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RESEARCH ARTICLE

Circadian rhythm and the influence of light on parameters related to calcium metabolism in stroke patients admitted for rehabilitation

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ABSTRACT

Hospitalized stroke patients are at high risk of developing circadian disruption due to lack of natural sunlight. This may affect the circadian rhythm of the calcium metabolism. This study is a secondary explorative analysis from a Randomized Controlled Trial. Acute stroke patients requiring a minimum of two weeks of rehabilitation were randomized to an Intervention unit (IU) equipped with naturalistic light or a Control unit (CU) with standard indoor lighting. Blood was drawn across 24 h at inclusion and discharge in 45 patients, 25 from the IU and 20 from the CU. Calcium showed significant rhythmicity at inclusion and discharge in both groups. Alkaline phosphatase, parathyroid hormone (PTH), and Vitamin D exhibited no significant rhythmicity at inclusion or discharge in either group while phosphate exhibited rhythmicity at discharge in the CU. PTH levels were elevated in the CU group compared to the IU group at time of discharge. Of the measured parameters, only calcium exhibited circadian rhythmicity after stroke. Naturalistic light did not have any influence on the rhythmicity, indicating that light may not be the main circadian regulator of the circadian oscillations that regulate calcium metabolism. PTH seems to be decreased by naturalistic light.

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Circadian rhythm; calcium; stroke; parathyroid hormone; light; vitamin D; circadian rhythm disorders; rehabilitation; chronobiology

Introduction

In humans, the circadian system is mainly entrained by sunlight containing the blue light spectrum. The essential parts of the circadian system are the hypothalamic suprachiasmatic nucleus (SCN; central circadian oscillator) and subordinate/peripheral oscillators in the brain and the body [1]. The SCN is directly entrained by light *via* input from the melanopsin containing intrinsically photosensitive retinal ganglion cells (ipRGCs) through the retino-hypothalamic tract [2,3].

Circadian disruption is mainly characterized by misalignment between the internal circadian rhythms and the external environmental conditions. Among those are inadequate light during the day [4], dependent on duration, wavelength and intensity of light [5] and reduced physical activity and immobilization because of reduced entrainment of the circadian system [6]. Also, food intake at abnormal times during the day, can induce circadian disruption due to the sensitivity of peripheral circadian oscillator stimulation in relation to food intake [7,8] However, dynamic and/or naturalistic light during hospitalization have shown positive regulation of circadian rhythm-regulated mechanisms if the admission period was more than five days [9].

Circadian disruption is often present after stroke [10–12] and stroke patients admitted for neurorehabilitation are at risk of developing circadian disruptions due to several reasons. They are indoor and lack stimulation by naturalistic light and are exposed to artificial hospital light at inappropriate times, which in turn can be associated with different metabolic and physiological outcomes. Furthermore, patients who need neurorehabilitation most often have reduced physical activity, often due to immobilization and a decreased food intake [6].

As parameters related to calcium metabolism normally follow a circadian rhythm [9] and are affected by immobilization, they too may be affected during immobilization after moderate to severe stroke and the presumed circadian misalignment obtained during the hospitalization for neurorehabilitation.

Prolonged immobilization may also result in hypercalcemia, hypercalciuria and osteoporosis [13]. One can speculate about the consequences of immobilization upon calcium metabolism following stroke hemiplegia. As immobilization induces significant and progressive bone loss [14,15], with elevated serum calcium, this in turn leads to an increase in urinary excretion and a decrease in intestinal absorption of calcium [14], all leading to negative calcium balance and the development of

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disuse osteoporosis. The negative calcium balance that follows immobilization in the human bed rest model is primarily the result of an increase in bone resorption. Likewise, immobilization can lower intestinal calcium absorption through down-regulating duodenal mRNA levels of calcium transport proteins [14]. All these elaborate that bedridden patients are at risk of osteoporosis and fractures [16–18].

Previous studies from our research group have shown positive influence of light on circadian rhythm-regulated mechanisms after stroke. We hypothesize that the circadian rhythm of the calcium metabolism is disturbed in stroke patients, and the presumed disturbance may be influenced by natural light [19–22].

To our knowledge, the circadian rhythm of calcium after stroke has not previously been investigated. This study examines the level and circadian rhythmicity of the calcium metabolism after stroke and whether naturalistic light can affect these outcomes.

Materials and methods

Study design and participants

The study is a secondary explorative analysis on blood samples collected as part of a randomized controlled trial investigating the effect of naturalistic light on melatonin and cortisol, sleep and mental parameters in stroke patients [23]. Participants with a need of more than 14 days of hospitalization were recruited from the acute stroke unit. They were randomized to either an intervention group admitted to a rehabilitation unit with naturalistic lighting, the intervention unit (IU), or to a control group admitted to a rehabilitation unit equipped with standard indoor lighting, the control unit (CU). The study was approved by the Danish scientific ethics committee (H-4-2013-114) and Danish Data Protection Agency (2007-58-0015). ClinicalTrials.gov Identifier: NCT02186392.

Naturalistic light intervention

A 24-h circadian-lighting scheme with multi-colored light-emitting diode–based luminaires was installed in every area of the IU. This naturalistic lighting scheme was always running, and the luminaires were placed in the ceiling. During the day the light slowly increased to reach maximum illuminance at noon with high content of blue light. After 14:00 h, the light started dimming and at the evening (18:00 h) the light changed to amber color with a minimal content of blue light. The light turned off at 22:00 h in the patient's rooms and continued as amber color in the rest of the unit. A more thorough description of the light intervention can be read in the study's method article [23].

Measurements

Blood sampling and biochemical analysis

Blood samples were drawn at inclusion and discharge with 4 h interval during a 24 h period at 08:00 h, noon, 16:00 h, 20:00 h, midnight and at 04:00 h. Registered and adjusted parameters before blood sampling are described in Table 2B in Online Appendix.

Blood samples for total calcium, total alkaline phosphatase and phosphate were drawn in lithium heparin tubes (Greiner Bio-One, Kremsmünster Austria). The other parameters were collected in serum clot activator tubes coated with silica particles (Greiner Bio-One, Kremsmünster Austria). All blood samples were immediately after collection centrifuged at 2500 g for 10 min. The analytical imprecision estimates (CV%) were assessed on internal quality control samples. Serum for the analysis of 25-hydroxy-vitamin D (D3 + D2) (vitamin D) was stored at −80 °C until batch analysis of all the samples on a Cobas e411 instrument (Roche Diagnostics, Basel, Switzerland) by a competitive electro-chemiluminescence immunoassay having an analytical between-run coefficient of variation of 10%. Serum for analysis of parathyroid hormone (PTH) was stored at −20 °C until analysis two weeks after sampling on a Vitros instrument (Ortho-Clinical Diagnostics, Rochester, New York, USA) by an immunometric immunoassay technique having an analytical between-run coefficient of variation of 5%. The rest of the biochemical parameters were almost immediately after centrifugation analyzed on a Vitros instrument (Ortho-Clinical Diagnostics, Rochester, New York, USA) total calcium and phosphate by colorimetric slide technologies, total alkaline phosphatase by multiple point rate technology, thyroid stimulating hormone (TSH) by an immunometric immunoassay technique and free triiodothyronine (FT3) and free thyroxine (FT4) by competitive immunoassays. All analytes had an analytical between-run coefficient of variation below 6%.

In Denmark the normal references of the parameters in healthy subjects have been analyzed (see Table 1). Regarding phosphate, the normal references in women aged 8–125 were 0.76–1.41 mmol/L, while in men aged 18–49 were 0.71–1.53 mmol/L, and those aged 50–125 were 0.71–1.23 mmol/L.

Statistical analysis

All statistical analyzes were performed by using SAS (SAS Inst. Inc., Cary, NC USA, 9.4) and a p value of <.05 was considered significant.

Basic demographic parameters and the between-group differences were calculated using t test (due to normally distributed data) for continues variables and chi-square test for categorical variables.

Mixed model analyzes were performed in SAS to describe the variance between time-points of the diurnal rhythm where 04:00h was held as reference point. Data were logarithmically transformed due to non-normally distributed data and were transformed back to empirical fractiles and converted to percentage variance ($(2^x-1)^*100$). The percentage variance describes the difference in median levels between time points. The total inclusion value for both groups was used as a baseline to calculate the changes between inclusion and discharge in each group and the

further comparison in values between the two groups. Due to the small number of patients, we eliminated the within group differences of CU and IU by pooling the total inclusion values for both before calculating the difference between groups (see Table 2).

Cosinor rhythmicity was analyzed assuming a 24 h time cosinor period [24] and the divergence of the calculated rhythmicity to this assumed cosinor curve was expressed as standard error (*SE*). The 24 h rhythms of each group were further characterized by the following cosinor rhythm parameters: (1) Mesor (rhythm-adjusted average about which oscillation occurs), (2) Amplitude (difference between the highest and lowest values of the fitted cosinor curve) and (3) Times of peak and nadir [24,25]. Data analyzes were performed using the GPLOT procedure in SAS. Cosinor analysis was not performed on Ca/P ratio due to the Ca/P ratio not being a circadian controlled value.

The group difference of the 24 h delta mean values from inclusion to discharge were calculated by Wilcoxon signed-rank test.

Results

In our study, 256 patients who required in-hospital rehabilitation were screened for eligibility. Out of these, 90 fulfilled both the inclusion and exclusion criteria. Those who failed to be part of the study were considered ineligible mostly due to not meeting the inclusion criteria because they were expected to be discharged prior to completing two weeks of admission. Of the included 90 participants, 45 were missed from blood collection, whereby the remaining 45 patients completed the current study, among which 25 in the intervention unit and 20 in the control group (see Figure 1). The missed blood collection was mostly attributed to discomfort related to the procedure and thereby lack of consent. Other causes of exclusion were technical complications, fragile veins, or low hemoglobin concentration. Table 3 presents the demographic data of the included patients. Both groups were well matched.

Circadian rhythm

Cosinor curves

Calcium showed significant cosinor rhythmicity at both inclusion and discharge in both groups, whereas alkaline phosphatase, PTH, Vitamin D, FT3, FT4, TSH all showed no significant cosinor rhythmicity at inclusion and discharge in both groups. Phosphate showed significant rhythmicity at discharge in the control group. The results are summarized in Table 4. Best fitted curves to cosinor rhythmicity of calcium, alkaline phosphatase, PTH, Vitamin D, FT3, FT4, and TSH levels at inclusion and discharge in the IU and CU are presented in the Online Appendix Figures 1A–7A.

Table 2. Comparable variance between inclusion and discharge and between groups.

	Intervention unit (*p* value)	Control unit (*p* value)	Difference between groups (*p* value)
Analysis	Difference between inclusion and discharge	Difference between inclusion and discharge	Difference between inclusion and discharge
Calcium	.048	.034	NS
PTH	NS	NS	NS
Ca/P ratio	<.0001	<.0001	NS
Vitamin D	NS	NS	NS
Phosphate	NS	NS	NS
Alkaline phosphatase	.038	NS	NS

Notes: Variance between inclusion and discharge and between groups. The values were log transformed before calculation due to non-parametric distribution. The *p* values represents if there was seen a significant difference in circadian variance over all compared six times points (08:00h–04:00h) between inclusion and discharge and between groups. The results are calculated by SAS mix model *type 3 tests of fixed effects. NS: not significant.

Table 1. Mean level changes distributed over 24 h from inclusion to discharge in each unit.

Blood parameter	Healthy normal references and units	Inclusion blood Mean values (SD)	Discharge blood Mean values (SD)	Delta value	*p* Value
Calcium, 24-h	2.15–2.51 mmol/L				NS
Control unit (*N* = 19)		2.27 (0.08)	2.28 (0.08)	0.01	
Intervention unit (*N* = 22)		2.20 (0.09)	2.24 (0.08)	0.04	
PTH, 24-h	14–72 ng/L				**.02**
Control unit (*N* = 20)		50.07 (17.3)	49.67 (14.9)	−0.4	
Intervention unit (*N* = 25)		69.90 (33.7)	58.25 (32.0)	−11.65	
Ca/P ratio, 24-h	1.78–2.55				NS
Control unit (*N* = 19)		1.83 (0.20)	1.80 (0.14)	0.07	
Intervention unit (*N* = 22)		1.86 (0.21)	1.79 (0.25)	0.03	
Alkaline phosphatase, 24-h	35–105 U/L				NS
Control unit (*N* = 20)		83.2 (38.9)	77.9 (21.4)	−5.3	
Intervention unit (*N* = 25)		72.7 (18.9)	76.1 (20.7)	3.4	
Vitamin D, 24-h	>50 nmol/L				NS
Control unit (*N* = 20)		75.20 (44.4)	77.04 (38.6)	1.84	
Intervention unit (*N* = 25)		74.51 (36.2)	73.15 (30.1)	−1.36	
Mean phosphate, 24h	0.72–1.39 mmol/L				NS
Control group (*N* = 20)		1.25 (0.12)	1.30 (0.11)	0.033	
Intervention group (*N* = 25)		1.20 (0.15)	1.28 (0.21)	0.076	

Notes: Calculated differences for each unit between inclusion and discharge. Values are calculated from mean blood levels distributed across (24 h). Wilcoxon signed-rank test was used due to non-parametric distribution. Bold value indicates p-values that are below 0.05. NS = Not significant. No significant differences for calcium ($n = 41$ and p value .146), Alkaline phosphatase ($n = 45$ and p value .288), Vitamin D ($n = 45$ and p value .90), phosphate ($n = 45$ and p value .615) and Ca/P ratio ($n = 41$ and p value .6567) were found when comparing mean levels of the intervention and control groups by analysis of covariance. PTH levels in the intervention group were found to significantly decrease from inclusion to discharge. The levels decreased from a mean of 69.90 to a mean of 58.25 in the intervention group with a p value = .01.

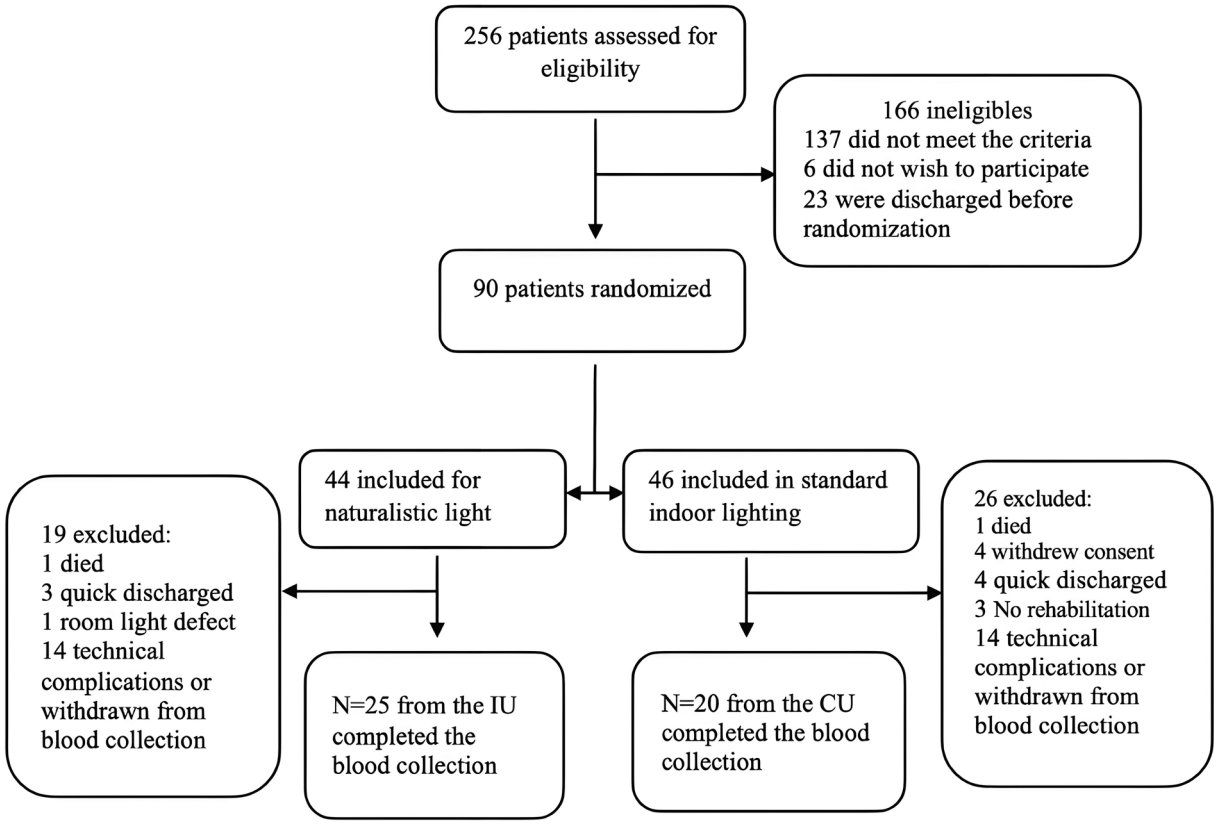

Figure 1. Flowchart.

Table 3. Patient characteristics.

Characteristic	Intervention unit ($N=25$)	Control unit ($N=20$)	p Value
Age, mean years (range)	73.7	70.9	.63
Sex,			.74
Male, *n* (%)	15 (68.2)	15 (75)	
Female, *n* (%)	7 (31.8)	5 (25)	
Smoker, *n* (%)	13 (59.1)	16 (80)	.10
Hypertension, *n* (%)[a]	15 (68.2)	12 (60)	.75
Diabetes			
Type 1, *n* (%)	0 (0)	2 (10)	.22
Type 2, *n* (%)	3 (13.6)	2 (10)	1.00
Hypercholesterolemia, *n* (%)	7 (31.8)	4 (20)	.49
Atrial fibrillation, *n* (%)	4 (18.2)	4 (20)	1.00
Barthel, mean score (±SD)	57.1 (±31.2)	62.0 (±24.5)	.30
NIHSS[b], mean score (±SD)	4.9 (±4.0)	4.6 (±4.1)	.95

[a]Hypertension defined as under medical treatment for hypertension at inclusion.
[b]National Institutes of Health Stroke Scale.

Variance between time-points in each group, respectively

Overall time points variations, calculated by variations between six different time points with 4 am as reference, showed significant variations regarding calcium, vitamin D, phosphate and alkaline phosphatase, except PTH. Calcium showed the highest level of significant variations between time points except midnight at both inclusion and discharge in both units. With elevated levels from 8 am to noon and from 20:00 h to midnight as illustrated by Table 5 and Figure 1A in Online Appendix.

Our study demonstrated significant variations during different time points in the calcium to phosphorus (Ca/P) ratio both at inclusion and discharge in the IU and CU. These variations corresponded closely with changes observed in the levels of calcium and phosphate. However, no significant differences in the Ca/P ratio were observed between the two units.

Variance between time points with combined inclusion value

Calcium levels showed a significant difference in diurnal time points variance from inclusion to discharge in both units, however it did not display a significant difference in variance between the two groups. Alkaline phosphatase levels showed a significant difference in diurnal time points variance between inclusion and discharge in the intervention group only.

If we only focus on the mean difference between groups and not the circadian element, the PTH is the only parameter which significant change between groups from inclusion to discharge (see Figure 2).

Mean level differences between groups

PTH plasma mean levels was the only parameter that exhibit a significant difference between the two group from inclusion to discharge. PTH level decreased significantly from inclusion to discharge in the IU group compared to the CU group calculated by Wilcoxon rank-sum test with a p value of .019.

Table 4. Cosinor rhythmicity of calcium, alkaline phosphatase, vitamin D, phosphate, parathyroid hormone, free triiodothyroinine 3, free triiodothyroinine 4 and thyroid-stimulating hormone.

Group	*N*	Cosinor *p* value	Mesor (*SE*) (pg/mL)	Amp (*SE*) Peak–Nadir	Peak time	Nadir time
Calcium control						
Inclusion	112	**.0006**	2.27 (0.009)	0.11 (0.02)	15:53	03:53
Discharge	112	**.0005**	2.29 (0.009)	0.10 (0.02)	13:53	01:53
Intervention						
Inclusion	120	**.013**	2.21 (0.01)	0.07 (0.015)	14:44	02:44
Discharge	120	**<.0001**	2.25 (0.01)	0.11 (0.015)	14:20	02:20
Alkaline-phosphatase control						
Inclusion	117	.80	83.69 (3.61)	8.13 (6.31)	17:19	05:19
Discharge	117	.56	77.85 (2.02)	5.49 (3.53)	16:18	04:18
Intervention						
Inclusion	138	.34	73.52 (1.62)	6.78 (2.84)	19:10	07:10
Discharge	138	.66	76.85 (1.76)	4.54 (3.08)	16:17	04:17
Vitamin D control						
Inclusion	115	.57	75.76 (4.13)	11.31 (7.13)	16:37	04:37
Discharge	117	.30	76.79 (3.61)	15.90 (6.31)	15:43	03:43
Intervention						
Inclusion	135	.66	72.98 (3.24)	8.29 (5.67)	17:46	05:46
Discharge	137	.14	70.83 (2.64)	14.84 (4.6)	15:04	03:04
Phosphate control						
Inclusion	117	.22	1.25 (0.01)	0.06 (0.02)	00:53	12:53
Discharge	117	**.04**	1.29 (0.01)	0.10 (0.02)	02:27	14:27
Intervention						
Inclusion	138	.25	1.19 (0.01)	0.07 (0.03)	23:51	11:53
Discharge	138	.16	1.24 (0.01)	0.07 (0.02)	01:50	13:50
PTH control						
Inclusion	114	.52	49.52 (1.75)	5.66 (3.04)	21:32	09:32
Discharge	117	.64	48.99 (1.44)	3.88 (2.51)	20:01	08:01
Intervention						
Inclusion	133	.78	69.42 (3.08)	6.75 (5.41)	20:42	08:42
Discharge	138	.68	57.13 (2.70)	6.75 (4.72)	00:51	12:51
FT3 control						
Inclusion	118	.82	5.63 (0.09)	0.14 (0.17)	07:44	19:44
Discharge	118	.90	5.80 (0.09)	0.09 (0.15)	05:21	17:21
Intervention						
Inclusion	132	.72	5.41 (0.07)	0.16 (0.12)	07:18	19:18
Discharge	132	.87	5.74 (0.09)	0.14 (0.17)	08:30	20:30
FT4 control						
Inclusion	117	.84	16.48 (0.31)	0.59 (0.53)	13:05	01:05
Discharge	118	.80	14.54 (0.20)	0.33 (0.36)	11:28	23:28
Intervention						
Inclusion	132	.68	16.76 (0.25)	0.62 (0.44)	12:01	00:01
Discharge	132	.85	17.09 (0.33)	0.55 (0.58)	10:35	22:35
TSH control						
Inclusion	118	.39	2.14 (0.13)	0.47 (0.22)	03:08	15:08
Discharge	118	.08	1.88 (0.9)	0.60 (0.16)	04:16	16:16
Intervention						
Inclusion	132	.15	1.64 (0.09)	0.49 (0.15)	03:25	15:25
Discharge	132	.17	1.52 (0.09)	0.49 (0.16)	03:32	15:32

Notes: Circadian rhythm of calcium, alkaline phosphatase, vitamin D, phosphate, PTH, FT3, FT4 and TSH measured by cosinor rhythmicity. Values were fitted to the best-fitting cosinor curve, and the 24 h rhythm of was further characterized by the following rhythm parameters: mesor (rhythm-adjusted average about which oscillation occurs), amplitude and times of peak and nadir.

Table 5. Variance between blood collection time-points.

	Intervention unit (*p* value)			Control unit (*p* value)		
Analysis	Inclusion	Discharge	In vs out	Inclusion	Discharge	In vs out
Calcium	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
PTH	NS	NS	NS	NS	NS	NS
Ca/P ratio	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Vitamin D	.0029	<.0001	<.0001	<.0001	<.0001	.0012
Phosphate	.0002	<.0001	.0013	.0058	.0018	.0459
Alkaline phosphatase	.0003	<.0001	.0002	<.0001	<.0001	.0004

Notes: Variance between blood collection time-points. The values were log2 transformed before calculation due to non-parametric distribution. The *p* values represents if there was seen a significant variance over all six times points (08:00h–04:00h). The results are calculated by SAS mix model *Type 3 tests of fixed effects. In vs. out: difference in variance between inclusion and discharge; NS: not significant.

Discussion

This study is the first to investigate the effect of naturalistic light exposure on the parameters related to calcium metabolism in stroke patients during at least two weeks of hospitalization for neurorehabilitation. Both the IU and CU stroke patient group exhibited significant circadian variations of calcium at both inclusion and discharge. This was proven by calculating significant variance between timepoints and cosinor rhythmicity with peak levels around 14:30 h. Our findings show similarity in calcium rhythm compared to healthy subjects. Several previous studies have showed diurnal rhythmicity of calcium in healthy subjects [26–33]. Calvo [34] measured serum calcium every 2 h for 26 h in 25 women (aged 21–69 years) and 24 men (aged 20–67 years) consuming self-selected diets and the calcium levels followed a circadian rhythm in both sexes ($p \leq .01$), while the patterns differed between sexes, notably during early morning, when serum calcium levels were lower in women. Additionally, it was found that in postmenopausal women the plasma levels of calcium exhibited a circadian

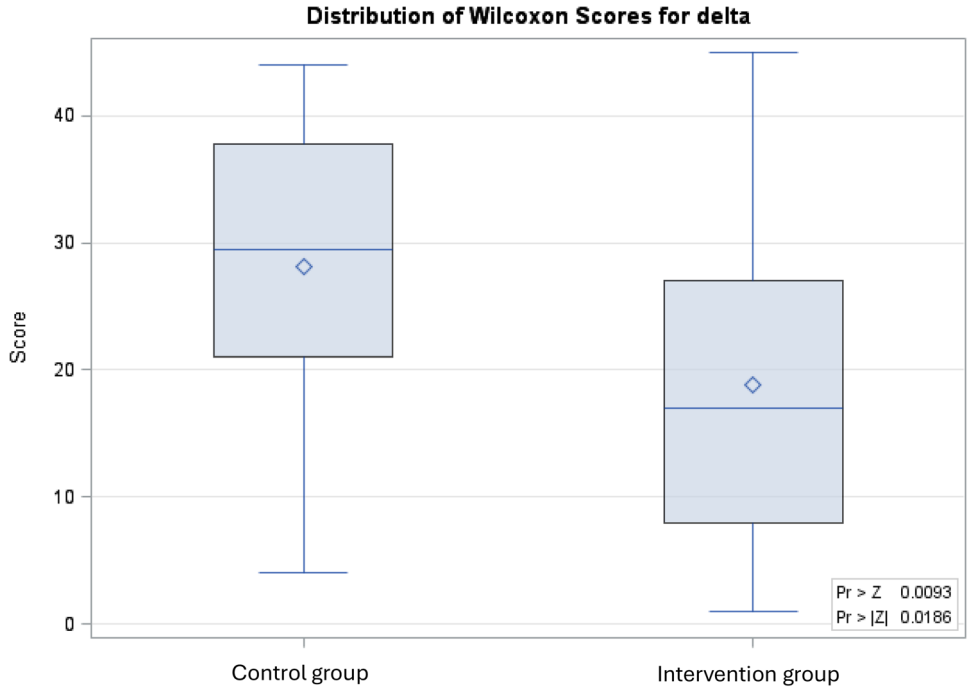

Figure 2. Comparison between group of PTH mean level. Comparison between two groups in regards to PTH mean level. Differences between groups is calculated by a non-parametric test - Wilcoxon rank-sum test which is based on the difference between inclusion and discharge values in the two groups. PTH was significantly lower in the intervention group with a *p* value of .018.

pattern of variation [35] with a diurnal pattern like that reported in previous studies with low levels during the night, a peak in the forenoon and high levels during the day [31,36,37]. Whilst our data showed different peak levels (14:30 h vs 10:00 h). This non similarity across study results, indicate difference in cohorts, however a similarity in exhibition of a circadian rhythmicity of calcium. We therefore cannot conclude with certainty that stroke patients have a different and/or disturbed circadian rhythm in calcium compared with healthy people.

A diurnal rhythmicity has been reported in plasma phosphate with an increase during the day, a peak late in the evening and night and a nadir in the early morning in both post-menopausal women [35] and healthy adult men and women [36] Furthermore, alkaline phosphatase also showed a circadian rhythm with nighttime peak and daytime nadir [38]. In contrast to this, PTH, vitamin D and alkaline phosphatase did not vary in a statistically significant circadian rhythm in respects to cosinor in this study, however phosphate, showed significance at discharge in the control group.

PTH was the only parameter showing no significant variation between time-points during the day in our study. These results are contrary to those reported in healthy subjects. Different studies [39,40] reported that PTH levels in 11 healthy men and 18 healthy individuals of both men and women respectively, showed a biphasic circadian pattern with two peaks. Similarly, PTH in healthy individuals of different ethnicities, Gambian, Chinese and British adults, respectively (aged 60–75 years), showed a circadian rhythm with two different peaks, one in the early morning and one in the late evening [38]. Several other studies in healthy people found a well-documented diurnal rhythm of PTH with two peaks, one in the afternoon/evening and one at night [33,35,39,41]. While others, however, have reported only one maximum for the PTH diurnal rhythm [27,28,36,42], most often in the early morning hours [36,42,43].

However, a circadian pattern of variation for PTH was found [35], with the exact times for peak and nadir levels of PTH as in our study in both inclusion and discharge in the control group. While at discharge, the intervention unit showed different peak level times of PTH. Despite the same peak and nadir values as in [35], our PTH oscillation was not strong enough to show a significant circadian rhythm in both the cosinor and variation calculations. The reason for the latter is not clear.

In the present study we found no significant cosinor rhythmicity at inclusion and discharge of 25-hydroxyvitamin D (25(OH)D). Previous studies in healthy subjects have reported conflicting results. No distinct pattern in 24 h variations of 25(OH)D was found when blood was collected every 2 h for 24 h in 10 healthy adult Maasai men and women from Tanzania [44]. While a marked circadian variation was demonstrated for 25(OH)D in 10 healthy volunteers of 7 men and 3 women ($p = .030$) and a circadian variation of borderline statistical significance was demonstrated in 10 newly diagnosed diabetic patients of 6 men and 4 women ($p = .083$) when measured by population mean cosinor analysis [45] with peak levels at 12:00h and nadir at 06:00h in both groups. Similarly, in 91 healthy men and women aged 60–75 y and resident in the UK (n 30), Gambia (n 31) and China (n 30), it was reported that 25(OH)D exhibited significant diurnal rhythms [46]. 25-OH-vitamin D was also shown to have significant diurnal rhythmicity in 12 postmenopausal women [35].

Nevertheless, it is essential to mention that an explanation for this difference in findings across different studies

could be that vitamin D levels are complexly regulated through different mechanisms, in addition to small sample sizes and difference in measurement methods.

Our data indicate that stroke patients may exhibit impaired circadian rhythmicity of the parameters related to calcium metabolism except calcium which showed similarity with healthy subjects. Additionally, our findings also indicate, apart from PTH, that calcium metabolism did have a significant diurnal variance between our time points, but not enough that could be expressed as a significant circadian rhythm over 24 h measured by cosinor rhythmicity.

In our study PTH plasma levels decreased significantly from inclusion to discharge in the IU group compared to the CU group. In a study of 111 hypertensive patients, it was found that sun light exposure seems to lower the PTH level [47]

This implies that naturalistic light environment may exhibit inhibitory effect on PTH levels through SCN and long-term inhibition of PTH secretion induced by light stimulation may therefore prevent bone loss [48,49] and may be a way to reduce bone loss in bedridden patients. Studies indicate also that high PTH levels are associated with calcific aortic stenosis, high-density lipoprotein cholesterol and cancer risk [50–53].

We analyzed the calcium-phosphate ratio (Ca/P) in our study, a well-established and reliable marker for detecting parathyroid dysfunctions, such as primary hyperparathyroidism (PHPT) and hypoparathyroidism. Previous studies have demonstrated that the Ca/P ratio offers superior sensitivity and accuracy compared to serum calcium and phosphorus levels, particularly when using cut-off values above 2.55 and below 1.78, as shown in a cohort of 1038 patients [54].

In our study, we observed significant diurnal variations during different time-points except midnight in the Ca/P ratio at both inclusion and discharge for patients in both units, which were consistent with changes in calcium and phosphate levels. Our results seems to relate to what is found in a study which have found a borderline statistically significant variation for serum Ca/P ratio in healthy volunteers [45]. However, no significant differences were found between the two groups as neither calcium or phosphate showed variations, suggesting that light exposure did not influence the rhythmicity of the Ca/P ratio. These findings imply that parathyroid hormone (PTH) dysfunction is unlikely in either patient unit, as the Ca/P ratio remained within the normal physiological range.

In contrast to PTH, no change in the plasma levels of other parameters in either group were seen regardless of the naturalistic light exposure, this could imply that light is not the most important zeitgeber of the circadian peripheral oscillations that regulate calcium metabolism. Activity, food intake and posture changes are other potential entrainment factors. Furthermore, in elderly patients with dementia peripheral oscillations were found to not respond to light but to still maintain a normal circadian rhythm despite dysfunction of the systemic SCN controlled circadian rhythms, also suggesting that the circadian clocks of peripheral tissues are entrained by other factors than light [55] This hypothesis was also supported by the results in the original trial by West et al. where melatonin circadian rhythm in stroke patients were changed by exposure to naturalistic light but not the circadian rhythm of cortisol [12]. It is therefore important to conduct studies emphasizing the effect of other potential entrainment factors including activity levels and eating patterns known as chorononutrition regarding calcium metabolism. Some studies have already shown that frequency of feeding may influence the circadian changes in bone resorption [56]. Whether this is true in humans is unknown and requires further study. Another important Zeitgeber is posture, as prolonged bed rest leads to an increase in bone resorption [57]. Of the described negative health effects that can be correlated to the disrupted circadian rhythmicity of calcium parameters, and overall calcium homeostasis, are bone loss as well as fractures. Additionally, an important factor that may have significant effects on calcium homeostasis, including PTH secretion [58] and renal calcium handling [59,60], is cortisol. In another study [61] the effects of circadian cortisol pattern on serum calcium, PTH and urinary calcium excretion were assessed. The results indicated that the circadian variation in serum cortisol has significant effects on urinary calcium excretion. Thus, calcium excretion declined after the cortisol peak compared to the day without the cortisol peak, when there was a rise in calcium excretion.

The inconsistency in relation to the lack of cosinor circadian rhythmicity in alkaline phosphatase, PTH, Vitamin D, FT3, FT4 and TSH at inclusion and discharge in both units, may be an indication of circadian disruption after stroke, however further studies should elaborate on this.

Strengths and limitations

The strengths of this study include the power of having two comparable units as patients were randomly allocated following the normal procedure for an equal distribution of patients to the two rehabilitation units and the conditions in the two rehabilitation units were equal with regards to size, form and professional staffs. The IU unit had blackout curtains, that went up at 08:00 h and down at 20:00 h during all four seasons. It was estimated that the light significantly differed between beds during the 40% of the meteorological time, over a 5 h period during the peak summer season and disappeared outside of this period. Therefore, another strength of this study is the ability to include data for all four seasons since sunlight exposure in Denmark significantly changes throughout the year. This study was performed in a real-world hospital setting therefore, the results reflect the real-life situation in a rehabilitation hospital ward.

In contrast, the limitations were that only 45 patients completed the blood collection, and the sample size was therefore relatively small. The patients dropped out of the blood tests due to various reasons, among them discomfort to the procedure, feeling of tiredness, quick discharge, death and technical complications during the blood collection. NIHSS and Barthel scores significantly differed between the included and excluded participants, expectedly since the most severely impaired patients had the most difficulties participating in blood collection.

As mentioned before in the discussion, blue-spectrum light is not the only zeitgeber as food intake, activity levels and posture function also are entraining elements. In this study the participants were offered the same meals at the same time of the day regardless of units, however it was not monitored whether the participants did eat the offered meals.

This study was part of an exploratory investigational study in a relatively new scientific area. Thus, more specific studies are needed to further address the effects of naturalistic light on the levels and rhythmicity of parameters related to calcium metabolism.

Conclusion

The results indicate that only calcium exhibited a clear circadian rhythm and a variance during the day in our post stroke cohort while the other parameters related to calcium metabolism did not. Other than PTH, the parameters did not show variations in response to naturalistic light intervention, therefore it seems that light is not the main regulator of the circadian peripheral oscillations that regulate calcium metabolism, but other factors such as activity, posture and food intake may be the main regulators.

The decreased PTH secretion in patients exposed to naturalistic lightning, may prevent bone resorption and could be a way of preventing bone loss during long time hospitalizations, however, further studies should elaborate on this.

When comparing our data to healthy individuals from other studies, the results seem to differ regarding the circadian rhythmicity suggesting that stroke may depress the circadian rhythm of the calcium metabolism. These findings demonstrate a rationale for further investigations of circadian rhythm alterations after stroke and to differentiate between central and peripheral circadian regulation mechanism regarding the examination of the long-term effects of circadian light intervention.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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