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Long-Term Monitoring and Analysis of Age-Related Changes on Autonomic Nervous Function

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Abstract

This study used a small wearable heart rate sensor to monitor the daily autonomic function of 600 subjects from across all age groups over a prolonged period of time. The results indicated that the LF/HF ratio (Heart Rate Variability, LF: frequencies between 0.04 Hz - 0.15 Hz, HF: frequencies between 0.15 Hz - 0.4 Hz) an indicator of balance in the autonomic nervous system, tended to peak for subjects in their 40's and decline thereafter. This conceivably may be partially due to the causes for concern and stress changing and/or declining for the group aged 50-plus. A decline in diurnal variation of autonomic nervous activity was also exhibited in subjects aged 50 and up, showing a tendency for decline in the function of rising sympathetic nerve activity particularly in the morning. It is conceivable that this stems from a decline in the responsiveness of the autonomic nervous system. Subjects in the 50-plus group furthermore exhibited a tendency for declining variation in autonomic nervous activity between sleeping and waking hours. This phenomenon was consistent with the tendency for there to be a rise in wake after sleep onset coupled with a decline in slow-wave sleep in middle- to old-age.

Keywords

Heart Rate Variability, Autonomic Nervous System, Stress, Age, Circadian Rhythm, Sleep

1. Introduction

Having breached the threshold of being a hyper-aging society, the prevalence of lifestyle-related diseases and soaring medical costs have emerged as issues of dire consequence in Japan. Modern-day society, in particular, is referred to as a "stress society" and according to statistics from the Health, Labour, and Welfare Ministry [1], nearly half of all people are living with some kind of stress as 46.5% of people reported experiencing worries or stress in their daily lives (**Figure 1**).

It is said that this kind of stress suffered by many Japanese people is closely related to autonomic nervous function [2]. Seen structurally and functionally, the autonomic nerves are positioned to connect the internal environment and the external environment, harmonize the functions of the body, regulate homeostasis (cardiovascular/respiratory control, body temperature regulation, gastrointestinal motility, urine/feces excretion function, reproductive function, metabolism/endocrine function), and perform adaptive response to stress ("fight or flight" reaction). Thus, the autonomic nervous system is doing what could be called indefatigable work in order to ensure the survival and proliferation of the species [3].

In 1921 Langley, a physiologist at the University of Cambridge, published "Autonomic Nervous System" and classified the autonomic nervous system into three systems: sympathetic nerves, parasympathetic nerves, and enteric nerves, establishing the current concept of autonomic nerves [3].

The sympathetic nerves together with parasympathetic nerves form the autonomic nervous system, and are the nerves that control the secretory glands, blood vessels, internal organs, and so on. During mental stimulation or exercise, they work to increase the activity of the whole body, such as secreting saliva, increasing blood pressure/blood sugar, constricting blood vessels in the skin and internal organs, and collecting blood in the muscles/brain [3].

On the other hand, the parasympathetic nerves emanate from the brain region and the sacral region and, mainly through the vagus nerve, secrete acetylcholine as a neurotransmitter. It acts to suppress the heart and excite the gastrointestinal tract. It also acts to promote bile secretion, promote secretion of tears and saliva, dilate the pupils, etc. These autonomic nervous functions are affected not only by physical stress but also by psychological stress [3].

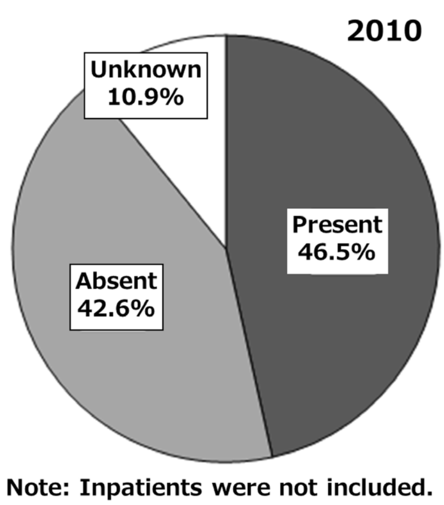

Figure 1. Presence of worries or stress (age 12 and over).

Traditionally, evaluation based on subjective values has been used as the means for quantifying stress. The Beck Depression Inventory (BDI) developed by Beck *et al*. [4] is a self-administered system of 21 question items that makes it possible to evaluate the severity of depression symptoms in the past two weeks. The adequate reliability and validity of the Japanese version of BDI have been confirmed by Sameshima *et al*. [5]. Developed by Zung [6], SDS (Self-rating Depression Scale) is a measure consisting of 20 items for evaluating emotional, physiological and psychological aspects of depression symptoms. The reliability and validity of the Japanese version of SDS have been confirmed by Fukuda *et al*. [7]. CES-D consists of 20 items, and is answered based on a 4-point scale [8]. The reliability and validity of the Japanese version of CES-D are being studied by Shima *et al*. [9].

Meanwhile, there are measurement methods using saliva, blood, and brain waves to evaluate stress based on objective values. Regarding saliva, it is possible to measure substances such as cortisol, which is an indicator of endocrine activity, SIgA, which is an indicator of immune activity, and α-amylase, which is an indicator of sympathetic nerve activity, and all of these are known to reflect states of acute and chronic stress [10].

Regarding blood, it is said that cytokines are produced in the brain when under stress, and cytokines are produced from the immune cells in the blood through acceleration of the hypothalamus and autonomic nerves. There are reports that patients with depression have increased levels of such cytokines [11].

Regarding brain waves, the term alpha waves refers to the 8 - 13 Hz component of the electrical signal (brain waves) generated by the human or animal brain, and in the brain waves at the time of relaxation or when the eyes are closed, the proportion occupied by alpha waves is higher than that of other frequency components. They decrease when the eyes are open or during visual stimulation, physical exercise, or mental activity such as arithmetic memorization, or when nervous or asleep. At the present time, it has been found that alpha waves increase when relaxing [12].

However, these objective indices are, in the case of saliva measurement, time-consuming to collect and evaluate, and in the case of blood measurement, they are invasive, so there is a considerable physical burden on the test subject. Also, in the case of brain wave measurement, it is necessary to mount electrodes in multiple places, and it becomes a large-scale measurement. Therefore, it is difficult to evaluate stress easily using these methods, and they are not suitable for obtaining long-term data from a large number of test subjects.

Therefore, heart rate variability analysis can be included as a method for evaluating autonomic nervous function more simply and non-invasively. In recent years, it has become possible to measure the fluctuation of the heartbeat (interval between heartbeats) by using a small wearable heart rate sensor that can be worn in everyday life, and use heart rate variability analysis to calculate the indices of sympathetic nerve activity and parasympathetic nerve activity.

Generally, there is fluctuation of the heartbeat in a living body, and when this fluctuation is frequency analyzed, a peak can be seen at a certain frequency. In the case of a person, there appear a high frequency component (0.15 - 0.40 Hz: HF) reflecting the variation of the respiratory cycle and a low frequency component (0.05 - 0.15 Hz: LF) reflecting fluctuation in blood pressure, both of which reflect autonomic nerve activity. It is said that HF is regulated by the parasympathetic nerves, and LF is regulated by both sympathetic and parasympathetic nerves [3].

There are several prior studies of the relationship between autonomic nervous indices and physiological phenomena that used such heart rate variability analysis.

Previous research has explored the relationship between the severity of depression based upon the SDS ("Self-rating Depression Scale") and autonomic activity obtained by analyzing heart rate variability from a Holter ECG monitor in 31 subjects (12 males and 19 females) diagnosed with mood disorders. In association with the severity of depression, the results demonstrated a significant rise in sympathetic nerve function along with significantly diminishing parasympathetic nerve function. Accordingly, in assessments of depression severity conducted with the use of Holter ECG monitors, these results support the onset of abnormalities in autonomic nerve function brought on by depression [13].

In another paper examining the effects of depression on the autonomic nervous system in 16 male subjects diagnosed with mild heart attacks, a trend was observed of elevated sympathetic nerve function and suppressed parasympathetic nerve function as subjects' scores rose on the SDS (denoting increasingly severe depression) [14]. Thus, abnormalities in autonomic nervous function wherein the sympathetic nerves are excited and the parasympathetic nerves are suppressed may conceivably have an effect on the pathogenesis and prognosis of heart attacks.

Also, in the Multiethnic Study of Atherosclerosis (MESA), a leading epidemiological study in the United States, as a result of investigating the relationship between depression symptoms, anger, anxiety, social support scores and parasympathetic nerve functions, evaluated in questionnaire form, it was observed that there was a significant negative association with symptoms of depression. Therefore, it is said that continuing depression in particular affects autonomic nervous function, especially parasympathetic function [15].

In addition to those people who have specific diseases as described above, there are several reports that the balance of the autonomic nervous system also changes with age. This is because the internal environment of the human body is kept constant by the homeostasis mechanism, but with age the balance of the autonomic nervous system deteriorates, so it is said to become difficult for the body to respond appropriately to changes in the external environment. In previous reports, a gradual decline in parasympathetic function due to aging in females and predominance of sympathetic function due to aging in males have been recognized, but many reports are based on the results of short-term measurement [16].

Consequently, in this study we used a small wearable heart rate sensor capable of long-term monitoring to test how age-related changes in levels of autonomic activity and diurnal variations in the autonomic nerves change as a function of age. We also attempt to reveal variations across age groups, diurnal variations, and variations between sleeping and waking states by monitoring a large pool of subjects ranging across all age groups as they went about their daily lives.

2. Methodology

In this study, we measured biological data from 600 subjects (329 males, 271 females) for a prolonged period of time (24 hours)using a small wearable heart rate sensor (WHS-1) produced by Union Tool Co., shown in Figure 2. Regarding the experiment, it was carried out after obtaining approval from the ethics committee of the medical institution and informed consent from the test subjects.

The small wearable heart rate sensor can measure RR intervals, body surface temperature, and triaxial acceleration. The sensor's sampling frequency for heart-rate intervals, body surface temperature, and triaxial acceleration is 1000 Hz, 1 Hz, and 31.25 Hz, respectively. Table 1 shows the distribution of gender and ages of the subjects.

Fast Fourier Transform (FFT) was used for the frequency analysis of the measured RR intervals, defining the low-frequency component (LF) as the

Figure 2. Small wearable heart rate sensor.

Table 1. Subjects' age and sex distribution.

Age	Man	Female	Total
10's	26	23	49
20's	59	39	98
30's	95	47	142
40's	77	87	164
50's	37	44	81
Over 60's	35	31	66
Total	329	271	600

frequencies between 0.04 Hz - 0.15 Hz and the high-frequency component (HF) as the frequencies between 0.15 Hz - 0.4 Hz. The sum of the LF and HF components is referred to as total power (TP), and is considered to be an indicator of overall autonomic nervous activity. Our method for computing indicators of autonomic nerve activity adhered to procedures from paper [17] in the references. These indicators of autonomic nerve activity are also said to correlate with fatigue [18].

In this experiment, we use solely the data where subjects exhibit little body movement from among the long-term measurement data. To analyze the data, body movement levels were determined on the basis of the acceleration sensors embedded in the small wearable heart rate sensor using the supplied software (produced by WINFrontier Co.). It has been reported that, in general, the composite value of triaxial acceleration correlates to body activity indicators and energy consumption, and the values change while walking, running, and resting [19] [20] [21].

The threshold for the composite value of acceleration was configured in alignment with each of the activities (walking, running, resting) on the sensor to distinguish the activity. Only the data determined as being recorded at rest when there is little body movement was used. IBM SPSS Statistics Version 22 was used for statistical processing in this study, with the level of statistical significance being 5%. We used the games-howell method for multiple comparison.

3. The Relationship between Autonomic Nerve Function and Aging

We investigated the relationship between age and the LF component of the autonomic nerve indicators. It is said that the LF component is a low frequency component (0.05 - 0.15 Hz) of heart rate variability analysis and is regulated by both sympathetic and parasympathetic nerves [3], but as it decreases with administration of β blockers (propranolol: 0.15 mg/kg), sympathetic nerves are said to be heavily involved [22].

Subjects were divided into six groups from teenagers to subjects aged 60-plus and the Games-Howell method was used to conduct multiple comparisons in order to examine the variances between groups. The results are shown in **Figure 3** and **Table 2**. The results demonstrate a tendency for LF to peak in the 30's and trend downward thereafter.

Subsequently, the HF component was studied. Since the HF component is considered to be the high frequency component (0.15 - 0.40 Hz) of heart rate variability analysis and disappears with a parasympatholytic drug (atropine: 0.04 mg/kg), the cardiac vagus nerve is thought to be involved, and is said to display parasympathetic nervous activity [23].

The Games-Howell method was used to conduct multiple comparisons to examine the variances between the groups. The results are shown in **Figure 4** and **Table 3**. The results indicate a tendency for HF to peak in the 20's and decline thereafter, reversing to an upward trend in the 60's.

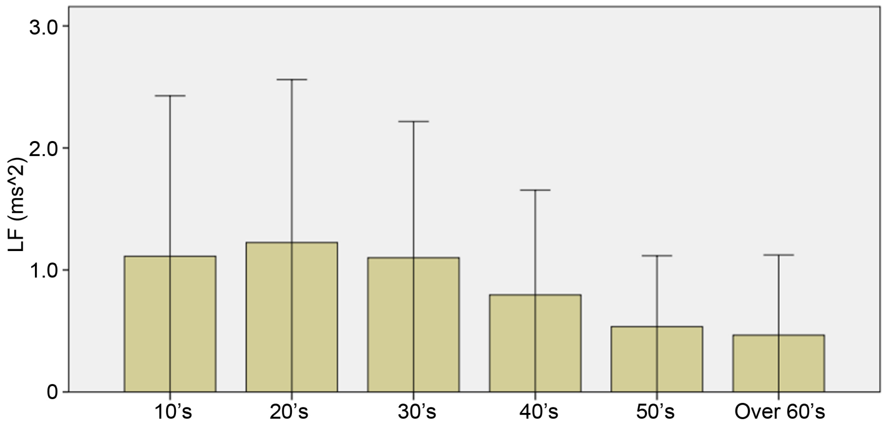

Figure 3. Relationship between LF and age.

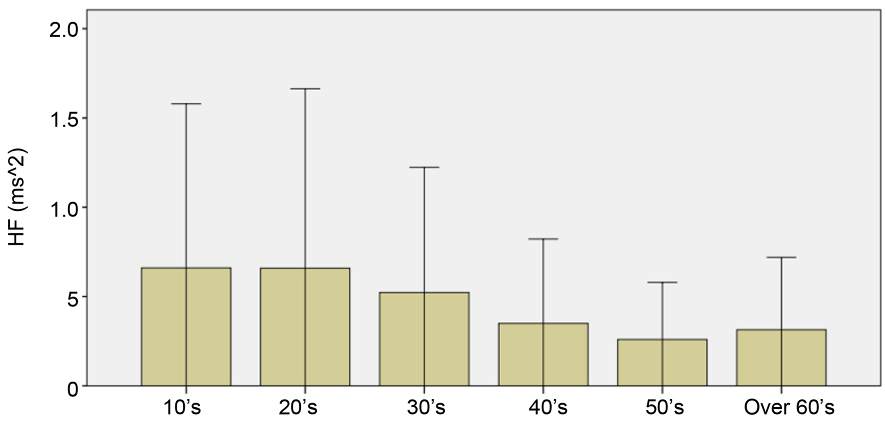

Figure 4. Relationship between HF and age.

Next, we investigated the relationship between total power (TP), an indicator of the autonomic nerves, and age. Total power is the sum of the LF and HF components and is considered to be an indicator representative of the volume of autonomic nerve activity [17]. Total power is said to be correlated with fatigue, and smaller values of total power are thought to indicate higher levels of fatigue accumulation [18].

The subjects were divided into six groups from teenagers to subjects aged 60-plus, and the Games-Howell method was used to conduct multiple comparisons in order to examine the variances between groups. The results are shown in **Figure 5** and **Table 4**. The results indicate a tendency for TP to peak in the 20's with a declining trend thereafter.

Earlier studies also showed a tendency for LF, HF, and total power to decrease with aging [16] [24], and the same trend was observed in this study as well. In addition, it is possible that the trend of HF increasing in subjects in their sixties is influenced by the lifestyle changes that result from retirement, etc.

Finally, we examine the relationship between age and the LF/HF ratio, an indicator of the autonomic nerves. The LF/HF ratio is considered to be an indicator of the balance between the sympathetic and parasympathetic nerves, or an

Table 2. Multiple comparisons (relationship between LF and age).

Dependent variable: LF (ms^2)

Games-Howell

(I) Age flag	(J) Age flag	Mean difference (I-J)	Std. error	Sig.	95% Confidence interval	
					Lower bound	Upper bound
10's	20's	−0.112159369*	0.0140548007	0.000	−0.152218228	−0.072100510
	30's	0.0120527425	0.0137553960	0.952	−0.027153352	0.0512588369
	40's	0.316947065*	0.136172453	0.000	0.2781344368	0.3557596934
	50's	0.576613248*	0.0136477862	0.000	0.5377136369	0.6155128598
	Over 60's	0.646600454*	0.0138285632	0.000	0.6071859647	0.6860149426
20's	10's	0.112159369*	0.0140548007	0.000	0.0721005100	0.1522182285
	30's	0.124212112*	0.0050304089	0.000	0.4098767908	0.1385474326
	40's	0.429106434*	0.0046393388	0.000	0.4158855298	0.4423273389
	50's	0.688772618*	0.0047282304	0.000	0.6752983878	0.7022468475
	Over 60's	0.758772618*	0.0052271649	0.000	0.7438637352	0.7736559105
30's	10's	−0.01205742	0.0137553960	0.952	−0.051258837	0.0271533519
	20's	−0.124212112*	0.0050304089	0.000	−0.138547433	−0.109876791
	40's	0.304894323*	0.0036327621	0.000	0.2945419477	0.3152466975
	50's	0.564560506*	0.0037456186	0.000	0.55388647914	0.5452345204
	Over 60's	0.634547711*	0.0043585260	0.000	0.6221269835	0.6469684388
40's	10's	−0.316947065*	0.0136172453	0.000	−0.355759693	−0.278134437
	20's	−0.429106434*	0.0046393388	0.000	−0.442327339	−0.415885530
	30's	−0.304894323*	0.0036327621	0.000	−0.315246697	−0.294541948
	50's	0.259666193*	0.0032012668	0.000	−0.2505434069	0.2687889597
	Over 60's	0.329653389	0.0039006665	0.000	−0.3185373741	0.3107694029
50's	10's	−0.546613248*	0.0136477862	0.000	−0.614412860	−0.537713637
	20's	−0.688772618*	0.0047282304	0.000	−0.702246847	−0.575298388
	30's	−0.564560506*	0.0037456186	0.000	−0.575234520	−0.553886491
	40's	−0.259666183*	0.0032012668	0.000	−0.268788960	−0.250543407
	Over 60's	0.069987205*	0.0040059828	0.000	0.0585710597	0.0814033508
Over 60's	10's	−0.646600454*	0.0138285632	0.000	−0.686014943	−0.607185965
	20's	−0.758459823*	0.0052271649	0.000	−0.773655911	−0.743863735
	30's	−0.634547711*	0.0043585260	0.000	−0.646968439	−0.622126983
	40's	−0.329653389*	0.0039006665	0.000	−0.340769403	−0.318537374
	50's	−0.069987205*	0.0040059828	0.000	−0.081403351	−0.508571060

*The mean difference is significant at the 0.05 level.

Table 3. Multiple comparisons (relationship between HF and age).

(I) Age flag	(J) Age flag	Mean difference (I-J)	Std. error	Sig.	95% Confidence interval	
					Lower bound	Upper bound
10’s	20’s	0.0012325036	0.0098947422	1.000	−0.026969272	0.0294342789
	30’s	0.137519813'	0.0095776040	0.000	0.1102213130	0.1648183128
	40’s	0.310538135*	0.0094768242	0.000	0.2835266626	0.3375496078
	50’s	0.400229014*	0.0094902572	0.000	0.3731792839	0.4272787447
	Over 60’s	0.346629397*	0.0096120152	0.000	0.3192328882	0.3740259052
20’s	10’s	−0.001232504	0.0098947422	1.000	−0.029434279	0.0269692717
	30’s	0.136287309*	0.0035862520	0.000	0.1260674358	0.1465071828
	40’s	0.309305632*	0.0033077032	0.000	0.2998795191	0.3187317442
	50’s	0.398996511*	0.0033459955	0.000	0.3894612762	0.4085317452
	Over 60’s	0.345396893*	0.0036771648	0.000	0.3349179180	0.3558758682
30’s	10’s	−0.137519813"	0.0095776040	0.000	−0.164818313	−0.110221313
	20’s	−0.136287309'	0.0035862520	0.000	−0.146507183	−0.126067436
	40’s	0.173018322*	0.0021829970	0.000	0.1667973767	0.1792392680
	50’s	0.262709201*	0.0022405938	0.000	0.2563241089	0.2690942939
	Over 60’s	0.209109584*	0.0027103720	0.000	0.2013856925	0.2168334751
40’s	10’s	−0.310538135'	0.0094768242	0.000	−0.337549608	−0.283526663
	20’s	−0.309305632*	0.0033077032	0.000	−0.318731744	−0.299879519
	30’s	−0.173018322*	0.0021829970	0.000	−0.179239268	−0.166797377
	50’s	0.089690879*	0.0017606697	0.000	0.0846734292	0.0947083290
	Over 60’s	0.036091261'	0.0023293375	0.000	0.0294531592	0.0427293637
50’s	10’s	−0.400229014'	0.0094902572	0.000	−0.427278745	−0.373179284
	20’s	−0.398996511'	0.0033459955	0.000	−0.408531745	−0.389461276
	30’s	−0.262709201'	0.0022405938	0.000	−0.269094294	−0.256324109
	40’s	−0.089690879'	0.0017606697	0.000	−0.094708329	−0.084673429
	Over 60’s	−0.053599618'	0.0023834006	0.000	−0.060391786	−0.046807450
Over 60’s	10’s	−0.346629397*	0.0096120152	0.000	−0.374025905	−0.319232888
	20’s	−0.345396893*	0.0036771648	0.000	−0.355875868	−0.334917918
	30’s	−0.209109584*	0.0027103720	0.000	−0.216833475	−0.201385692
	40’s	−0.036091261'	0.0023293375	0.000	−0.042729364	−0.029453159
	50’s	0.053599618'	0.0023834006	0.000	0.0468074498	0.0603917855

*The mean difference is significant at the 0.05 level.

indicator of sympathetic nerve activity, although as mentioned earlier, interpretations are split. The subjects were divided into six groups from teenagers to subjects aged 60-plus, and the Games-Howell method was used to conduct multiple comparisons in order to examine the variances between groups. The results are shown in Figure 6 and Table 5. The results indicate a tendency for the

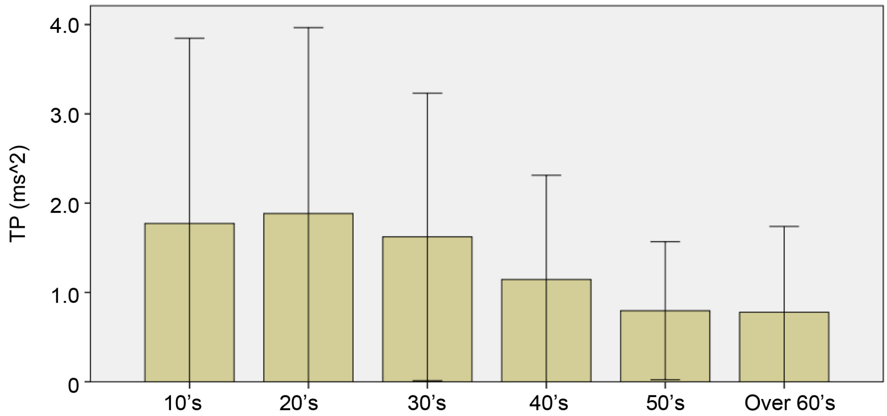

Figure 5. Relationship between TP and age.

Table 4. Multiple comparisons (relationship between TP and age).

Multiple comparisons
Dependent variable: TP
Games-Howell

(I) Age flag	(j) Age flag	Mean difference (I-J)	Std. error	Sig.	95% Confidence interval Lower bound	95% Confidence interval Upper bound
10's	20's	−0.110926866*	0.0221587508	0.000	−0.174083568	−0.047770163
	30's	0.149572555*	0.0216270603	0.000	0.0879302055	0.2112149052
	40's	0.627485200*	0.0214196776	0.000	0.5664334909	0.6885369097
	50's	0.976842263*	0.0214451137	0.000	0.9157181096	1.037966416
	Over 60's	0.993229850*	0.0217387010	0.000	0.9312695297	1.055190171
20's	10's	0.110926866*	0.0221587508	0.000	0.0477701630	0.1740835683
	30's	0.260499421*	0.0076507554	0.000	0.2386968006	0.2823020414
	40's	0.738412066'	0.0070432171	0.000	0.7183407143	0.7584834177
	50's	1.08776913*	0.0071201982	0.000	1.067478395	1.108059862
	Over 60's	1.10415672*	0.0079608695	0.000	1.081470275	1.126843157
30's	10's	−0.149572555*	0.0216270603	0.000	−0.211214905	−0.087930206
	20's	−0.260499421*	0.0076507554	0.000	−0.282302041	−0.238696801
	40's	0.477912645*	0.0051309270	0.000	0.4632909081	0.4925343818
	50's	0.827269707*	0.0052360984	0.000	0.8123482271	0.8421911876
	Over 60's	0.843657295'	0.0063320570	0.000	0.8256124836	0.8617021062
40's	10's	−0.627485200*	0.0214196776	0.000	−0.688536910	−0.566433491
	20's	−0.738412066*	0.0070432171	0.000	−0.758483418	−0.718340714
	30's	−0.477912645*	0.0051309270	0.000	−0.492534382	−0.463290908
	50's	0.34935706*	0.0042999506	0.000	0.3371033265	0.3616107982
	Over 60's	0.365744650*	0.0055828125	0.000	0.3498348727	0.3816544272
50's	10's	−0.976842263*	0.0214451137	0.000	−1.03796642	−0.915718110
	20's	−1.08776913*	0.0071201982	0.000	−1.10805986	−1.06747839
	30's	−0.827269707*	0.0052360984	0.000	−0.842191188	−0.812348227
	40's	−0.349357062*	0.0042999506	0.000	−0.361610798	−0.337103327
	Over 60's	0.016387588*	0.0056796224	0.045	0.0002019182	0.0325732570
Over 60's	10's	−0.993229850*	0.0217387010	0.000	−1.05519017	−0.931269530
	20's	−1.10415672*	0.0079608695	0.000	−1.12684316	−1.08147028
	30's	−0.843657295*	0.0063320570	0.000	−0.861702106	−0.825612484
	40's	−0.365744650*	0.0055828125	0.000	−0.381654427	−0.349834873
	50's	−0.016387588*	0.0056796224	0.045	−0.032573257	−0.000201918

*The mean difference is significant at the 0.05 level.

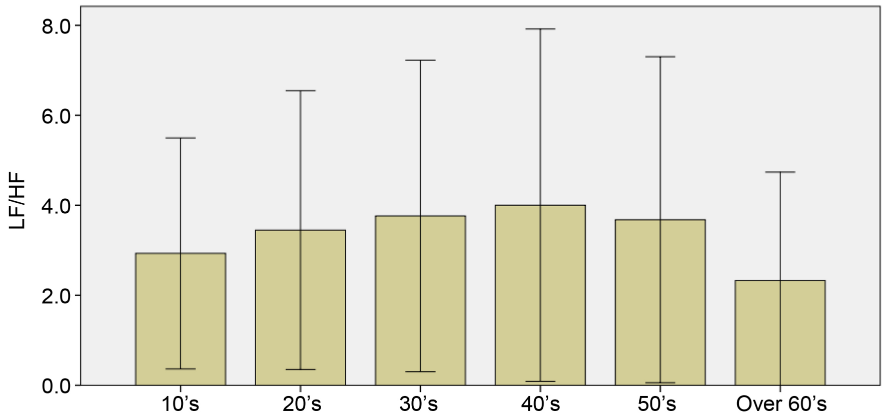

Figure 6. Relationship between LF/HF and age.

Table 5. Multiple comparisons (relationship between LF/HF and age).

Multiple comparisons
Dependent variable: LF/HF
Games-Howell

(I) Age flag	(J) Age flag	Mean difference (I-J)	Std. error	Sig.	95% Confidence interval	
					Lower bound	Upper bound
10's	20's	−0.518237016*	0.0279154228	0.000	−0.597800398	−0.438673634
	30's	−0.832669919*	0.0277558174	0.000	−0.911778684	−0.753561154
	40's	−1.07084570*	0.0280485861	0.000	−1.15078837	−0.990903024
	50's	−0.747990162*	0.0300676722	0.000	−0.833684432	−0.662295891
	Over 60's	0.604431896*	0.0288130236	0.000	0.5223116679	0.6865521250
20's	10's	0.518237016*	0.0279154228	0.000	0.4386736344	0.5978003984
	30's	−0.314432902*	0.0131144537	0.000	−0.351805533	−0.277060272
	40's	−,552608683*	0.0137232167	0.000	−0.591716104	−0.513501262
	50's	−0.229753145*	0.0174833751	0.000	−0.279576482	−0.179929809
	Over 60's	1.12266891*	0.0152247767	0.000	1.079281900	1.166055925
30's	10's	0.832669919*	0.0277558174	0.000	0.7535611539	0.9117786837
	20's	0.314432902*	0.0131144537	0.000	0.2770602722	0.3518055326
	40's	−0.238175780*	0.0133955682	0.000	−0.276349449	−0.200002112
	50's	0.084679757*	0.0172273902	0.000	0.0355858975	0.1337736169
	Over 60's	1.43710182*	0.0149301170	0.000	1.394554496	1.479649135
40's	10's	1.07084570*	0.0280485861	0.000	0.9909030245	1.150788374
	20's	0.552608683*	0.0137232167	0.000	0.5135012615	0.5917161041
	30's	0.238175780*	0.0133955682	0.000	0.2000021120	0.2763494489
	50's	0.322855538*	0.0176952184	0.000	0.2724285569	0.3732825184
	Over 60's	1.67527760*	0.0154675847	0.000	1.631198717	1.719356475
50's	10's	0.747990162*	0.0300676722	0.000	0.6622958908	0.8336844324
	20's	0.229753145*	0.0174833751	0.000	0.1799298088	0.2795764816
	30's	−,084679757*	0.0172273902	0.000	−0.133773617	−0.035585897
	40's	−0.322855538*	0.0176952184	0.000	−0.373282518	−0.272428557
	Over 60's	1.35242206*	0.0188835352	0.000	1.298608520	1.406235596
Over 60's	10's	−0.604431896*	0.0288130236	0.000	−00.686552125	−0.522311668
	20's	−1.12266891*	0.0152247767	0.000	−1.16605593	−1.07928190
	30's	−1.43710182*	0.0149301170	0.000	−1.47964913	−1.39455450
	40's	−1.67527760*	0.0154675847	0.000	−1.71935647	−1.63119872
	50's	−1.35242206*	0.0188835352	0.000	−1.40623560	−1.29860852

*The mean difference is significant at the 0.05 level.

LF/HF ratio to peak in the 40s and trend downward thereafter.

In previous studies by Zhang *et al.* [24], there was a tendency for this LF/HF decrease from a peak in the subject's 50's, which is about 10 years different from this study, but the tendency for stress to decrease with is consistent.

In addition, looking at the Ministry of Health, Labor and Welfare statistics shown in **Figure 7** [1], there is a tendency for the proportion of anxiety and stress in everyday life to decrease from a peak in the subject's 40's, and it is consistent with the LF/HF changes with age of this study.

Also, in the Ministry of Health, Labor and Welfare statistics shown in **Figure 8** [1], there are two anxieties that increase in those in their 50's and above—"my illness and nursing care" and "family illness and nursing care"—and the trend is for other anxieties to decrease. Thus, the number of anxieties for people in their 50's and above is reduced compared with the younger generation, they are mentally mature, and can be considered possible that by acquiring methods to cope with stress they have a higher stress tolerance.

4. The Relationship between Autonomic Nervous Function and Circadian Rhythm/Sleep

In this section, we analyzed the relationship between the autonomic nerve indicator LF/HF ratio and diurnal variations in order to assess the relationship between autonomic nerve function and diurnal variations. The analysis divided one day into eight three-hour sections. **Figure 9**, **Table 6**, and **Table 7** show the aggregate results for subjects under the age of 50. **Figure 10**, **Table 8**, and **Table 9**

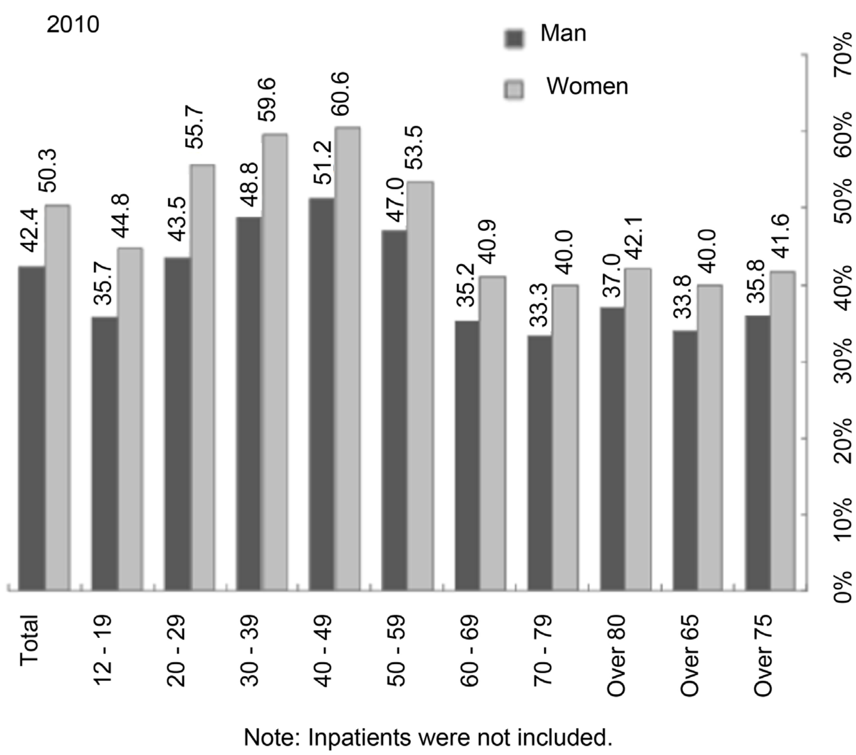

Figure 7. Presence of worries or stress by age and sex (age 12 and over).

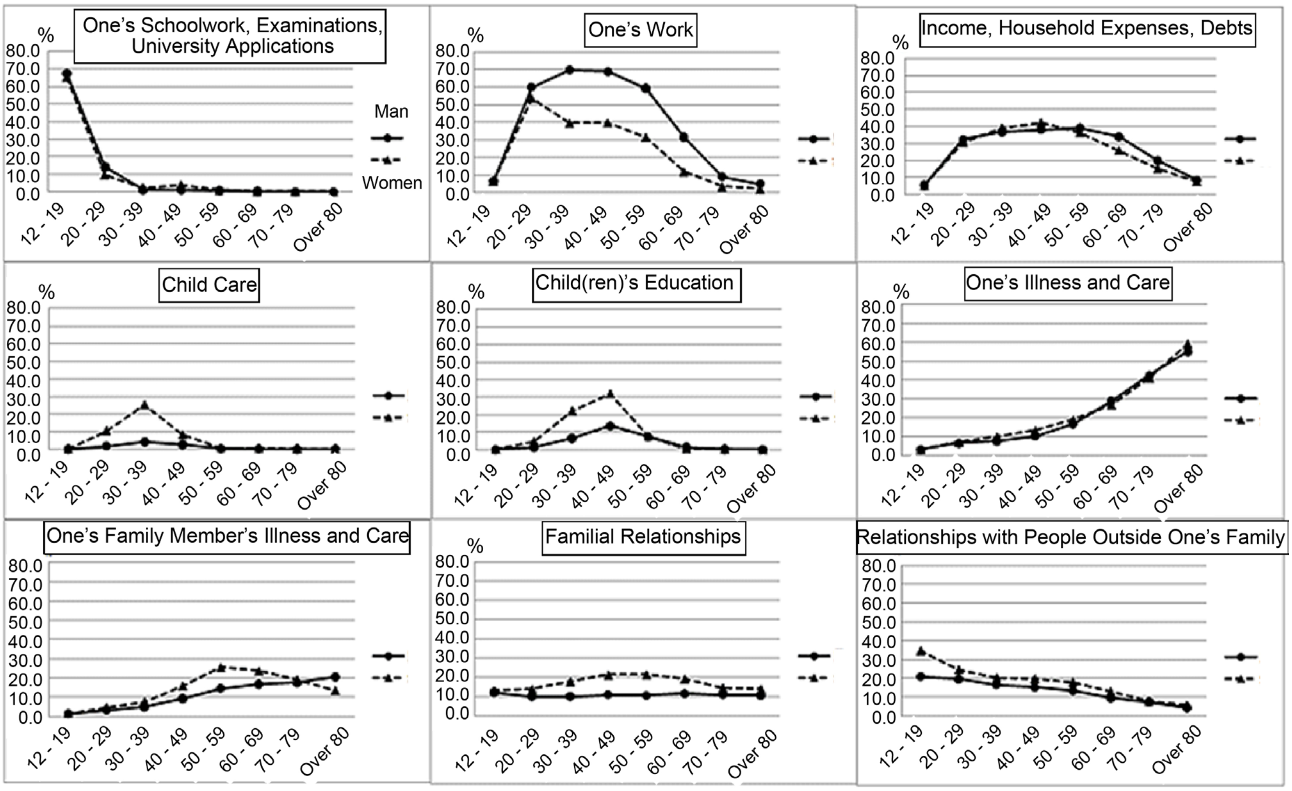

Figure 8. Primary cause of worries or stress by age and sex (age 12 and over).

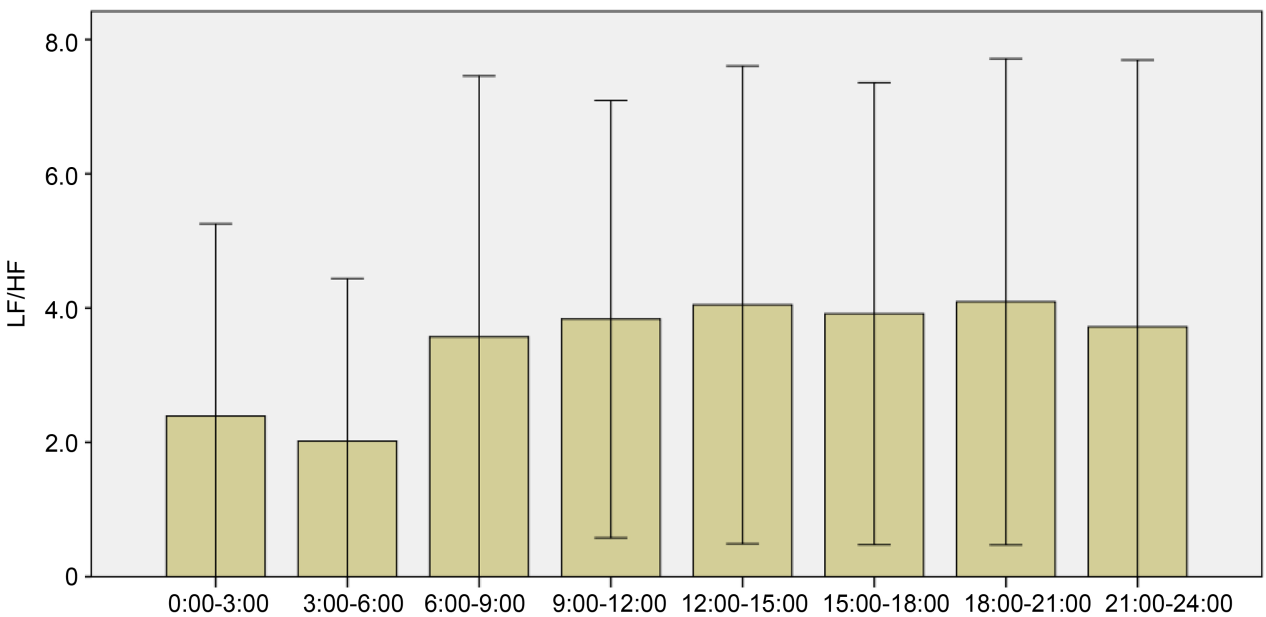

Figure 9. Relationship between LF/HF and circadian rhythm in subjects under 50 years of age.

show the aggregate results for subjects aged 50-plus.

Comparing the diurnal variations of the LF/HF ratio for subjects below 50 against subjects aged 50-plus reveals striking differences in the elevation rates of the LF/HF ratio from between 3:00 and 6:00 in the morning to between 6:00 and 9:00 in the morning.

Table 6. Relationship between LF/HF and circadian rhythm in subjects under 50 years of age (descriptive statistics).

Descriptives LFHF								
	N	Mean	Std. deviation	Std. error	95% Confidence interval for mean		Minimum	Maximum
					Lower bound	upper bound		
0:00-3:00	67,836	2.393469930	2.863404234	0.0109939194	2.371921866	2.415017993	0.0207066132	50.54979966
3:00-6:00	64,337	2.020522403	2.419858802	0.0095402471	2.001823516	2.039221289	0.0310185554	71.04799683
6:00-9:00	59,273	3.573768236	3.883893100	0.0159528695	3.542500558	3.605035915	0.0536916576	69.86762657
9:00-12:00	75,585	3.835712749	3.256889566	0.0118463681	3.812493930	3.858931568	0.0150149330	56.80034183
12:00-15:00	74,545	4.048507535	3.558050522	0.0130317533	4.022965361	4.074049709	0.0806316874	60.71608144
15:00-18:00	75,799	3.916584321	3.440740240	0.0124974138	3.892089457	3.941079185	0.0541924435	60.96557143
18:00-21:00	68,882	4.094753570	3.621127728	0.0137972003	4.067711088	4.121796053	0.0466757865	58.15724576
21:00-24:00	63,486	3.720298319	3.975522798	0.0157781204	3.689373191	3.751223446	0.0382549455	124.3444459
Total	549,743	3.476204212	3.483718151	0.0046985425	3.466995221	3.485413204	0.0150149330	124.3444459

Looking at the standard deviation of the average value of LF/HF in each time period, it is 0.739 for those under 50 years old, but it is 0.419 for those 50 years old and above, and the trend is for the fluctuation of autonomic nervous function to decrease in the elderly.

Sympathetic nerve activity should become vigorous in the morning in preparation for activity during the day because of human circadian rhythms; however, this switch is faint for subjects aged 50 and up. In other words, this may indicate the possibility of a decline in responsiveness of the autonomic nerves and a drop off in adaptability to the external environment.

There are several previous studies on the decline in responsiveness of the autonomic nervous system with aging, and when healthy people of different generations were examined using the orthostatic hypotension test, the deep breath test, and the perspiration reaction, the average reaction amount statistically significantly decreased as the age group increased, and the response of the autonomic nerves to changes in the environmental or the body tended to be slower [25]. Shimazu *et al.* also report increased sympathetic function and decreased responsiveness of the autonomic nervous system associated with aging [26].

Thus, the tendency of LF/HF in this study appears the same as the trend for autonomic nerves to change with age seen in previous studies, and a tendency was seen that suggests a decrease in responsiveness with aging.

Next, we analyzed the relationship between the autonomic nerve indicator LF/HF ratio while sleeping and waking.

Figure 11, **Table 10**, and **Table 11** show a comparison of the LF/HF ratio while sleeping and waking for subjects under the age of 50.

Figure 12, **Table 12** and **Table 13** show a comparison of the LF/HF ratio while sleeping and waking for subjects aged 50-plus.

Comparing the variations while sleeping and waking of the LF/HF ratio for subjects under 50 and subjects aged 50 and up, although both groups exhibit significant differences while sleeping and while waking, the variations between

Table 7. Relationship between LF/HF and circadian rhythm in subjects under 50 years of age (multiple comparisons).

Multiple comparisons
Dependent variable: LFHF
Games-Howell

(I) Time flag	(J) Time flag	Mean Difference (I-J)	Std. error	Sig.	95% Confidence interval Lower bound	Upper bound
0:00-3:00	3:00-6:00	0.372947527*	0.0145562	0	0.3288288	0.4170663
	6:00-9:00	−1.18029831*	0.0193742	0	−1.2390204	−1.1215763
	9:00-12:00	−1.44224282*	0.0161618	0	−1.4912279	−1.3932577
	12:00-15:00	−1.65503761*	0.0170497	0	−1.706714	−1.6033612
	15:00-18:00	−1.52311439*	0.0166449	0	−1.5735637	−1.4726651
	18:00-21:00	−1.70128364*	0.0176417	0	−1.7547543	−1.647813
	21:00-24:00	−1.32682839*	0.0192306	0	−1.385115	−1.2685418
3:00-6:00	0:00-3:00	−0.372947527*	0.0145562	0	−0.4170663	−0.3288288
	6:00-9:00	−1.55324583*	0.0185879	0	−1.6095848	−1.4969069
	9:00-12:00	−1.81519035*	0.0152103	0	−1.8612916	−1.7690891
	12:00-15:00	−2.02798513*	0.0161506	0	−2.0769365	−1.9790337
	15:00-18:00	−1.89606192*	0.0157226	0	−1.9437161	−1.8484077
	18:00-21:00	−2.0742311	0.0167744	0	−2.1250731	−2.0233892
	21:00-24:00	−1.69977592*	0.0184382	0	−1.7556609	−1.643891
6:00-9:00	0:00-3:00	1.18029831*	0.0193742	0	1.1215762	1.2390204
	3:00-6:00	1.55324583*	0.0185879	0	1.4969069	1.6095848
	9:00-12:00	−0.261944513*	0.0198703	0	−0.3221702	−0.2017188
	12:00-15:00	−0.474739299*	0.020599	0	−0.5371736	−0.412305
	15:00-18:00	−0.342816085*	0.0202652	0	−0.4042386	−0.2813936
	18:00-21:00	−0.520985334*	0.0210916	0	−0.5849126	−0.4570581
	21:00-24:00	−0.146530082*	0.0224375	0	−0.2145367	−0.0785234
9:00-12:00	0:00-3:00	1.44224282*	0.0161618	0	1.3932577	1.4912279
	3:00-6:00	1.81519035*	0.0152103	0	1.7690891	1.8612916
	6:00-9:00	0.261944513*	0.0198703	0	0.2017188	0.3221702
	12:00-15:00	−0.212794786*	0.0176114	0	−0.2661737	−0.1594159
	15:00-18:00	−0.080871572*	0.0172198	0	−0.1330635	−0.0286797
	18:00-21:00	−0.259040821*	0.0181851	0	−0.3141586	−0.203923
	21:00-24:00	0.115414430’	0.0197303	0	0.0556132	0.1752157
12:00-15:00	0:00-3:00	1.65503761*	0.0170497	0	1.6033612	1.706714
	3:00-6:00	2.02798513*	0.0161506	0	1.9790337	2.0769365
	6:00-9:00	0.474739299*	0.020599	0	0.412305	0.5371736
	9:00-12:00	0.212794786’	0.0176114	0	0.1594159	0.2661737
	15:00-18:00	0.131923214*	0.0180558	0	0.0771975	0.1866489
	18:00-21:00	−0.046246	0.0189787	0.224	−0.1037689	0.0112768
	21:00-24:00	0.328209216*	0.020464	0	0.2661843	0.3902342
15:00-18:00	0:00-3:00	1.52311439*	0.0166449	0	1.4726651	1.5735637

Continued

	3:00-6:00	1.89606192*	0.0157226	0	1.8484077	1.9437161
	6:00-9:00	0.342816085*	0.0202652	0	0.2813936	0.4042386
	9:00-12:00	0.080871572*	0.0172198	0	0.0286797	0.1330635
	12:00-15:00	−0.131923214*	0.0180558	0	−0.1866489	−0.0771975
	18:00-21:00	−0.178169249*	0.0186158	0	−0.2345923	−0.1217462
	21:00-24:00	0.196286002*	0.020128	0	0.1352796	0.2572924
18:00-21:00	0:00-3:00	1.70128364*	0.0176417	0	1.647813	1.7547543
	3:00-6:00	2.07423117*	0.0167744	0	2.0233892	2.1250731
	6:00-9:00	0.520985334*	0.0210916	0	0.4570581	0.5849126
	9:00-12:00	0.259040821*	0.0181851	0	0.203923	0.3141586
	12:00-15:00	0.046246	0.0189787	0.224	−0.0112768	0.1037689
	15:00-18:00	0.178169249*	0.0186158	0	0.1217462	0.2345923
	21:00-24:00	0.374455252*	0.0209598	0	0.3109277	0.4379828
21:00-24:00	0:00-3:00	1.32682839*	0.0192306	0	1.2685417	1.385115
	3:00-6:00	1.69977592*	0.0184382	0	1.643891	1.7556608
	6:00-9:00	0.146530082*	0.0224375	0	0.0785234	0.2145367
	9:00-12:00	−0.115414430*	0.0197303	0	−0.1752157	−0.0556132
	12:00-15:00	−0.328209216*	0.020464	0	−0.3902342	−0.2661843
	15:00-18:00	−0.196286002*	0.020128	0	−0.2572924	−0.1352796
	18:00-21:00	−0.374455252*	0.0209598	0	−0.4379828	−0.3109277

*The mean difference is significant at the 0.05 level.

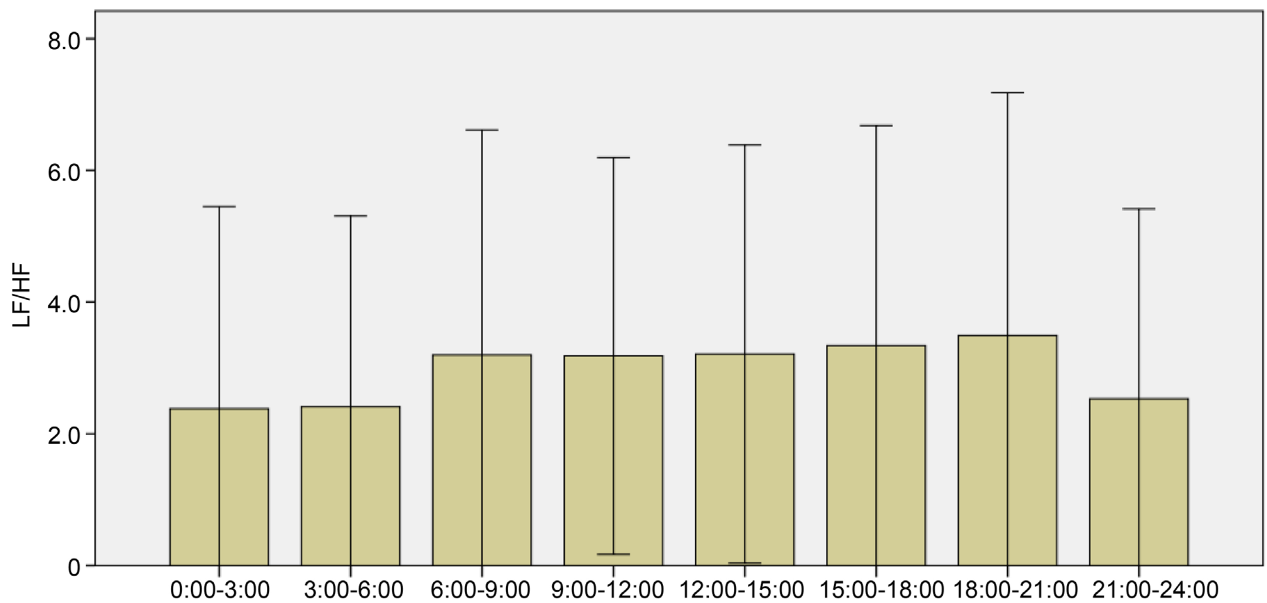

Figure 10. Relationship between LF/HF and circadian rhythm in subjects over 50 years of age.

waking and sleeping decline markedly for subjects aged 50-plus relative to subjects under 50. This supports the trends of previous research indicating growth in sleep time occupied by wake after sleep onset and declining levels of slow-

Table 8. Relationship between LF/HF and circadian rhythm in subjects over 50 years of age (descriptive statistics).

Descriptives LFHF								
	N	Mean	Std. deviation	Std. error	95% Confidence interval for mean		Minimum	Maximum
					Lower bound	Upper bound		
0:00-3:00	17,108	2.380588839	3.069294877	0.0234659961	2.334593077	2.426584600	0.0052378176	51.12275387
3:00-6:00	17,066	2.411597093	2.897219037	0.0221776466	2.368126621	2.455067564	0.0085205484	40.38270518
6:00-9:00	15,535	3.197607645	3.417184907	0.0274165530	3.143868002	3.251347289	0.0078901472	49.81408403
9:00-12:00	18,385	3.182214489	3.011838897	0.0222126268	3.138675674	3.225753304	0.0316005655	31.97811159
12:00-15:00	18,928	3.211938122	3.175350653	0.0230801872	3.166698893	3.257177351	0.0651674696	64.45880871
15:00-18:00	18,440	3.339790544	3.341343045	0.0246059766	3.291560550	3.388020538	0.0520169243	44.92429550
18:00-21:00	17,843	3.492774616	3.688529619	0.0276133653	3.438649742	3.546899489	0.0141719790	44.61341598
21:00-24:00	16,892	2.531507033	2.883233297	0.0221839689	2.488024137	2.574989929	0.0100680885	43.78371076
Total	140,197	2.978154844	3.223821687	0.0086099703	2.961279472	2.995030216	0.0052378176	64.45880871

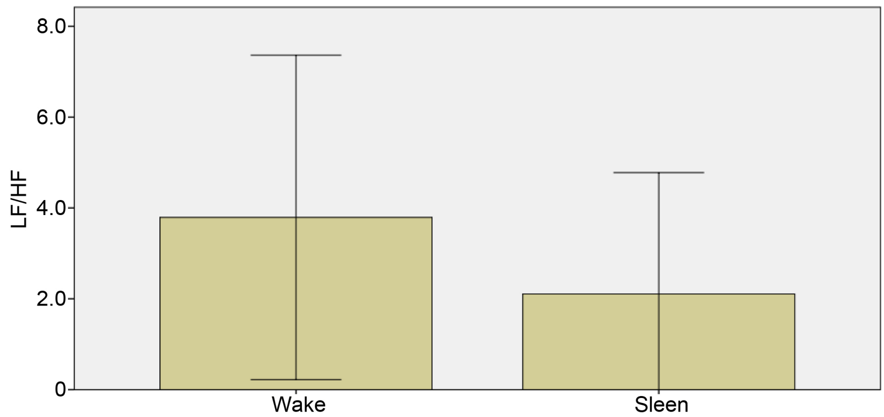

Figure 11. Comparison of LF /HF during sleep and waking in subjects under 50 years of age.

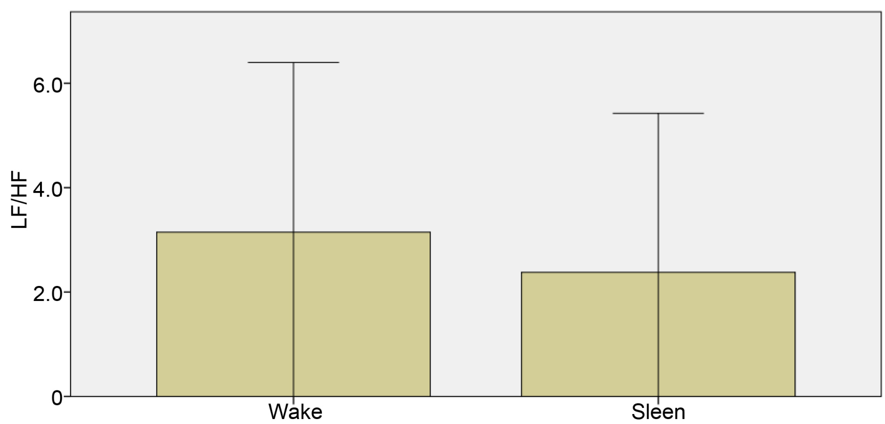

Figure 12. Comparison of LF/HF during sleep and waking in subjects over 50 years of age.

wave sleep in middle- to old-age [27] (**Figure 13**).

Also, just as changes in autonomic nervous system and endocrine system rhythms, such as reduced secretion of melatonin and cortisol, are reported to be

Table 9. Relationship between LF/HF and circadian rhythm in subjects over 50 years of age (multiple comparisons).

Multiple comparisons
Dependent variable: LFHF Games-Howell

(1) Time flag	(j) Time flag	Mean difference (I-J)	Std. error	Sig.	95% Confidence interval	
					Lower bound	Upper bound
0:00-3:00	3:00-6:00	−0.031008254	0.0322877838	0.980	−0.128874716	0.0668582088
	6:00-9:00	−0.817018806'	0.0360876758	0.000	−0.926403592	−0.707634021
	9:00-12:00	−0.801625650'	0.0323118208	0.000	−0.899564774	−0.703686527
	12:00-15:00	−0.831349283'	0.0329142525	0.000	−0.931114302	−0.731584265
	15:00-18:00	−0.959201705*	0.0340015743	0.000	−1.06226252	−0.856140894
	18:00-21:00	−1.11218578’	0.0362374242	0.000	−1.22202383	−1.00234773
	21:00-24:00	−0.150918195*	0.0322921267	0.000	−0.248797849	−0.053038540
3:00-6:00	0:00-3:00	0.0310082538	0.0322877838	0.980	−0.066858209	0.1288747165
	6:00-9:00	−0.786010553'	0.0352635135	0.000	−0.892897413	−0.679123692
	9:00-12:00	−0.770617396’	0.0313886731	0.000	−0.865758369	−0.675476423
	12:00-15:00	−0.800341030'	0.0320084841	0.000	−0.897360593	−0.703321466
	15:00-18:00	−0.928193451*	0.0331255505	0.000	−1.02859901	−0.827787889
	18:00-21:00	−1.08117752*	0.0354167468	0.000	−1.18852817	−0.973826877
	21:00-24:00	−0.119909941'	0.0313683995	0.003	−0.214989707	−0.024830174
6:00-9:00	0:00-3:00	0.817018806*	0.0360876758	0.000	0.7076340211	0.9264035917
	3:00-6:00	0.786010553'	0.0352635135	0.000	0.6791236922	0.8928974129
	9:00-12:00	0.0153931562	0.0352855235	10.000	−0.091560263	0.1223465749
	12:00-15:00	−0.014330477	0.0358380024	10.000	−0.122958306	0.0942973519
	15:00-18:00	−0.142182899'	0.0368391295	0.003	−0.253845082	−0.030520716
	18:00-21:00	−0.295166970'	0.0389122772	0.000	−0.413112904	−0.177221037
	21:00-24:00	0.666100612*	0.0352674900	0.000	0.5592016777	0.7729995458
9:00-12:00	0:00-3:00	0.801625650"	0.0323118208	0.000	0.7036865267	0.8995647738
	3:00-6:00	0.770617396’	0.0313886731	0.000	0.6754764234	0.8657583694
	6:00-9:00	−0.015393156	0.0352855235	10.000	−0.122346575	0.0915602626
	12:00-15:00	−0.029723633	0.0320327307	0.983	−0.126816488	0.0673692220
	15:00-18:00	−0.157576055'	0.0331489800	0.000	−0.258052451	−0.057099659
	18:00-21:00	−0.310560127*	0.0354386615	0.000	−0.417977045	−0.203143208
	21:00-24:00	0.650707456'	0.0313931405	0.000	0.5555529112	0.7458619999
12:00-15:00	0:00-3:00	0.831349283"	0.0329142525	0.000	0.7315842652	0.9311143016
	3:00-6:00	0.800341030*	0.0320084841	0.000	0.7033214665	0.8973605926
	6:00-9:00	0.0143304770	0.0358380024	10.000	−0.094297352	0.1229583059
	9:00-12:00	0.0297236331	0.0320327307	0.983	−0.067369222	0.1268164883
	15:00-18:00	−0.127852422’	0.0337364659	0.004	−0.230109409	−0.025595435
	18:00-21:00	−0.280836493*	0.0359887897	0.000	−0.389920726	−0.171752261
	21:00-24:00	0.680431089*	0.0320128649	0.000	0.5833982183	0.7774639592
15:00-18:00	0:00-3:00	0.959201705*	0.0340015743	0.000	0.8561408944	1.062262516
	3:00-6:00	0.928193451'	0.0331255505	0.000	0.8277878894	1.028599013
	6:00-9:00	0.142182899*	0.0368391295	0.003	0.0305207160	0.2538450818

Continued

	9:00-12:00	0.157576055'	0.0331489800	0.000	0.0570996592	0.2580524509
	12:00-15:00	0.127852422’	0.0337364659	0.004	0.0255954350	0.2301094088
	18:00-21:00	−0.152984071'	0.0369858355	0.001	−0.265090334	−0.040877809
	21:00-24:00	0.808283511*	0.0331297836	0.000	0.7078650920	0.9087019292
18:00-21:00	0:00-3:00	1.11218578’	0.0362374242	0.000	1.002347727	1.222023826
	3:00-6:00	1.08117752"	0.0354167468	0.000	0.9738268772	1.188528169
	6:00-9:00	0.295166970*	0.0389122772	0.000	0.1772210370	0.4131129037
	9:00-12:00	0.310560127'	0.0354386615	0.000	0.2031432078	0.4179770453
	12:00-15:00	0.280836493*	0.0359887897	0.000	0.1717522608	0.3899207260
	15:00-18:00	0.152984071'	0.0369858355	0.001	0.0408778094	0.2650903336
	21:00-24:00	0.961267582"	0.0354207061	0.000	0.8539049150	1.068630249
21:00-24:00	0:00-3:00	0.150918195*	0.0322921267	0.000	0.0530385404	0.2487978489
	3:00-6:00	0.119909941'	0.0313683995	0.003	0.0248301745	0.2149897072
	6:00-9:00	−0.666100612*	0.0352674900	0.000	−0.772999546	−0.559201678
	9:00-12:00	−0.650707456'	0.0313931405	0.000	−0.745862000	−0.555552911
	12:00-15:00	−0.680431089'	0.0320128649	0.000	−0.777463959	−0.583398218
	15:00-18:00	−0.808283511'	0.0331297836	0.000	−0.908701929	−0.707865092
	18:00-21:00	−0.961267582"	0.0354207061	0.000	−1.06863025	−0.853904915

*The mean difference is significant at the 0.05 level.

Table 10. Comparison of LF/HF during sleep and waking in subjects under 50 years of age (group statistics).

Group statistics					
	Sleep flag	N	Mean	Std. deviation	Std. error mean
LFHF	Wake	446,392	3.793270748	3.572033285	0.0053463489
	Sleep	103,351	2.106735460	2.671197896	0.0083089993

Table 11. Comparison of LF/HF during sleep and waking in subjects under 50 years of age (verified by independent sample).

		Levene’s test for equality of variances		t-test for equality of means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean difference	Std. error difference	95% Confidence interval of the difference	
									Lower	Upper
LFHF	Equal variances assured	8745.187	0.000	142.823	549,741	0.000	1.686535286	0.0118085402	1.663390929	1.709679644
	Equal variances not assured			170.695	198,754.628	0.000	1.686535286	0.0098804309	1.667169886	1.705900687

Table 12. Comparison of LF/HF during sleep and waking in subjects over 50 years of age (group statistics).

Group statistics					
	Sleep flag	N	Mean	Std. deviation	Std. error mean
LFHF	Wake	109,651	3.145304198	3.252340387	0.0098217685
	Sleep	30,546	2.378138678	3.044673392	0.0174206172

Table 13. Comparison of LF/HF during sleep and waking in subjects over 50 years of age (verified by independent sample).

		Levene's test for equality of variances		t-test for equality of means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean difference	Std. error difference	95% Confidence interval of the difference: Lower	Upper
LFHF	Equal variances assured	380.367	0.000	36.960	140,195	0.000	0.7671655196	0.020756365	0.7264833132	0.8078477260
	Equal variances not assured			38.361	51,597.812	0.000	0.7671655196	0.0199986260	0.7279680259	0.8063630133

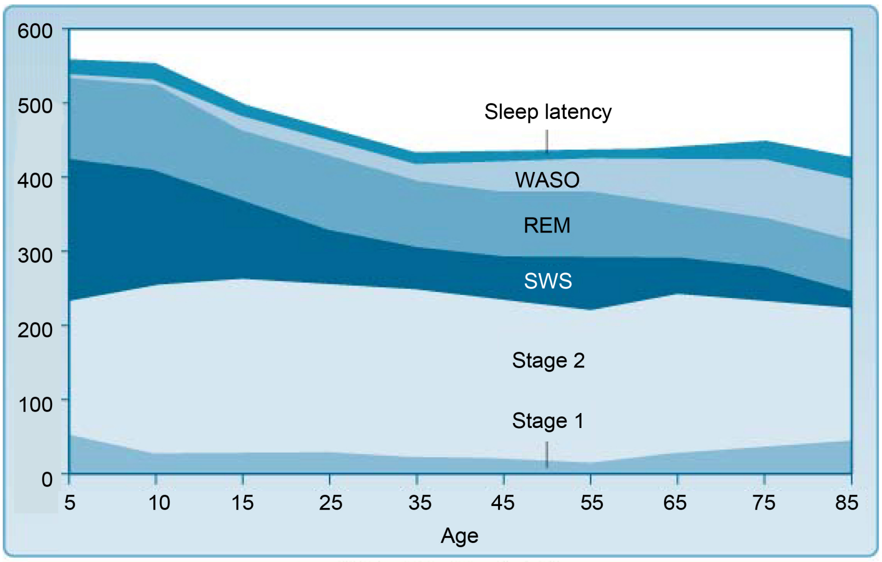

Figure 13. Relationship between age and sleep.

age-related [28], in this study also there was a trend that suggests a decrease with age in autonomic nervous function while sleeping as one of the causes.

5. Conclusions

Long-term monitoring of autonomic nerve function of 600 subjects across all age groups as they went about their daily lives revealed the following:

- The LF/HF ratio, an indicator of autonomic nerve balance, demonstrated a tendency to peak in the 40's and decline thereafter, conceivably due partially to causes of concern and stress changing and declining for individuals aged 50-plus.
- Diurnal variation of autonomic nerve activity tended to decline for subjects aged 50-plus, exhibiting a tendency for a drop in rising sympathetic nerve activity function particularly in the morning, which may conceivably stem from declining responsiveness of the autonomic nervous system.
- Differences in autonomic nerve activity between sleeping and waking hours exhibited a tendency to decline in subjects aged 50-plus, which is consistent

with the tendency for rising levels of wake after sleep onset and declining levels of slow-wave sleep in middle- to old-age.

Moving forward, we hope to examine what kind of differences emerge in these trends as well as see how the conclusions reached herein change as the result of adding correlational analysis with other biological indicators such as saliva and brain waves.

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