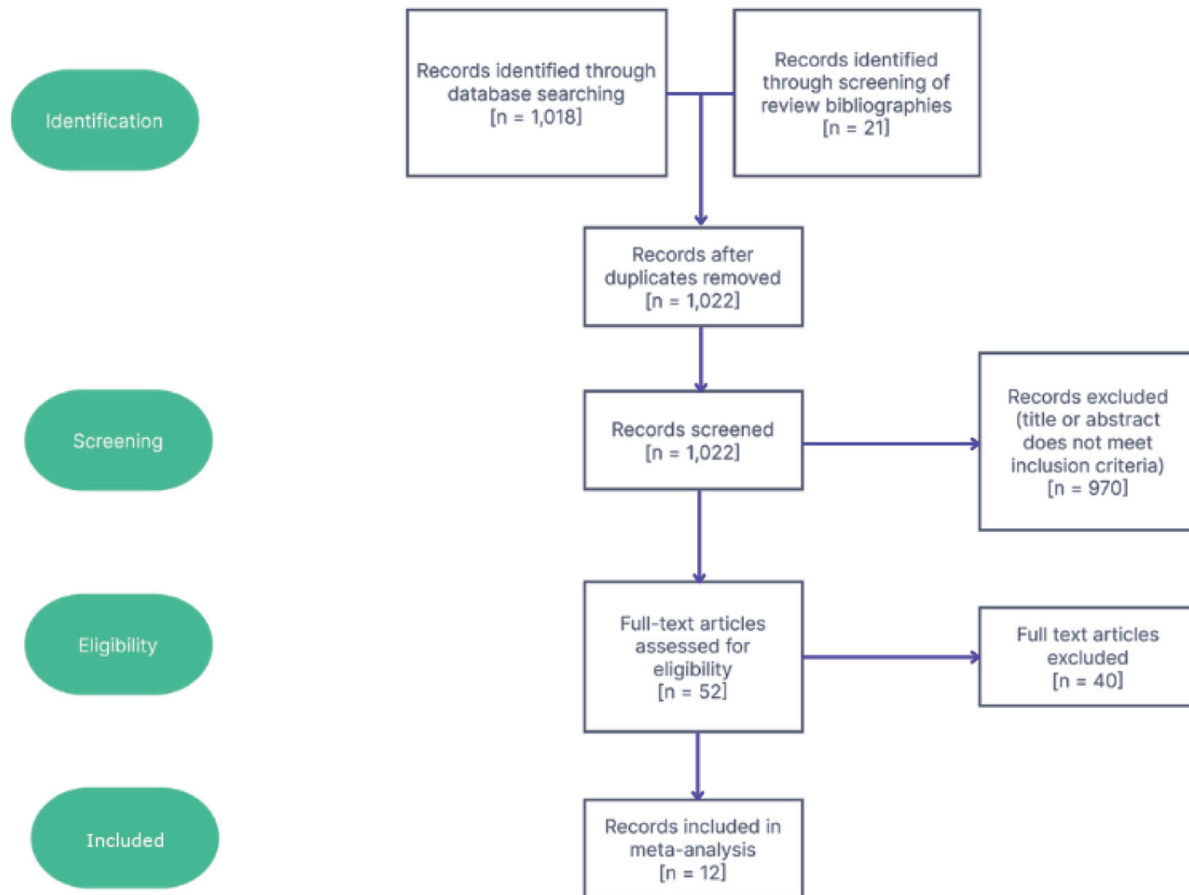
**Methods**

**Literature search**

A systematic literature search was performed using Embase, Scopus, JAMA Network, and PubMed. Google Scholar was used as a secondary search tool to identify grey literature and other studies, until 19 July 2022. The bibliographies of manuscripts were also screened to obtain additional back-referenced material. Search terms used for each database are collected in Table 1.

All inclusion and exclusion criteria were chosen *a priori*.

To be included, articles must measure levels of PTH in either real or simulated microgravity. Articles utilizing thyroid cancer cells were excluded, as malignant thyroid cells show a different gene expression profile as well as altered aggregation and proliferative behavior in microgravity compared to nonmalignant cells [34]. Animal subject studies were excluded. Non-English articles were translated and not excluded. Articles greater than 20 years of age at the time of reading were excluded using a minimum year of publication of 2002. Studies utilizing both simulated and real microgravity conditions were included. Studies must also have been peer-reviewed. Experimental designs lacking control groups or pre-flight measurements of hormone levels were excluded.



**Figure 1.** Flowchart showing screening and selection process for studies along with numbers of studies excluded at each step.

**Table 2.** Data Extracted for Each of the 12 Studies That Passed the Screening Process, Including Year, Name of First Author, Sample Size (n), Microgravity Condition (Real Versus Simulated), Time Points Collected, and Databases Where the Article Is Indexed

Ref.	Year	First author	n	Real/simulated	Timepoints	Database
[37]	2022	Linossier	9	Simulated	In-flight, R+2-7	Embase, Scopus, Google Scholar
[38]	2015	Smith	7	Real	In-flight	Scopus, Google Scholar
[39]	2012	Morgan	12	Simulated	In-flight, R+2-7	Embase
[27]	2012	Smith	6	Real	In-flight, R+0, > R+7	Scopus, PubMed, Google Scholar
[40]	2010	Armbrecht	10	Simulated	In-flight, R+1, R+2-7, >R+7	Embase, Scopus
[41]	2007	Zerwekh	10	Simulated	In-flight	Embase, Scopus, PubMed
[42]	2007	Zwart	7	Simulated	In-flight, R+0, R+1, R+2-7, >R+7	Scopus, PubMed
[28]	2005	Smith	6, 16*	Real	In-flight, R+0, R+2-7, >R+7	Embase, Scopus, Google Scholar
[43]	2005	Rittweger	25	Simulated	In-flight, R+1, R+2-7, >R+7	Google Scholar (grey)
[44]	2005	Morukov	9	Real	R+1, R+2-7, >R+7	Scopus
[45]	2004	Shackelford	18	Simulated	In-flight	Embase, Scopus, PubMed
[46]	2003	Smith	8	Simulated	In-flight, R+1, R+2-7	Scopus, PubMed

\*Smith et al report data from two separate studies, one with a sample size of six and another with 16 subjects.

Of the initial 1,022 articles retrieved, 12 were kept and analyzed. Figure 1 depicts the selection process for these studies.

### Data extraction

The following data were extracted: author name(s), year of publication, whether the microgravity experimental conditions were real or simulated, size of treatment groups, mean or median PTH levels and variance in these measurements, mean or median calcium levels in serum and/or urine as well as variance in these measurements, mean serum vitamin D levels as both calcitriol (1,25(OH)<sub>2</sub>D) and calcifediol (25(OH)D) and variance, and timepoint of measurement: pre-, in-, or post-flight. Post-flight values were binned into categories depending on the time after removal from microgravity: < 24 h after recovery (R+0), 24 - 48 h after (R+1), between 2 and 7 days after (R+2-7), and more than 7 days after (>R+7). Data extraction from graphical data was performed using MetaLab for MATLAB R2016b [35]. In cases where both levels of intact PTH (iPTH) and mid-molecule PTH are reported, iPTH is recorded in line with more modern techniques [36]. Baseline hormone measurements were taken as either hormone levels measured before microgravity exposure or as measurements in control groups.

Table 2 [27, 28, 37-46] shows the characteristics and data extracted from the 12 studies.

### Analysis

Analytical methods were chosen *a priori*. To standardize measurements across many different units, Cohen's *d* was calculated for each timepoint in an individual study, as given by the formula below [47]. Variances could reasonably be assumed to be equal between timepoints within a single study. All extracted data, along with calculated Cohen's *d* for each

study, are available in the Supplementary Materials 1-3 ([www.jofem.org](http://www.jofem.org)) to this article.

$$d = \frac{\text{Difference between baseline and outcome}}{\text{Pooled standard deviation of baseline and outcome}}$$

A weighted average of the *d* statistic ( $\bar{x}$ ) was computed using sample size to weight studies, as in the formula below. Heterogeneous variance among all studies precluded the use of inverse variance as a weighting factor.

$$\bar{x} = \frac{\sum_{i=1}^i (n_i \times d_i)}{\sum n_i}$$

Two-way analysis of variance (ANOVA) was then conducted to analyze the effect of experimental condition (i.e., real or simulated microgravity) and timepoint on PTH hormone levels. Tukey *post hoc* testing was then conducted to further characterize differences.

### Results

Simple main effects analysis identified a significant difference between timepoints ( $P < 0.01$ ) as well as a significant difference between experimental conditions ( $P < 0.01$ ). These main effects were qualified by an interaction between experimental condition and timepoint ( $P < 0.05$ ). The results are summarized in Table 3.

*Post hoc* testing identified significant differences from baseline in in-flight and R+7 PTH levels, as visualized in Figure 2. No significant differences from baseline were identified for R+0, R+1, and R+2-7. >R+7 shows a trend to significant increase from baseline ( $P < 0.1$ ). In-flight PTH data for individual studies are depicted in Figure 3, showing the consistency of observed decreases from baseline across all studies. In both fixed and random effects models, there is also evidence of a decrease by a weighted average of one standard deviation.

**Table 3.** Summary of Results From Two-Way ANOVA for a Relationship Between PTH Level and Timepoint, Experimental Condition, and the Combination of These Factors

Source	Sum of squares	DF	Mean square	F	P
Time	202.825	4	50.706	92.602	< 0.01
Condition	51.013	1	51.013	93.162	< 0.01
Time × condition	40.502	4	10.125	18.491	< 0.05
Residual	215.197	144	1.4944		

ANOVA: analysis of variance; DF: degrees of freedom; PTH: parathyroid hormone.

There is evidence for differences between experimental conditions within timepoints, as seen in Table 4, which summarizes the results of Tukey honestly significant difference (HSD) testing for two-way ANOVA. In-flight and >R+7 measurements were not significantly different between real and simulated conditions. However, there is evidence for significant differences between experimental conditions for other post-flight timepoints. Real microgravity shows significant increases from baseline at R+0, R+1, and R+2-7, displayed in Figure 4. However, simulated microgravity shows significant decreases from baseline at these same timepoints, as visualized in Figure 5. These opposite findings may explain the apparent lack of difference at these timepoints when both conditions are incorporated.

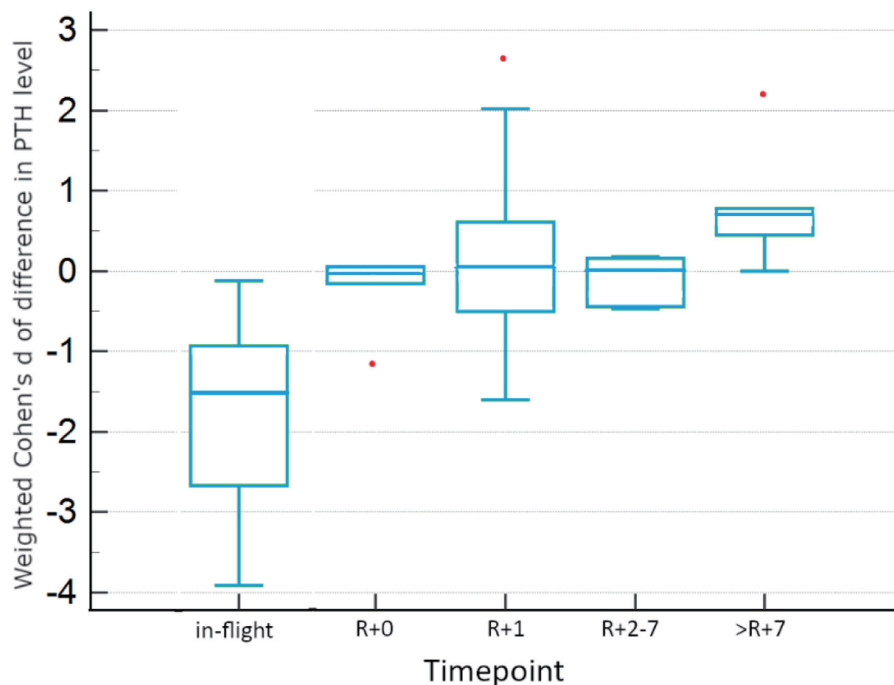
## Discussion

Consistent throughout the data are significant increases between in-flight and post-recovery values, suggesting a com-

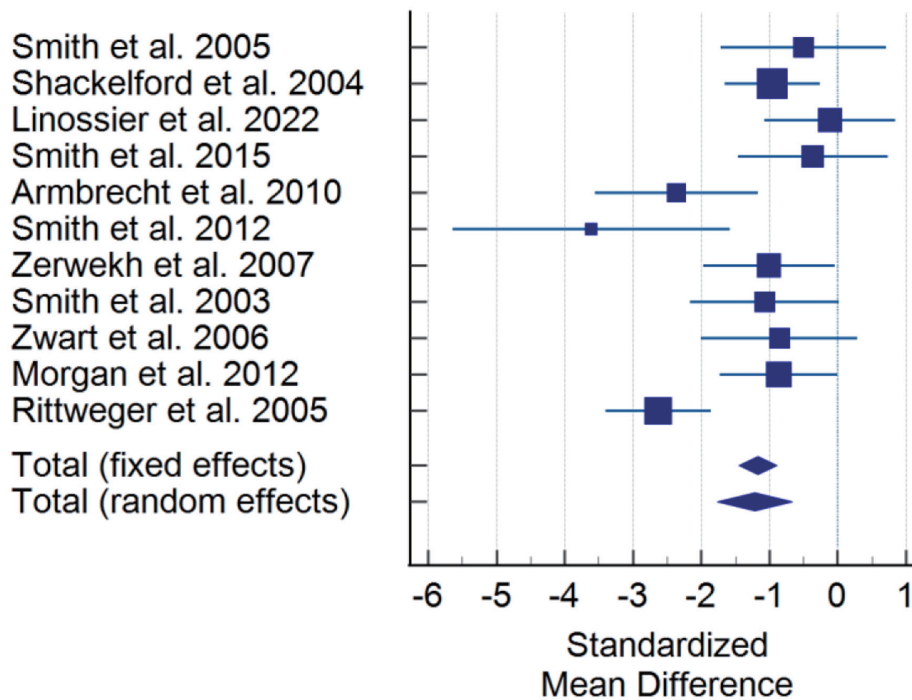
pensatory rebound in PTH levels after removal from microgravity. Notably, there is a significant difference between PTH levels in-flight and within a few hours of recovery. This rebound pattern is paralleled in studies on other hormones. This provides evidence that results from in-flight and R+0 measurements should not be conflated.

All studies save for two reported a statistically significant decrease in PTH while in flight. However, the magnitude of this difference appears heterogeneous between studies, even when normalized to a statistic like Cohen's d. This heterogeneity is characteristic of both experimental conditions. Between-study variability should be considered when attempting to pinpoint an average effect size of microgravity. Some of this may be due to the newness of gravitational physiology, where measurement methods and protocols may not be standardized.

The recovery timeline for PTH is difficult to establish in this meta-analysis, as post-flight results appear heterogeneous between studies. Furthermore, results appear to be



**Figure 2.** Tukey HSD plot of confidence intervals, inclusive of all studies. Red points indicate outlier data. HSD: honestly significant difference.



**Figure 3.** Forest plot for in-flight levels of PTH, inclusive of all studies. Squares with lines show 95% confidence intervals for a single study. Diamonds at the bottom indicate weighted average confidence interval computed from all studies. PTH: parathyroid hormone.

dependent on experimental conditions: studies in real microgravity show a significant increase in hormone levels post-flight, whereas simulated studies show decreases post-flight. Continued study examining microgravity research techniques is needed to clarify the effect of different protocols on experimental results.

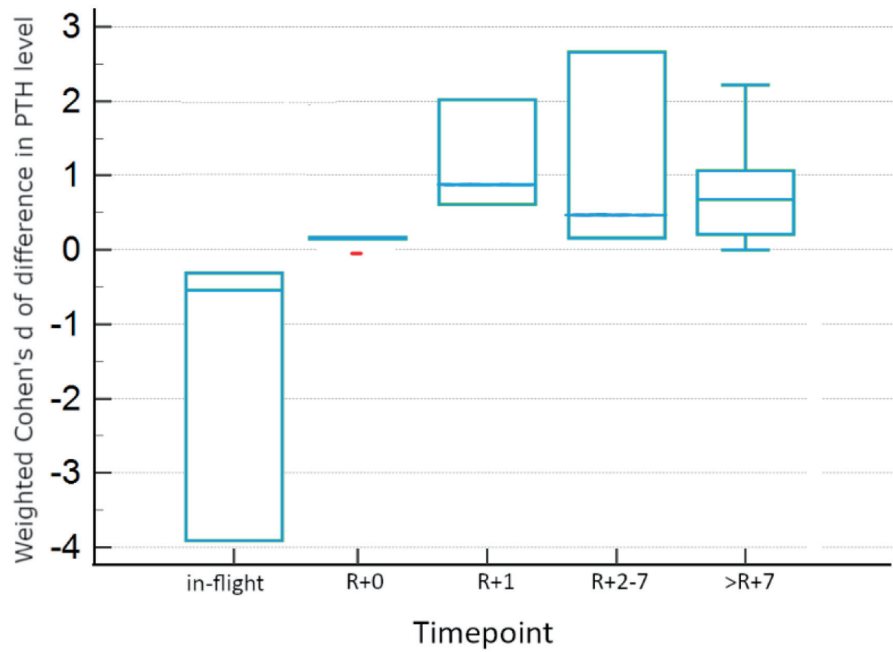
Overall, these findings for PTH echo what has been observed in other hormones. For example, in the thyroid, T3 and T4 [5, 33, 48] as well as CT levels [28, 49, 50] have been observed to decrease while in-flight with a post-flight rebound. These reduced hormone levels match physical changes in thyroid tissue morphology, such as increases in follicle size, cell

membrane composition [51], changes in cytoskeletal elements regulating the 3D structure and cell-cell interactions [34], as well as increased expression of apoptotic proteins [31]. Thyroid carcinoma cells reduce their T3 and T4 production as they undergo greater rates of apoptosis [31, 32], and C cells that produce CT have been observed to become less active in space [52, 53] and to decrease in number while in-flight [50]. Decreased T3 and T4 levels also correlate with changes in tissue morphology. An in-depth study of parathyroid tissue is forthcoming and may follow this same pattern seen in many other endocrine tissues. Still, there is only a relatively small body of research on in-flight hormone production in human subjects,

**Table 4.** Tukey HSD Testing With 95% Confidence Intervals for Each Timepoint, Separated by Experimental Condition

Time	Condition	n	Mean	Standard error	95% confidence interval
In-flight	Real	19	-1.5195	0.1698	-1.8532 to -1.1857
	Simulated	99	-1.4803	0.07437	-1.6265 to -1.3341
R+0*	Real	28	0.1207	0.1398	-0.1542 to 0.3956
	Simulated	8	-1.1400	0.2616	-1.6544 to -0.6256
R+1*	Real	25	1.1176	0.1480	0.8266 to 1.4086
	Simulated	50	-0.7226	0.1046	-0.9283 to -0.5169
R+2-7*	Real	31	0.8897	0.1329	0.6284 to 1.1510
	Simulated	71	-0.3001	0.08782	-0.4728 to -0.1275
>R+7	Real	37	0.8681	0.1217	0.6289 to 1.1073
	Simulated	35	0.6857	0.1251	0.4398 to 0.9316

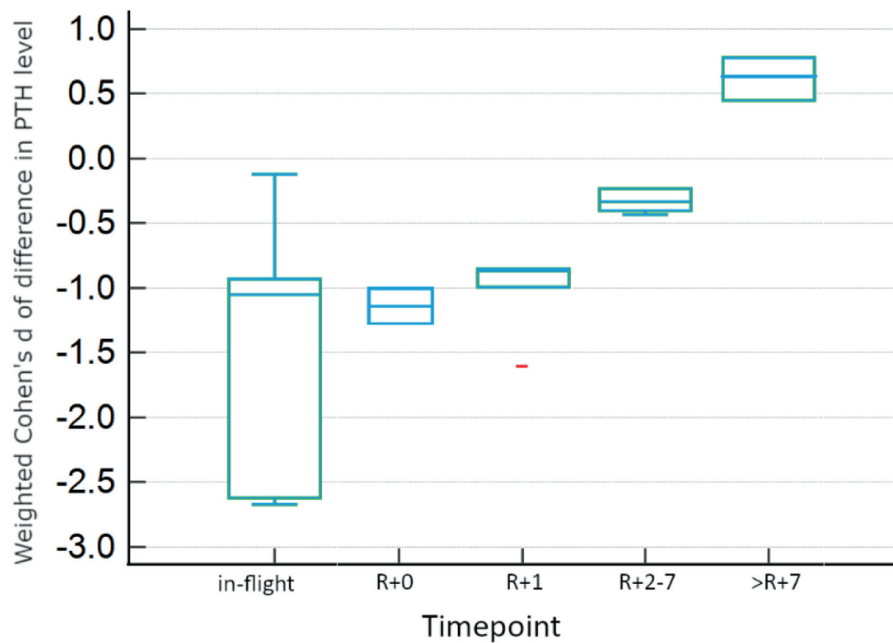
\*Timepoints differ significantly (P < 0.05) by experimental condition. HSD: honestly significant difference.



**Figure 4.** Tukey HSD plot showing 95% confidence intervals for real microgravity studies for each timepoint. Red points indicate outlier data. HSD: honestly significant difference.

particularly in real microgravity conditions. Confounding factors also hinder the ability to draw conclusions; for example, there is evidence to suggest that the loss of C cells is at least in part due to confinement stress and not microgravity [54]. Still, the evidence that PTH production is truly reduced in mi-

crogravity is compelling. Future research will help clarify the magnitude of change in parathyroid function in microgravity, the effects on gene expression and tissue morphology, and the effect of confounding variables on observed changes in hormone secretion.



**Figure 5.** Tukey HSD plot showing 95% confidence intervals for simulated microgravity studies for each timepoint. Red points indicate outlier data. HSD: honestly significant difference.

**Table 5.** Summary Data for In-Flight Serum and Urinary Calcium Levels

Serum calcium			Urinary calcium		
Cond. (n)	# significant	In-flight $\bar{x}$	Cond. (n)	# significant	In-flight $\bar{x}$
All (11)	3	0.300	All (6)	6	0.803
Real (3)	0	-0.460	Real (2)	2	0.797
Simulated (8)	3	0.589	Simulated (4)	4	0.806

Results are included from all studies as well as separated by microgravity condition. Data include the weighted average  $\bar{x}$  of Cohen's  $d$  and number of studies reporting significant change. Ionized calcium is not included due to a lack of data.

## Relationships with calcium and vitamin D

### Calcium

Exposure to microgravity is correlated with increased rates of bone resorption and elevated calcium excretion in urine, producing a negative calcium balance that reduces bone density over time [1]. Naturally, almost all studies included in this analysis report a significant increase in urinary calcium during flight. As calcium is a major regulator of PTH levels, it would be expected that bone demineralization during spaceflight is correlated with decreases in PTH levels [7]. Again, data from the included studies concur with this expectation, where increases in excreted calcium occur at the same time as the observed drop in PTH. A recovery timeline for urinary calcium is difficult to define due to low data availability, though many studies reporting post-flight levels appear to return to baseline by R+2-7.

As for serum calcium, data from the included studies are conflicted; a majority report no significant difference during flight while others report significant albeit modest increases, and two studies find slight decreases. A post-flight recovery timeline for serum calcium is unclear, but there is some evidence that serum calcium is depressed after spaceflight. Very limited data are available for ionized calcium, as only three of the 12 studies initially screened report any data on this factor.

However, separating real and simulated microgravity conditions suggests differences between the two regarding calcium levels. No studies conducted in real microgravity report significant changes in serum calcium, different from the near-unanimous in-flight drop in PTH seen across both types of experimental conditions. Real microgravity studies also appear to produce small magnitudes of negative change in serum calcium as opposed to the positive change that trends to significance in simulated studies. Both conditions appear to affect urinary calcium similarly, with all studies reporting a significant increase in urinary calcium and a similar magnitude of change (Table 5).

### Vitamin D

PTH stimulates the formation of calcitriol, the active form of vitamin D, facilitating the absorption of calcium from the intestines and increasing bone turnover to raise bone density

[7]. In turn, data from five of the eight studies reporting calcitriol levels show reductions in calcitriol during flight, correlated with the in-flight drop in PTH levels. These decreases are present in both real and simulated microgravity. Reduced calcitriol prevents the effective absorption of calcium into the blood, contributing to negative calcium balance in the body and furthering bone demineralization in weightless conditions.

There is scattered evidence of a post-flight rebound in calcitriol levels, but the majority of studies report no significant difference from baseline after exposure to microgravity. As with calcium, there is a lack of data in post-flight vitamin D data to establish a clear timeline for recovery.

There is possibly an effect of experimental condition on calcitriol levels. Subjects exposed to real microgravity show no significant difference from baseline calcitriol levels during flight, reporting a far lower magnitude change in calcitriol levels than in simulation. While more data are required to clarify these distinctions, this may represent emerging evidence that real and simulated microgravity have differential effects on calcitriol levels.

Calcifediol, on the other hand, does not appear to change significantly during or after spaceflight: only one study reports a change in calcifediol levels at any timepoint. This may be due to vitamin D supplementation in both real and simulated conditions, as is common in both microgravity research subjects and astronauts outside of experiments [7]. Still, there seems to be a difference between real and simulated microgravity in calcifediol levels: real microgravity seems to decrease calcifediol, while simulated conditions appear to increase it. This conclusion is hindered by high intra-study measurements of variance and relatively small magnitudes of change, however.

The observed changes to calcitriol imply differences between real and simulated weightlessness, either as a result of experimental technique or a true physical difference between the two conditions. As calcifediol plays a major role in recovering bone mineral density, more data are needed on calcitriol in the context of decreased PTH levels to obtain a clearer picture of bone mineral density during spaceflight (Table 6).

### Analysis of bias and limitations

A possible source of bias is the small sample sizes of most individual studies. Furthermore, the selection of astronauts for spaceflight introduces nonrandom sampling error in real microgravity conditions. This also hinders the generalization of these results to a broader population. Reporting issues such



**Table 6.** Summary Data for In-Flight Calcifediol and Calcitriol Levels

Calcifediol			Calcitriol		
Cond. (n)	# significant	In-flight $\bar{x}$	Cond. (n)	# significant	In-flight $\bar{x}$
All (8)	1	0.411	All (9)	6	-0.960
Real (4)	0	-0.313	Real (4)	1	-0.208
Simulated (4)	1	0.738	Simulated (5)	5	-1.220

Results are included from all studies as well as separated by microgravity condition. Data include the weighted average  $\bar{x}$  of Cohen's  $d$  and number of studies reporting significant change.

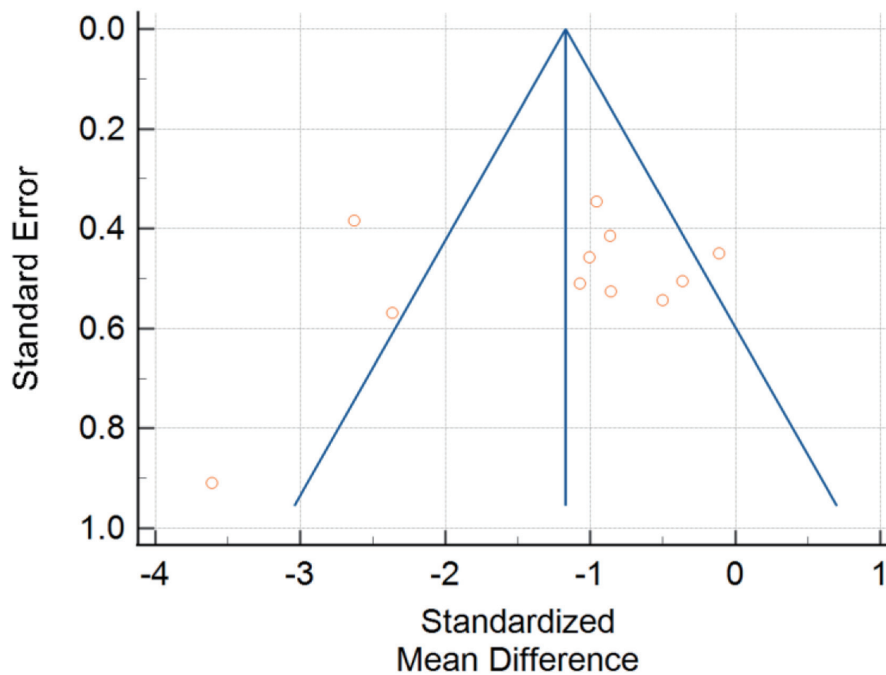
as unreported mean differences and measures of variance led to the exclusion of otherwise suitable studies, reducing the amount of available data. Searching and screening of articles were conducted carefully and all articles that met inclusion criteria were able to be accessed. None were unable to be located or inaccessible behind a paywall.

For in-flight changes in PTH levels, there was a trend to significance for evidence of publication bias (Egger's test intercept = -3.4695;  $P = 0.07$ ). Visual analysis of the funnel plot for all PTH studies, shown in Figure 6, reveals an asymmetrical distribution. In turn, there is potential for reporting bias in this meta-analysis.

Allowing the inclusion of studies in both real and simulated microgravity strengthened the power of the statistical tests but possibly introduced heterogeneity. Substudies on real and simulated conditions explore this and indicate that this could potentially bias conclusions from post-flight measurements, especially when summarizing studies from both conditions.

## Future Research

A major challenge in gravitational research is isolating the effect of microgravity from a ubiquity of possible confounds. Confinement stress, loss of privacy, and disruption of social and familial ties are a few factors that contribute to physiological stress in space [41, 45]. In real microgravity, background radiation and loss of circadian cues are additional factors that may play a role in decreased endocrine function [55, 56]. Another example is common plastics: bisphenol A and phthalates, two well-known endocrine disruptors, have been shown to alter the function and tissue of the parathyroid gland and contribute to tumor formation, hinting at effects on gene expression [57]. These plastics have been known to leach and contaminate spacecraft components [58]. Also common in astronauts is a hypocaloric diet and negative energy balance [59], which may further reduce PTH levels during flight [60].



**Figure 6.** Funnel plot with standard error of studies on the y-axis plotted against standardized mean difference on the x-axis. Yellow circles represent single studies.

Bone loss is also subject to many confounding factors. For example, as opposed to reductions in PTH, lack of UV light on spacecraft may contribute to osteopenia [61]. Other studies find evidence that confinement stress negatively impacts calcitonin production, further reducing bone density [54]. Thus, many factors affect bone density that may exacerbate the effect of reduced PTH. With the ubiquity of potential confounds that remain unexplored, identifying and exploring these confounds is critical to isolate the effect of microgravity on parathyroid function.

The difference between real and simulated microgravity has not been thoroughly explored in endocrine literature. There is evidence that experimental condition affects the recovery timeline for PTH and may provide evidence that the experimental setting is yet another of many possible confounds affecting gravity research. Corroborating these differences appears to be the trends in vitamin D and calcium, which seem to change differently based on the experimental condition. One such example is how serum calcium appears to change less in real microgravity than in simulated microgravity, though both real and simulated conditions produce similar magnitudes of change in PTH. As the parathyroid gland is regulated by serum calcium, this may suggest that real microgravity impacts parathyroid function itself. Still, several technical obstacles such as low sample sizes and discrepancies between research techniques must be closely analyzed to better define conclusions regarding PTH and other markers of bone formation in microgravity.

This analysis provides evidence to support the hypothesis that microgravity exposure affects parathyroid gland function and raises new questions in regard to the effect of experimental condition. The implications of these issues affect not just the results of endocrine research, but all research exploring the effects of weightless conditions and should be addressed. Continuing to understand the effect of gravity on physiology is critical to preserving astronaut health; as the goals of aerospace medicine move farther into space, ensuring the safety of astronauts both during and after long-duration spaceflight is critical for manned spaceflight to continue pressing forward.

## Supplementary Material

**Suppl 1.** PTH Data.

**Suppl 2.** Calcium Data.

**Suppl 3.** Vitamin D Data.

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## Conflict of Interest

The author declares there is no conflict of interest to report.

## Author Contributions

BSB performed all research, analysis, and preparation of the manuscript.

## Data Availability

The author declares that the data supporting the findings of this study are available within the article and supplementary files.

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