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Article

Insufficiency of the Hypothalamus-Pituitary-Adrenal (HPA) Axis in Patients with Long COVID

Running Head: HPA Insufficiency in Long COVID

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Abstract

Endocrine dysfunctions of the hypothalamo-pituitary-adrenal (HPA) axis have been reported as a cause of chronic fatigue in Long COVID. We investigated HPA axis abnormalities in 361 patients with Long COVID participating in this study. Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) was diagnosed according to the international diagnostic criteria. We evaluated circadian rhythms of ACTH, cortisol, and pituitary hormones, and urinary excretion of cortisol for 24 h. Pituitary loading test was performed with GRH, CRH, LHRH, and TRH, and ACTH loading test was performed to stimulate the adrenal glands. Plasma levels of ACTH were lower in hypothalamic adrenal insufficiency (AI) alone and hypothalamic AI with ME/CFS than in no endocrinological abnormality in ME/CFS (Control). Plasma levels of ACTH were relatively lower in hypothalamic AI with ME/CFS than Control. Plasma levels of ACTH were higher in primary AI with ME/CFS than Control. Serum cortisol after pituitary loading test was lower in hypothalamic AI with ME/CFS and primary AI with ME/CFS than Control. Serum cortisol was lower in primary AI with ME/CFS after ACTH loading test than Control. Urinary excretions of cortisol were lower in hypothalamic AI alone and hypothalamic AI and primary AI with ME/CFS than Control. We found that 95% of the patients with Long COVID had ME/CFS, among whom 63% had hypothalamic AI and 12% had primary AI. To improve chronic fatigue in patients with ME/CFS, detailed endocrine evaluation is necessary, and appropriate doses of corticosteroid replacement therapy are required to treat this patient population.

Keywords: Long COVID; ME/CFS; chronic fatigue; adrenal insufficiency; thypothalamus

1. Introduction

Some patients note several symptoms such as chronic fatigue, systematic muscle pain, severe headache, sleep insufficiency, and autonomic nerve dysfunction a few months after infection with COrona VIrus Disease of 2019 (COVID-19), which is now named Long COVID. The National Academies of Sciences, Engineering, and Medicine（NASEM）officially have broadly defined Long COVID as signs, symptoms, and conditions that are present four weeks or more after the initial phase of infection of COVID-19. Long COVID is not a single condition but represents many potentially overlapping entities, likely with different biological causes and different sets of risk factors and outcomes [1].

Pathophysiologies of the general fatigue experienced in Long COVID have been reported to include two types: myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and hypothalamic adrenal insufficiency (AI) [2].

ME/CFS is a heterogeneous condition with a multifactorial etiology involving immune, virologic, psychological, and other factors [3]. The post-exertion fatigue occurring in ME/CFS is associated with the energy deficiency of cellular mitochondria, which leads to lowered ATP production, impaired oxidative phosphorylation, and mitochondrial damage in brain cells [4,5]. Moreover, ME/CFS in Long COVID is accompanied by cognitive impairments, sleep disturbances, brain fog,

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concentration/memory impairments, and muscle pain and weakness. These symptoms are partially associated with mitochondrial dysfunction that leads to impairment of brain energy metabolism [6].

In contrast, endocrine dysfunctions of the hypothalamo-pituitary-adrenal (HPA) axis have been reported as a cause of chronic fatigue in patients with Long COVID. Hypocortisolism has reportedly been seen in patients with chronic fatigue over 3 months after infection with COVID-19 [7]. Decreases in levels of cortisol and adrenocorticotropic hormone (ACTH) have been reported in patients with Long COVID who experience severe fatigue [8].

ME/CFS is caused by corona virus invasion into the entire brain, whereas hypothalamic AI is induced by the virus's invasion into the hypothalamus [9]. It is possible that patients with ME/CFS complaining of chronic fatigue may also have insufficiency of the HPA axis after COVID-19 infection.

In this study, we investigated HPA axis abnormalities endocrinologically in patients with Long COVID including ME/CFS

2. Methods

The study protocol was approved by the Ethics Committee of Ukima Central Hospital. This was a prospective, randomized, and controlled open-label clinical trial at Ukima Central Hospital (clinical trial authorization No. 1/2024). We obtained written informed consent from all participants.

2.1. Participants

In total, 361 patients with Long COVID (151 men and 210 women aged 15 to 84 years (average 42.1 years) complaining of chronic fatigue were randomly hospitalized for endocrinological examination in the Long COVID Center of Ukima Central Hospital. They had not taken corticosteroids and benzodiazepines for at least one week before the hospitalization.

2.2. Brain Magnetic Resonance Imaging (MRI)

Patients underwent brain MRI with voxel-based specific regional analysis system for Alzheimer's disease (VSRAD; Eisai Co., Tokyo, Japan). For brain imaging, the patients' entire brain was scanned with 3-D T1-weighted MRI, and these sagittal imaging data were analyzed with VSRAD® advance 2 (Eisai Co.), which uses brain MRI data to assess morphological abnormalities of the brain [10]. Empty sella sign was evaluated by the degree of suprasellar herniation of cerebrospinal fluid into the pituitary gland in the sagittal plane after applying a cut-off of ≥ 1/3 of the sella height to indicate moderate herniation [11].

2.3. Examinations

After hospitalization, the patients' blood was sampled at 0600, 1200, 1800 and 2400 h to evaluate circadian rhythms of ACTH, cortisol, thyroid stimulating hormone (TSH), growth hormone (GH), prolactin (PRL), luteinizing hormone (LH), and follicle stimulating hormone (FSH) from hospital day 1 to 2. Urine was sampled for 24 h to evaluate urinary free cortisol excretion from hospital day 1 to 2. Pituitary loading test was performed to stimulate the pituitary gland with 0.1 mg of growth hormone-releasing hormone (Kaken Inc., Tokyo, Japan), 0.1 mg of corticotropin-releasing hormone (CRH; Tanabe Inc., Tokyo, Japan), 0.1 mg of luteinizing hormone-releasing hormone (Tanabe Inc.), and 0.5 mg of thyrotropin-releasing hormone (Tanabe Inc.) infused intravenously at 1000 h, and blood was sampled before and 30, 60, 90, and 120 min after the infusion on hospital day 2. ACTH loading test was performed to stimulate the adrenal glands with 250 g of ACTH (Cortrosin; Alfresa Pharma, Osaka, Japan) at 1000 h, and blood was sampled before and 30 and 60 min after the infusion on hospital day 3.

2.4. Measurements

Plasma level of ACTH and serum levels of cortisol, GH (Tosoh, Tokyo, Japan), PRL, LH, and FSH (Abbott, Tokyo, Japan) were measured by chemiluminescence enzyme immunoassays. Serum

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levels of free thyroxine (FT4) and TSH were determined by electrochemiluminescence immunoassays (ECLusys; Roche Diagnostics, Mannheim, Germany).

2.5. Diagnostic Criterias

ME/CFS

A diagnosis of ME/CFS is indicated by positivity more than five following criteria [12].

1. Fatigue at rest.
2. Post-exertional fatigue: difficulty of rescue.
3. Sleep disorders: abnormal sleeping rhythm, insomnia.
4. Pain: muscle pain, joint ache, severe headache.
5. Neurological/cognitive manifestations: confusion, impairment of concentration and short-term memory consolidation, disorientation.
6. Autonomic manifestations: orthostatic intolerance, postural orthostatic tachycardia syndrome, light-headedness, nausea, irritable bowel syndrome, pollakisuria.
7. Neuroendocrine disorders: loss of thermostatic stability, sweating episodes, cold hands and feet.
8. Immune manifestations: recurrent sore throat, recurrent flu-like symptoms, general malaise, new food sensitivities, medication and/or chemical sensitivities.

Hypothalamic AI

The diagnostic criteria indicating hypothalamic AI include the following.

1. Plasma levels of ACTH below 25 pg/mL with serum levels of cortisol below 12 μg/dL at 0600 h [13].
2. Normal or over response of plasma levels of ACTH to Pituitary loading test.

Pituitary AI

The diagnostic criterion for pituitary AI is low or no response of plasma levels of ACTH to Pituitary loading test.

Primary AI

The diagnostic criteria indicating primary AI include the following.

1. Plasma levels of ACTH over 25 pg/mL with serum levels of cortisol below 12 μg/dL at 0600 h.
2. Response of serum levels of cortisol is within a range of 15 to 18 μg/dL in an ACTH loading test; Addison's disease (below 15 μg/dL of cortisol) is an exclusionary illness.
3. Serum levels of cortisol are below 18 μg/dL in response to a CRH loading test.

2.6. VSRAD of MRI

We used VSRAD [10] of MRI in the patients with Long COVID. We evaluated the Z-score (0–6) for morphological degeneration of the olfactory bulbus and hypothalamic area.

2.7. Statistical Analyses

Values are reported as means ± SEM. After one-way ANOVA, Dunnett's multiple comparison test was applied to compare each treatment group with the Control group. P values < 0.05 were considered to indicate statistical significance.

3. Results

3.1. Grouping of Patients with Long COVID

In total, 361 patients with Long COVID who complained of chronic fatigue participated in this study. Grouping by ME/CFS diagnostic criteria and endocrinological examinations are shown in Table 1. These data indicate that around 74% of the patients with ME/CFS also had AI.

Table 1. Grouping of patients with Long COVID (Total 361).

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1	**No abnormality：3**
2	Primary adrenal insufficiency: 3
3	Pituitary adrenal insufficiency: 0
4	Hypothalamic adrenal insufficiency alone：12 (3%/Total)
5	ME/CFS:343 (94%/ Total)
	1) No endocrinological abnormality: 84 (25%/ME/CFS)
	2) Hypothalamic adrenal insufficiency：213 (62%/ ME/CFS)
	3) Pituitary adrenal insufficiency: 4 (1.2%/ ME/CFS)
	4) Primary adrenal insufficiency: 42 (12%/ ME/CFS)

3.2. Empty Sella in Long COVID

Table 2 shows classification of endocrinological abnormalities in the patients with empty sella by MRI. In total, 49/266 (18%) patients with Long COVID showed empty sella: 1 (20.1%) patient with hypothalamic insufficiency alone, 9 (13.2%) patients with ME/CFS without endocrinological abnormality, 32 (20.1%) patients with hypothalamic AI with ME/CFS, and 7 (20.6%) patients with ME/CFS with primary adrenal dysfunction.

Table 2. Classification of endocrinological abnormalities in the patients with empty sella and degeneration of brain by magnetic resonance imaging findings.

	Number	**Empty sella**	**Z-score of olfactory bulbus**	**Z-score of hypothalamus**
Control	11	0 (0%)	0.7 ± 0.4	0.9 ± 0.3
Hypothalamic insufficiency alone	5	1 (20.1%)	3.4 ± 0.9	1.0 ± 0.6
No endocrinological abnormality in ME/CFS	68	9 (13.2%)	3.0 ± 0.2*	1.3 ± 0.2
Hypothalamic AI in ME/CFS	159	32 (20.1%)	3.3 ± 0.1*	1.8 ± 0.1
Primary AI in ME/CFS	34	7 (20.6%)	3.3 ± 0.3*	2.1 ± 0.4

Abbreviations: AI, adrenal insufficiency; ME/CFS, myalgic encephalomyelitis/chronic fatigue syndrome.
Z-score: 2 to 6 levels of atrophy of gray matter in brain. Control: No Long COVID.
* $P < 0.05$ vs. Control.

3.3. VSRAD Analysis

Figure 1 shows typical brain MRI findings analyzed by VSRAD in a patient with Long COVID. A high Z-score indicating a decrease in cerebral gray matter volume is shown at the bilateral olfactory bulbus (red arrow in panel A). High Z-score areas indicating a decrease in cerebral gray matter volume are present in the entire brain including the hypothalamus (red arrow in panel B).

Table 2 also shows Z-scores of the olfactory bulbus and hypothalamus. The Z-score of the olfactory bulbus was significantly higher ($P < 0.05$) in the patients with Long COVID than that in the Control. However, there were no significant differences in Z-scores of the olfactory bulbus or of the hypothalamus among the subgroups of patients with Long COVID.

3.4. Circadian Rhythms of ACTH and Cortisol

Figure 2A shows the circadian rhythm of plasma levels of ACTH in hypothalamic AI alone, in no endocrinological abnormality with ME/CFS, in hypothalamic AI with ME/CFS, and in primary AI with ME/CFS. Plasma levels of ACTH at 0600 h were below 25 pg/mL and significantly lower ($P < 0.05$) in hypothalamic AI alone and hypothalamic AI with ME/CFS than that in no endocrinological abnormality with ME/CFS. Plasma levels of ACTH were also significantly lower ($P < 0.05$) in hypothalamic AI with ME/CFS at 1200 and 1800 h than that in the Control. Plasma levels of ACTH at 0600 h were significantly ($P < 0.05$) higher in primary AI with ME/CFS than that in the Control. Figure 2B

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shows the circadian rhythm of plasma levels of cortisol in the four groups. Plasma levels of cortisol at 0600 h were significantly lower ($P < 0.05$) in hypothalamic AI alone, hypothalamic AI with ME/CFS, and in primary AI with ME/CFS than that in no endocrinological abnormality with ME/CFS.

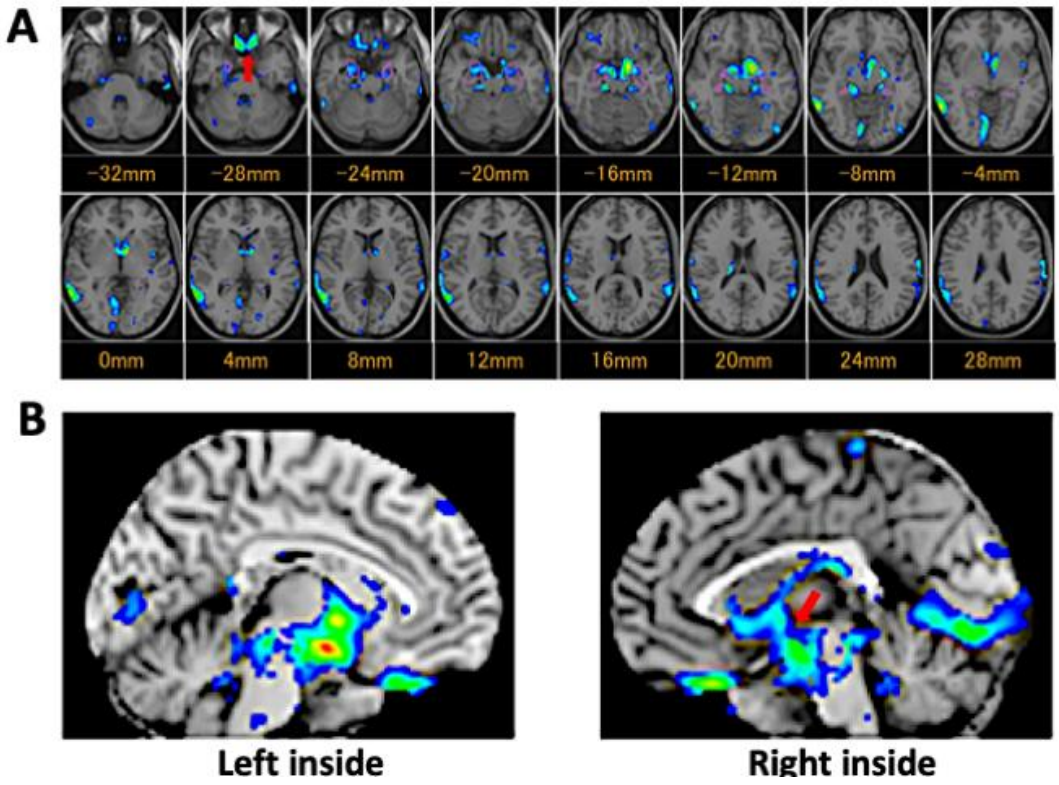

Figure 1. Typical brain magnetic resonance imaging findings analyzed by voxel-based specific regional analysis system for Alzheimer's disease in patients with Long COVID. Red arrow indicate olfactory bulbus in (A) and area of the hypothalamus in (B).

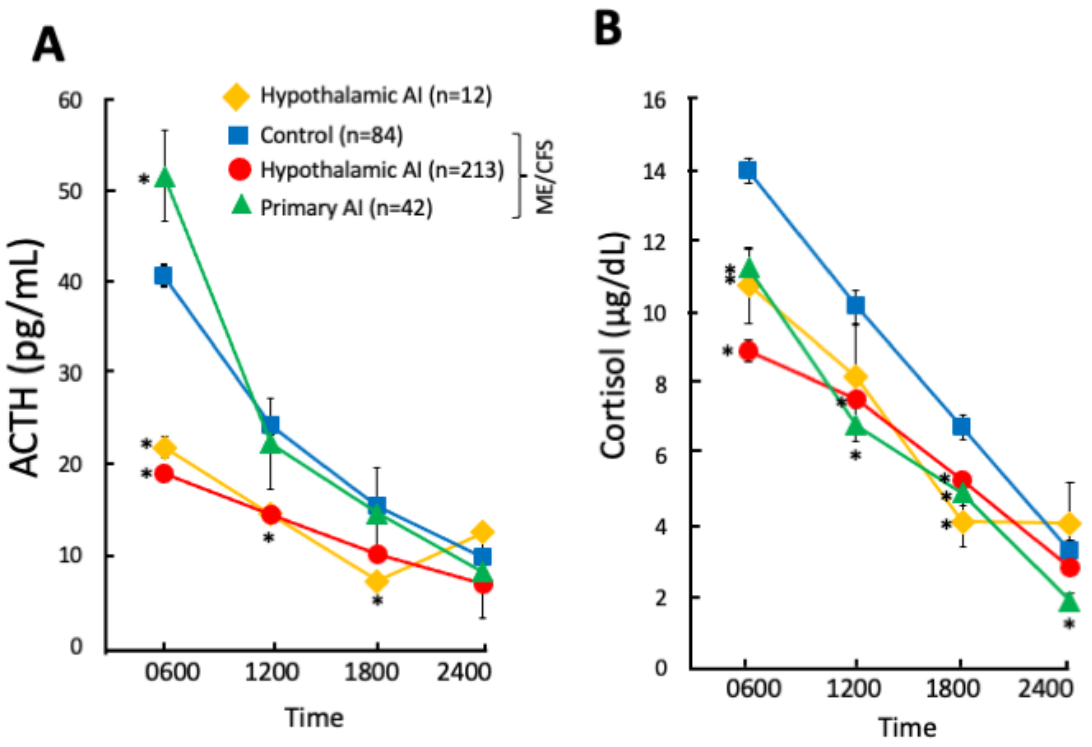

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Figure 2. Circadian rhythms of adrenocorticotropic hormone (ACTH) and cortisol. Blood was sampled at 0600, 1200, 1800, and 2400 h. * P < 0.05 vs. no endocrinological abnormality in patients with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) (Control). AI: adrenal insufficiency.

3.5. Circadian Rhythms of GH, TSH, PRL, LH, and FSH

Figure 3A–E shows the circadian rhythm of plasma levels of GH, TSH, PRL, LF, and FSH in the four groups. Plasma levels of GH showed an increasing circadian rhythm from 0600 to 2400 h, but there was no significant difference in plasma levels of GH among the four groups. There were no significant difference in plasma levels of TSH, PRL, LF, and FSH across their circadian rhythms.

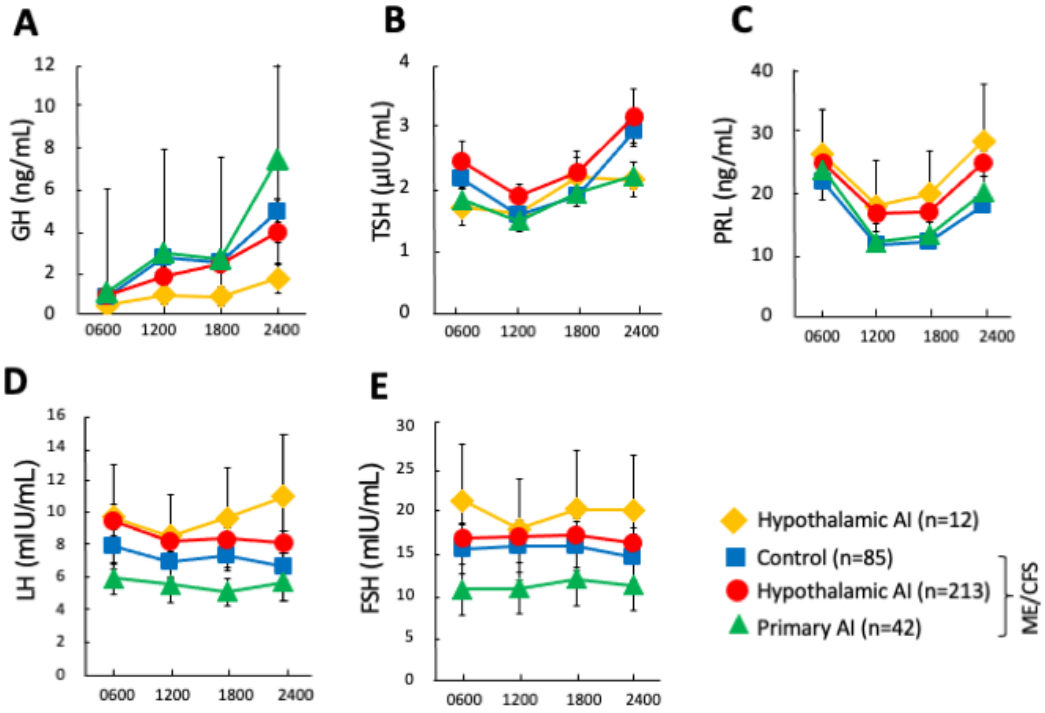

Figure 3. Circadian rhythms of growth hormone (GH), thyroid stimulating hormone (TSH), prolactin (PRL), luteinizing hormone (LH), and follicle stimulating hormone (FSH). * P < 0.05 vs. no endocrinological abnormality in patients with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) (Control). AI: adrenal insufficiency.

3.6. Responses of ACTH and Cortisol to Pituitary Loading Test

Figure 4A shows the responses of plasma levels of ACTH to the pituitary loading test in the four groups. Plasma levels of ACTH were significantly increased (P < 0.01) at 30 min from before CRH loading in all four groups. There were no significant differences in the responses of plasma levels of ACTH among the four groups after the pituitary loading test. Figure 3B shows responses of serum cortisol to CRH loading among the four groups. Serum cortisol level after CRH loading was significantly lower (P < 0.05) in hypothalamic AI with ME/CFS and primary AI with ME/CFS than that in no endocrinological abnormality with ME/CFS.

3.7. Responses of GH, TSH, PRL, LH, and FSH to Pituitary Loading Test

Figure 5A–E shows the responses of plasma levels of GH, TSH, PRL, LH, and FSH to the pituitary loading test in the four groups. Plasma levels of GH in hypothalamic AI alone were significantly lower (P < 0.05) than that in the Control after the pituitary loading test. There were no significant differences in plasma levels of TSH, PRL, and FSH among the four group after the pituitary loading

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test. Plasma levels of LH showed a delayed response in hypothalamic AI alone and in hypothalamic AI with ME/CFS.

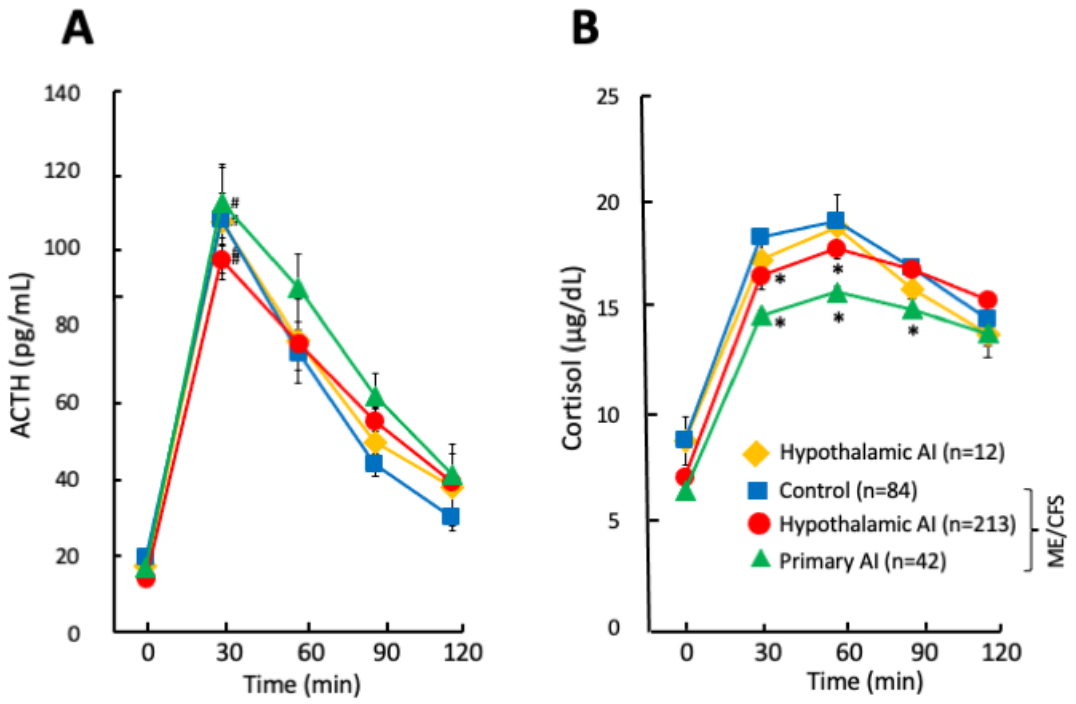

Figure 4. Responses of adrenocorticotropic hormone (ACTH) and cortisol to pituitary loading test. Pituitary loading test was performed to stimulate pituitary gland with 0.1 mg of growth hormone releasing hormone, 0.1 mg of corticotropin-releasing hormone, 0.1 mg of luteinizing hormone-releasing hormone, and 0.5 mg of thyrotropin-releasing hormone infused intravenously, and blood was sampled before and 30, 60, 90, and 120 min after the infusion. #: P< 0.05 vs. Time 0, * P < 0.05 vs. no endocrinological abnormality in patients with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) (Control). AI: adrenal insufficiency.

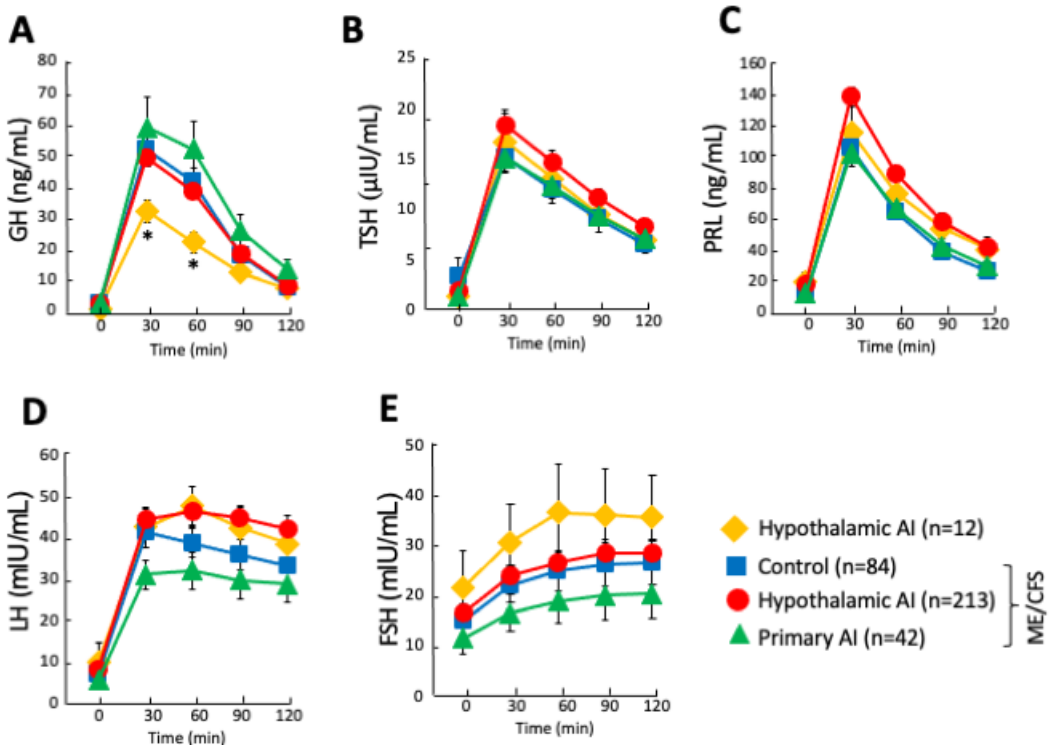

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Figure 5. Responses of growth hormone (GH), thyroid stimulating hormone (TSH), prolactin (PRL), luteinizing hormone (LH), and follicle stimulating hormone (FSH) to pituitary loading test. Pituitary loading test was performed to stimulate pituitary gland with 0.1 mg of growth hormone releasing hormone, 0.1 mg of corticotropin-releasing hormone, 0.1 mg of luteinizing hormone-releasing hormone, and 0.5 mg of thyrotropin-releasing hormone infused intravenously, and blood was sampled before and 30, 60, 90, and 120 min after the infusion. * $P < 0.05$ vs. no endocrinological abnormality in patients with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) (Control). AI: adrenal insufficiency.

3.8. Response of Cortisol to ACTH Loading

Figure 6A shows changes in serum cortisol after ACTH loading in the four groups. Serum cortisol levels at 30 and 60 min were significantly lower ($P < 0.05$) in primary AI with ME/CFS than that in no endocrinological abnormality with ME/CFS. Serum cortisol levels at 30 and 60 min were relatively but significantly lower ($P < 0.05$) in Hypothalamic AI with ME/CFS than that in no endocrinological abnormality with ME/CFS.

3.9. Urinary Excretion of Cortisol

Figure 6B shows urinary excretion of cortisol for 24 h in the four groups. Urinary excretion of cortisol was around 70 μg/day in no endocrinological abnormality with ME/CFS. Urinary excretions of cortisol in the other three groups were significantly lower ($P < 0.01$) than that in no endocrinological abnormality with ME/CFS.

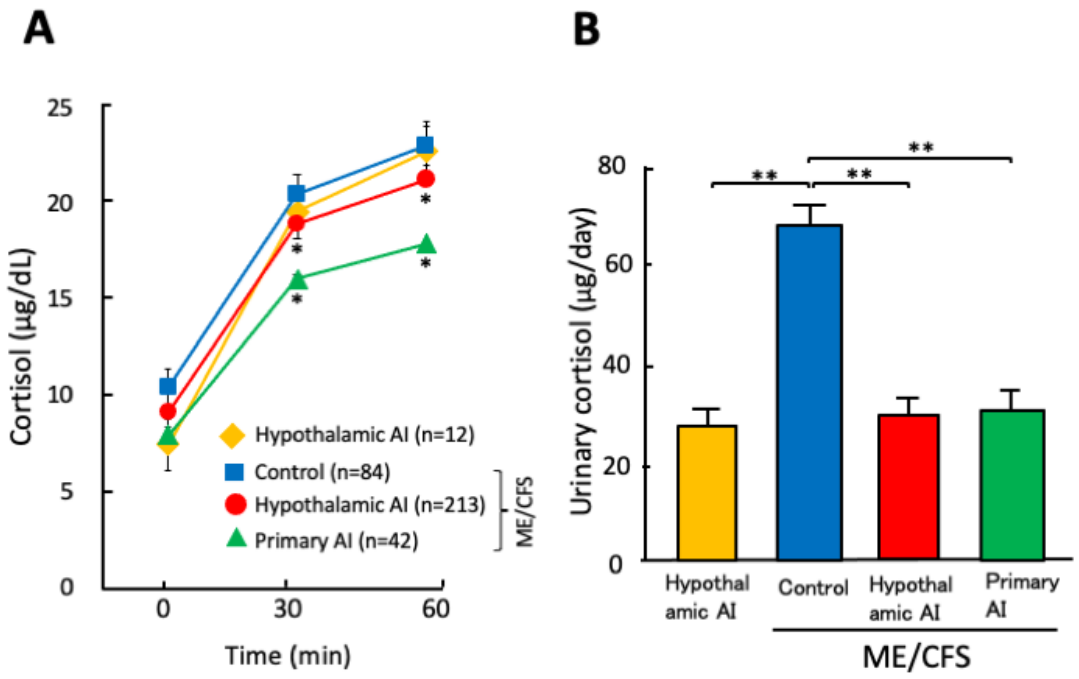

Figure 6. Response of cortisol to adrenocorticotropic hormone (ACTH) loading and urinary excretion of cortisol. (A) ACTH loading test was performed to stimulate the adrenal glands with 250 μg of ACTH, and blood was sampled before and 30 and 60 min after the infusion. (B) Urinary excretion of cortisol for 24 h. * $P < 0.05$, ** $P < 0.01$ vs. no endocrinological abnormality in patients with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) (Control). AI: adrenal insufficiency.

4. Discussion

Causes of chronic fatigue experienced in Long COVID have been reported to include two mechanisms: hypothalamic AI inducing chronic fatigue at rest [9] and brain mitochondrial dysfunction in ME/CFS inducing post-exertional malaise [14]. Endocrinological abnormalities have been reported in

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Long COVID. Severely affected patients complaining of general fatigue had subnormal levels of cortisol and ACTH [8]. Patients with Long COVID have an impaired HPA axis with decreased free cortisol excretion, increased sensitivity to ACTH, and attenuated response to CRH [15].

Morris et al.[16] reported that the activated immune system in brain induced the pathogenesis of ME/CFS. Activation of microglia as immune cells of the brain increases cytokines in brain to induce inflammations that cause the systematic pain, sleeping abnormality, abnormal neurological/cognitive manifestations, and autonomic manifestations [17]. Moreover, the activated brain has been reported to be associated with impaired neuroendocrine systems. De Bellis et al. [18] reported the presence of antibodies against the hypothalamus and pituitary that induce low levels of ACTH/cortisol and GH in patients with ME/CFS. Thus patients with ME/CFS possibly accompanie with AI. However, there have been no report for the detailed endocrinological abnormalities in ME/CFS.

In the present study, among the 361 patients with Long COVID complaining of chronic fatigue, the number of patients with hypothalamic AI alone was just 12, and that of the patients with ME/CFS was 341 (94%), of whom 213 (62%) patients had hypothalamic AI. In addition, 42 (12%) patients with ME/CFS had primary AI. Thus, 74% of the patients with ME/CFS also had AI. Byrne et al. [19] reported reduced hypothalamic volume in patients with ME/CFS, and Hollie et al. [20] reported hypothalamic abnormality observed on MRI of patients with ME/CFS. Thus, hypothalamic AI caused by invasion of COVID-19 induced chronic fatigue at rest in 63% of the patients with ME/CFS.

In the pituitary loading test, response of GH was attenuated in patients with hypothalamic AI alone. Urhan et al. [21] also reported that the response of GH to glucagon stimulating test was transiently attenuated in patients with Long COVID, suggesting that COVID-19 invasion into pituitary gland induces the low response of GH.

The 42 patients with ME/CFS complaining of chronic fatigue who had primary AI alone showed an impaired response of cortisol to ACTH loading, which differs from Addison disease blunting of adrenal function. Concerning the causes of the primary AI, some patients may originally have had lower adrenal gland function, and COVID-19 vaccine might have induced primary AI in some of these patients. COVID-19 vaccine is reported to frequently induce primary AI through degeneration of the adrenal glands [22]. Among these 42 patients with primary AI, 29 noted fever or fatigue after their COVID-19 vaccinations (data not shown), suggesting that COVID-19 infection induced high fever and severe general fatigue in these patients with primary AI. It has also been reported that COVID-19 virus itself induces primary AI. Autopsy studies in patients dying from COVID-19 showed necrosis and virus invasion in the adrenal glands [23], adrenal hemorrhage [24], and adrenal infarction [25,26]. Thus, the primary AI in this study appeared to be partially caused by the direct invasion of COVID-19 into the adrenal glands. Further, it is possible that long-term hypothalamic-pituitary insufficiency secondarily induces AI [27].

The response of serum cortisol to pituitary and ACTH loading tests in the present study was relatively but significantly lower in hypothalamic AI with ME/CFS than that in no endocrinological abnormality. This attenuated response of cortisol loading is considered to be a secondary phenomenon in hypothalamic AI that may be induced by sustained decreases in ACTH.

Interestingly, the frequency of hypopituitarism was low for AI in the patients with ME/CFS. The main mechanism of hypopituitarism is autoimmune lymphocytic hypophysitis [28]. Pal reported that autopsies of patients dying from COVID-19 infection showed neuronal degeneration of the hypothalamus [29]. Moreover, patients with previous infection in severe acute respiratory syndrome show decreased levels of ACTH and cortisol associated with direct hypothalamic damage [30]. Menotti et al. [31] conducted a comprehensive literature review of hypophysitis in COVID-19 and reported its rarity in patients with insufficiency of the HPA axis in Long COVID-19. Thus, the secondary AI in patients complaining of general fatigue in ME/CFS appears to be caused mainly by the hypothalamic insufficiency in ME/CFS.

In the present study, 24-h urinary excretion of cortisol was around 30 g/day in hypothalamic AI alone, hypothalamic AI, and primary AI with ME/CFS, which is significantly lower than that in no endocrinological abnormality. This level was higher than that in Addison's disease (below 5

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g/day of urinary cortisol), suggesting that the AI in Long COVID was relative but not complete AI like that seen in Addison disease. Patients with hypothalamic AI alone, hypothalamic AI, and primary AI with ME/CFS complained of general fatigue even with urinary cortisol levels of around 30 g/day.

In the present study, 49 patients with Long COVID showed empty sella on brain MRI, of whom 21% had hypothalamic insufficiency. Empty sella is a radiological finding not related the pituitary gland insufficiency, but as empty sella in some cases has been related to hypothalamus-pituitary AI [32], it is possible that COVID-19 virus invaded the hypothalamus and might have induced the hypothalamic insufficiency.

Angiotensin-converting enzyme 2 (ACE2) is reported to be ubiquitously present in brain vasculature. ACE2 is mainly expressed in capillaries in the olfactory bulb, the hypothalamic paraventricular, supraoptic, and mammillary nuclei, the midbrain substantia nigra and ventral tegmental area, and the hindbrain pontine nucleus [33]. All of the present patients with ME/CFS showed scattered degeneration in the brain on VSRAD analysis of MRI. It has been reported that COVID-19 invades the brain and attaches on ACE2, COVID-19 receptor, presence on brain tissues and induces the brain degeneration [34,35] that can be detected by VSRAD analysis. In the present study, ME/CFS induced by the random invasion of corona virus into brain including the hypothalamus was observed on the VSRAD analyses. Emmi et al. [36] investigated distribution of ACE2 in the brain stem obtained from patients at autopsy and found that ACE2 is expressed in the vagal nuclei of the medulla and the midbrain tegmentum. Immunohistochemical studies have shown that ACE2 is highly expressed in circumventricular organs connecting the hypothalamus [37]. ACE2 has also been reported to be present in the hypothalamic paraventricular nuclei [33].

We treated the present study patients with ME/CFS and with AI by administration of adequate amounts of hydrocortisone based on the circadian rhythm of the levels of ACTH-cortisol, which improved the chronic fatigue in these patients. Moreover, we administered *Saireito*, a herbal medicine, to the patients with hypothalamic AI because *Saireito* is known to stimulate the hypothalamus [38], and it allowed us to gradually reduce the amount of hydrocortisone administered. Thus, an adequate amount of hydrocortisone is needed to improve the chronic fatigue experienced by patients with Long COVID.

In conclusion, we found that 94% of our patients with Long COVID had ME/CFS, of whom 62% of them had hypothalamic AI and 12% had primary AI. Thus, the fatigue occurring in ME/CFS appeared to be associated with AI. To improve chronic fatigue in ME/CFS, detailed endocrine evaluation is necessary, and appropriate doses of corticosteroid replacement therapy are required to treat the chronic fatigue of patients with ME/CFS.

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Institutional Review Board Statement: The study protocol was approved by the Ethics Committee of Ukima Central Hospital. This was a prospective, randomized, and controlled open-label clinical trial at Ukima Central Hospital (clinical trial authorization No. 1/2024).

Informed Consent Statement: Informed consent was obtained from all participants involved in this study.

Data Availability Statement: The raw data supporting the findings of this study are available from the corresponding author N.F. upon reasonable request.

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