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Can the early bird catch the worm? Effects of early rising on leukocyte subsets via modification of autonomic nervous system and the effect on glucose levels

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ABSTRACT

The importance of sleep has been described in proverbs such as "the early bird catches the worm". However, there are few scientific reports on the effects of early rising. Therefore, early risers (Group E) and late risers (Group L) were compared and the number and ratio of leukocytes, body temperature, glucose and its associated hormones were studied. Furthermore, each group was divided into two groups by the duration of sleep and the following four groups were compared: early risers with short sleep (Group E-S); early risers with long sleep (Group E-L); late risers with short sleep (Group L-S); and late risers with long sleep (Group L-L). Then, compared with Group L, Group E showed lower numbers and ratio of granulocytes and a higher ratio of lymphocytes showing parasympathetic nerve dominance. Group E showed higher levels of glucose and its related hormones than Group L, indicating sympathetic nerve dominance. Compared with Groups E-S and L-S, Groups E-L and L-L showed lower glucose and cortisol levels, respectively. These results indicated that early rising might affect leukocyte subsets, and adequate duration of sleep could decrease levels of glucose via modification of the autonomic nervous system.

Keywords: Early Rising; Leukocyte; Autonomic Nervous System (ANS)

1. INTRODUCTION

Sleep is an indispensable action and its importance has been related in many proverbs such as "the early bird catches the worm" or the Japanese saying "sleep brings up a child well". However, there are few scientific reports that have investigated the influence of sleep on the number and the ratio of leukocytes and the autonomic nervous system (ANS). Adachi *et al.* reported that the ratio of lymphocytes in persons who went to bed early was higher than that of subjects who sleep late [1]; however, the effects of early rising were not reported.

Therefore, in this study 23 subjects were divided into two groups, early risers (Group E) and late risers (Group L), and the number and ratio of leukocytes, body temperature, glucose, and glucose-associated hormones [cortisol (CR), adrenocorticotropic hormone (ACTH), noradrenaline (NA) and growth hormone (GH)] were investigated. Furthermore, each group was divided into two groups to create the following four groups: early risers with short sleep (Group E-S); early risers with long sleep (Group E-L); late risers with short sleep (Group L-S); and late risers with long sleep (Group L-L). Through the comparison of these groups, the efficiency of early rising was revealed.

2. MATERIALS AND METHODS

2.1. Subjects

Twenty-three healthy volunteers (ages 20 to 59; avera-

ge 38.1 ± 13.5 years old) participated in this study. Written informed consent was obtained from all subjects and the study was approved by the institutional review board of Niigata University.

2.2. Early Risers (Group E) and Late Risers (Group L)

Subjects were divided into 2 groups by the time of their rising. Group E, the group of early risers, had 12 members who regularly rose before 8:00 am (including 8:00 am). Group L, the group of late risers, had 11 subjects who usually rose after 8:00 am (excluding 8:00 am). Group E was divided into the following two groups at the border of 6.20 hours: early risers with short sleep group (Group E-S); and early risers with long sleep group (Group E-L). In the same way, Group L was divided into the late risers with short sleep group (Group L-S) and the late risers with long sleep group (Group L-L). Details of the subjects are shown in **Table 1**.

2.3. Venous Blood Samples and Analysis

As mentioned in a previous study [1], fresh venous blood (25 mL) for analysis was obtained from the forearm median antebrachial vein. Body temperature was measured under the arm (axillary). Considering the effects of circadian rhythm and nutrition intake, these data were obtained between 16:30 and 17:30, more than 4 hours after eating. Leukocyte subsets were determined using a hemocytometer and the May-Grünwald Giemsa stain method. Blood glucose levels were measured by Precision Xtra TM (Abott Japan Co., Ltd., Chiba, Japan). Blood samples were sent to Mitsubishi Chemical Medience Corporation (Tokyo, Japan) for the analysis of CR, ACTH, GH and NA. The levels of CR, NA and ACTH and of GH were analyzed by radioimmunoassay (coated tube solid phase method), high performance liquid chromatography and immunoradiometric assay (beads solid phase method), respectively.

2.4. Statistical Analysis

All analyses were performed using the Statistical Package for the Social Sciences (SPSS) Version 20. The difference between values was determined by Student's *t*-test, Mann-Whitney's U test, Welch's *t*-test between two groups comparison (Group E vs. Group L) and Kruskal-Wallis test among four groups comparison (Group E-S, Group E-L, Group L-S and Group L-L). Figures are all shown as the mean ± one S.D. *P*-values < 0.05 were considered to indicate statistical significance, and all statistical tests were two-tailed.

3. RESULTS

3.1. Age, Glucose and Body Temperature

To study the effects of early rising, the average ages, glucose levels and body temperatures of subjects were compared between two groups. The average age of Group E (45.1 ± 12.0) was significantly older than that of Group L (30.5 ± 10.9) ($P < 0.05$), and the level of glucose of the former (95.1 ± 7.9 mg/dL) was remarkably higher than that of the latter (86.7 ± 10.4 mg/dL) ($P < 0.05$). The average of both groups stayed within the normal range. Subjects with hypothermia (<36.0˚C) were found only in Group L; however, there was no significant difference between the two groups in terms of body temperature (**Figure 1**).

3.2. The Number and the Ratio of Leukocytes

The number of granulocytes of Group E subjects was statistically lower than that of Group L subjects (3.3 ± 0.9 vs. 4.2 ± 1.3 × 10^3/μL) ($P < 0.05$). The number of lymphocytes of subjects in Group E tended to be higher than those in Group L; however, there was no significant difference (2.5 ± 0.6 vs. 2.1 ± 0.6 × 10^3/μL). The ratio of granulocytes of Group E was lower than that of Group L (54.0% ± 8.3% vs. 62.5% ± 7.7%) ($P < 0.05$), while the

Table 1. Details of subjects. Group E (early risers: rising up before 8:00 am, including 8:00 am), Group L (late risers, rising up after 8:00 am, excluding 8:00 am), Group E-S (early risers with short sleep), Group E-L (early risers with long sleep),Group L-S (late risers with short sleep), Group L-L (late risers with long sleep).

	n (m, f)	Age	Time to sleep	Time to rise up	Duration (H)
Group E	**12 (7, 5)**	**45.1 ± 12.0**	**23:50 ± 0:51**	**6:05 ± 1:05**	**6:15 ± 0:57**
Group ES	8 (5, 3)	45.1 ± 13.3	23:52 ± 1:01	5:33 ± 0:54	5:41 ± 0:27
Group EL	4 (2, 2)	45.0 ± 11.0	23:45 ± 0:30	7:07 ± 0:28	7:22 ± 0:37
Group L	**11 (7, 4)**	**30.5 ± 10.9**	**27:16 ± 1:07**	**9:35 ± 0:55**	**6:19 ± 0:58**
Group LS	5 (3, 2)	30.4 ± 12.2	27:54 ± 1:14	9:18 ± 0:39	5:24 ± 0:37
Group LL	6 (4, 2)	30.5 ± 10.9	26:45 ± 0:45	9:50 ± 0:45	7:05 ± 0:12

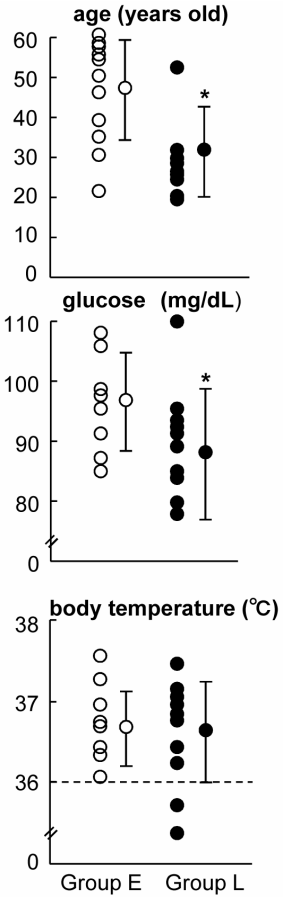

Figure 1. Age, glucose and body temperature. The average age of Group E was significantly older than that of Group L ($P < 0.05$), and the levels of glucose of the former were remarkably higher than those of the latter ($P < 0.05$). Subjects with hypothermia (<36.0˚C) were found only in Group L; however, there was no significant difference between the two groups.

ratio of lymphocytes was precisely the opposite (40.9% ± 8.5% vs. 31.4% ± 7.2%) ($P < 0.05$) (**Figure 2**).

3.3. CR, ACTH, NA and GH

There was a lot of individual variation among hormones, showing no significant differences between the two groups; however, subjects with high levels (over the normal range) of CR and GH were found only in Group E. The levels of ACTH in Group E subjects were higher than that of Group L, though without a significant difference (27.1 ± 19.0 vs. 21.4 ± 7.7 pg/mL). The levels of NA in both groups were roughly the same (**Figure 3**).

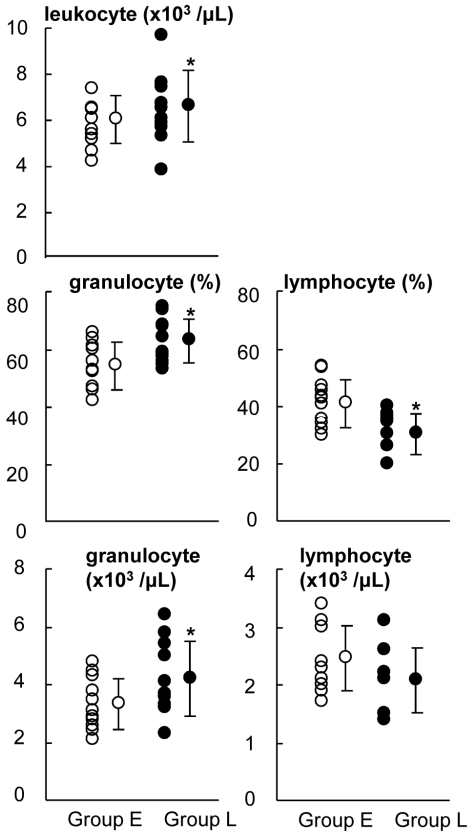

Figure 2. The number and ratio of leukocytes in venous blood. The number of leukocytes and granulocytes of Group E were statistically lower than that of Group L ($P < 0.05$). The number of lymphocytes in Group E tended to be higher; however, there were no significant differences. The ratio of granulocytes of Group E was lower than that of Group L ($P < 0.05$), while the ratio of lymphocytes was precisely the opposite ($P < 0.05$).

3.4. Preparation of Age Matched Groups

As described above, the average age of Group E was older than that of Group L. To avoid the possibility of the effects of aging [2,3] and the duration of sleep [4-6], each group was divided again and then the duration of sleep (6.20 hours) and age-matched four groups were prepared (**Table 1**).

3.5. Comparison among Four Groups-Glucose, CR and GH

The levels of glucose were as follows: Group ES (96.3 ± 6.6 mg/dL) > Group E-L (92.8 ± 10.8 mg/dL) > Group L-S (90.4 ± 11.1 mg/dL) > Group L-L (83.7 ± 9.7 mg/dL). Levels of CR in Group E-S (11.7 ± 5.2 μg/dL) were higher than that of Group E-L (9.4 ± 4.3 μg/dL). In the same way, levels of CR in Group L-S (12.6 ± 6.3 μg/dL) were higher than Group L-L (11.3 ± 3.7 μg/dL),

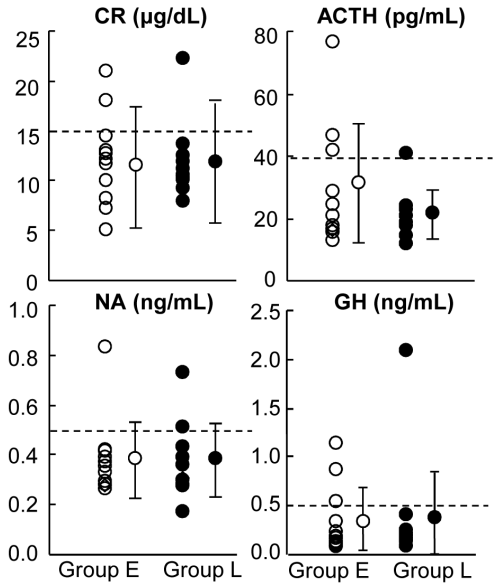

Figure 3. The plasma levels of various hormones. There was a lot of individual variation in the levels of hormones, with no significant differences. High levels of CR, ACTH, NA and GH were observed only in Group E. The dotted lines in the figures indicate normal levels.

without a significant difference. High levels of GH were shown only in the groups with longer sleep duration (1.21 and 2.04 ng/dL) (**Figure 4**).

4. DISCUSSION

4.1. Leucocyte Subsets and Body Temperature Indicated That Early Rising Induced PN Dominance (SN Suppression)

In this study, the effect of early rising was revealed, as well as that of going to bed early [1]. In fact, compared with Group L, Group E showed a lower number and ratio of granulocytes and a higher ratio of lymphocytes. The average age of Group E was older than that of Group L. It has been reported that aging inducing sympathetic nerve (SN) dominance, with a subsequent increase in granulocytes and decrease in lymphocytes [7-9]. The results of this study indicated Group E showed more parasympathetic nerve (PN) dominance (SN suppression) than Group L based on leukocyte subset levels. This hypothesis also was supported by body temperature because it has been reported that hypothermia can be induced by SN dominance [10,11].

4.2. Glucose Levels Were Higher in Early Risers than Late Risers

The levels of glucose of subjects in Group E were significantly higher than those in Group L. One reason

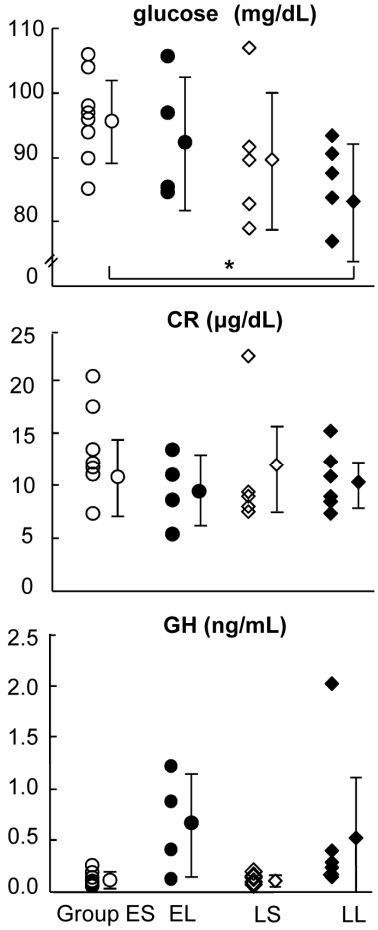

Figure 4. Comparison among four groups: glucose, CR and GH. The levels of glucose were as follows: Group E-S > Group E-L > Group L-S > Group L-L.

could be that early rising induces a mild SN dominance and it has been reported that SN dominance elevates the level of glucose [10,11]. This finding conflicts with the above mentioned hypothesis concerning subsets of leukocyte. The difference in the average age between the two groups must be considered (45.1 ± 12. 0 vs. 30.5 ± 10.8) because it is well known that elevated levels of glucose are directly affected by aging [2,3]. An association with the duration of sleep also was reported [4-6]. Therefore, it was required to evaluate the difference.

4.3. Sleep Lowered Glucose Levels via PN Dominance (SN Suppression)

The average age and sleep duration were matched between Groups E-S and E-L, and between Groups L-S and L-L (**Table 1**). This was because Group E-S showed higher levels of glucose and CR than Group E-L (**Figure 4**). In the same way, levels in Group L-S were higher than those of Group L-L. In short, it was understood that compared with short sleep (4.30 - 6.20 hours), long sleep

(6.20 - 8.00 hours) decreased the levels of glucose, irrespective of age and the time of rising. In the age-matched groups of this study the results agreed with former reports [4-6]. It was considered that sleep induced PN dominance (SN suppression) [12], which has been reported to decrease glucose levels [11]. However, further study in large-scale group is needed to investigate the possibility that early rising elevates the level of glucose via a mild SN dominance.

4.4. Early Rising with Adequate Sleep Duration Might Provide the Anti-Aging Effects via Modification of ANS Balance and Leukocyte Subsets.

It is well known the sleep curtailment has become a common behavior in modern society [13]. In modern society, there are many occupations that need to keep irregular hours with such efforts, such as doctors, nurses and healthcare workers [14,15]. The results of this study might provide a clue for their health promotion. Early rising sometimes requires efforts and strong will, and there is a possibility that it might induce a mild increase of glucose levels within the normal range. Therefore, maintaining an adequate sleep duration is recommended because it could suppress the possibility of SN dominance and its associated hormones such as CR, ACTH and NA, which were often induced by aging. In short, early rising with adequate sleep could be expected as anti-aging effects. At the same time, the possibility of an increase of GH, which is known to regulate carbohydrate metabolism and promote anabolism and lipolysis [16], must be studied in the future.

5. CONCLUSION

The early bird can catch the worm with the effects of anti-aging because in this study it revealed that early rising might affect leukocyte subsets and adequate duration of sleep and could decrease levels of glucose via modification of the ANS. To maintain adequate sleep duration, going to bed early was needed. In cases in which persons stayed up late, efforts and ingenuity to get additional longer sleep duration were necessary.

REFERENCES

[1] Adachi, K., Nishijo, K. and Abo, T. (2010) Those with the habit of going to sleep early show a higher ratio of lymphocytes while those with the habit of staying up late show a higher ratio of granulocytes. *Biomedical Research*, **31**, 143-149. http://dx.doi.org/10.2220/biomedres.31.143

[2] Barrett-Connor, E. and Ferrara, A. (1998) Isolated post-challenge hyperglycemia and the risk of fatal cardiovascular disease in older women and men. The Rancho Bernardo Study. *Diabetes Care*, **21**, 1236-1239. http://dx.doi.org/10.2337/diacare.21.8.1236

[3] Ren, J.P., Chen, D.N., Wang, S.H., Han, X.Q., Liang, R. and Wang, Z.P. (2012) Stratified analysis of blood lipids and glucose in annual check-ups for medical staff at a Beijing class A grade III hospital, and related intervention measures. *HEP*, **39**, 530-534. http://jglobal.jst.go.jp/public/20090422/201202272396374070

[4] Hancox, R.J. and Landhuis, C.E. (2012) Association between sleep duration and haemoglobin A1c in young adults. *Journal of Epidemiol Community Health*, **66**, 957-961. http://dx.doi.org/10.1136/jech-2011-200217

[5] Gottlieb, D.J., Punjabi, N.M., Newman, A.B., Resnick, H.E., Redline, S., Baldwin, C.M. and Nieto, F.J. (2005) Association of sleep time with diabetes mellitus and impaired glucose tolerance. *Archives of Internal Medicine*, **165**, 863-867. http://dx.doi.org/10.1001/archinte.165.8.863

[6] Najafian, J., Mohamadifard, N., Siadat, Z.D., Sadri, G. and Rahmati, M.R. (2013) Association between sleep duration and diabetes mellitus: Isfahan Healthy Heart Program. *Nigerian Journal of Clinical Practice*, **16**, 59-62. http://dx.doi.org/10.4103/1119-3077.106756

[7] Abo, T, Kawamura, T. and Watanabe, H. (2005) Immunologic status of autoimmune diseases. *Immunologic Research*, **33**, 23-34. http://dx.doi.org/10.1385/IR:33:1:023

[8] Abo, T. and Kawamura, T. (2002) Immunomodulation by the autonomic nervous system: Therapeutic approach for cancer, collagen diseases, and inflammatory bowel diseases. *Therapeutic Apheresis*, **6**, 348-357. http://dx.doi.org/10.1385/IR:33:1:023

[9] Miyaji, C., Watanabe, H., Minagawa, M., Toma, H., Kawamura, T., Nohara, Y., Nozaki, H., Sato, Y. and Abo, T. (1997) Numerical and functional characteristics of lymphocyte subsets in centenarians. *Journal of Clinical Immunology*, **17**, 420-429. http://dx.doi.org/10.1023/A:1027324626199

[10] Kainuma, E., Watanabe, M., Tomiyama-Miyaji, C., Inoue, M., Kuwano, Y., Ren, H. and Abo, T. (2009) Association of glucocorticoid with stress-induced modulation of body temperature, blood glucose and innate immunity. *Psychoneuroendocrinology*, **34**, 1459-1468. http://dx.doi.org/10.1016/j.psyneuen.2009.04.021

[11] Watanabe, M., Tomiyama-Miyaji, C., Kainuma, E., Inoue, M., Kuwano, Y., Ren, H., Shen, J. and Abo, T. (2008) Role of alpha-adrenergic stimulus in stress-induced modulation of body temperature, blood glucose and innate immunity. *Immunology Letters*, **115**, 43-49. http://dx.doi.org/10.1016/j.imlet.2007.09.010

[12] Suzuki, S., Toyabe, S., Moroda, T., Tada, T., Tsukahara, A., Iiai, T., Minagawa, M., Maruyama, S., Hatakeyama, K., Endoh, K. and Abo, T. (1997) Circadian rhythm of leucocytes and lymphocytes subsets and its possible correlation with the function of the autonomic nervous system. *Clinical & Experimental Immunology*, **110**, 500-508. http://dx.doi.org/10.1046/j.1365-2249.1997.4411460.x

[13] Morselli, L., Leproult, R., Balbo, M. and Spiegel, K.

(2010) Role of sleep duration in the regulation of glucose metabolism and appetite. *Best Practice & Research Clinical Endocrinology & Metabolism*, **24**, 687-702. http://dx.doi.org/10.1016/j.beem.2010.07.005

[14] Gangwisch, J.E., Feskanich, D., Malaspina, D., Shen, S. and Forman, J.P. (2013) Sleep duration and risk for hypertension in women: Results from the nurses' health study. *American Journal of Hypertension*, **26**, 903-911. http://dx.doi.org/10.1093/ajh/hpt044

[15] Ghalichi, L., Pournik, O., Ghaffari, M. and Vingard, E. (2013) Sleep quality among health care workers. *Archives of Iranian Medicine*, **16**, 100-103. http://www.ncbi.nlm.nih.gov/pubmed/23360632

[16] Amato, G., Carella, C., Fazio, S., Montagna, L.M., Cittadini, A., Sabatini, D., MarcianoMone, C., Sacca, L. and Bellastella, A. (1993) Body composition, bone metabolism, and heart structure and function in growth hormone (GH)-deficiend adults before and after GH replacement therapy at low doses. *JCE & M*, **77**, 1671-1976. http://www.cenegenicsfoundation.org/library/library_files/Body_composition__bone_metabolism__and_heart_structure_and_function_in_growth_hormone__GH___deficient_adults_before_and_after_GH_replacement_therapy_at_low_doses.pdf

