

# Quantitative regulation of peripheral leukocyte by light exercise and tailored scale for assessment\*

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## ABSTRACT

Two primary defense systems develop in vertebrates, innate and adaptive. However, every individual exposes to the risk of immunodeficiency in daily life with both internal and external stimuli. In this report, we tried to report two different stimuli for regulating the immune system modulate and discussed how we can compare and select each menu by evidence-based manner more than VAS. In a series of investigation, we have assessed the various leukocyte regulating menus such as hot spring hydrotherapy, acupuncture & moxibution, light walking, independent of the western medicine. In this report, we try to compare the regulatory effect of light exercise under the influence of hypothalamus system. Their effects of elements were evaluated by the total number of peripheral leukocyte, its subsets, granulocyte and lymphocyte ratio. The regulative effects were confirmed also by other menu such as hydrotherapy, acupuncture, walking etc. However, by simply comparing the group before with the group after CAM, we cannot catch the real regulatory effect by each menu. So we tried to show the effect by trying to indicate the constitution/condition dependent manner. The effect was not uniform to each individual but it was dependent on each condition/constitution before the start of the exercise. This was one of the main purposes of this report for summarizing that it is important to regulate

the number and levels of each factor as ideal value. In other words, the regulation of each factor affected under the hypothalamus system is regulated with each constitution, under the influence of daily circumstances. The mode of regulation was the same in each menu, indicating the higher level was down regular and lower level was up regular. Moreover, each vector of change was reversely correlated to the value of the day before. The other purpose of this study was designed to establish the regulatory effect of this light exercise which is not limited in the number of leukocyte in peripheral. Light exercise for a short duration was expected to influence the immune system in the blood quantitatively. Preparing two modes of impact for walking, 4 mets by walking exhibited more effective than that of 8 mets. We tried to discuss the suitable scale to know the best intensity to regulate whole body system other than VAS judgment.

**Keywords:** Light Exercise; Walking; Peripheral Leukocyte; Leukocyte Subsets Ratio; Metabolic Rate

## 1. INTRODUCTION

There are two primary systems, innate and adaptive. Despite this defense system the overwhelming problems of possessing this dual system, the innate and adoptive do not seem to guard or even prevent the development of one internal threat to survival. However, every indi-

\*Conflict of Interests: No conflict of interest exists in this work.

vidual in the world exposes to the risk of immunodeficiency in daily life with both internal and externals. The factors that influence the acquired immune activity are systemic metabolic disorder such as diabetes mellitus, malnutrition, extreme exhaustion, extensive stress, aging and medical side effect [1-12]. So we have to select appropriate menu to regulate immune function through leukocyte storage. The menu had been summarized and listed as CAM: complementary and alternative Medicine [13-18]. For example when the patients in diabetes, suggested to achieve some exercise without precise direction, how much speed and how long have to do. Because, none of the physicians has tailored scale for each patient. In other words, no tentative scale to evaluate the appropriate intense of exercise for each patient. The purpose of this study is to get best tailored scale by different walking exercise through leukocyte regulation in number. For this purpose, we tried to set up two different intense of menu for the same volunteers by both different intense of walking. The one was 4 km per hour and the other was 8 km per hour. Each value of impact was 4 mets and 8 mets. By the way, what is the mechanism of light walking that affects our immune system? It is known that the function of the immune system is closely related to the nervous and endocrine systems, and varies with physical conditions and changes in the surroundings [13-15]. It seems reasonable that light exercise should also influence the immune system, but so far no objective studies have been made. Finding the answer to this query became the focus of our investigation. In order to investigate the influence of light walking on the immune system, we set up the different exercise to the same volunteers at different time with two weeks of cooling in early spring, 5 - 27, May, 2010. Our results showed that within 24 hours after light exercise, the white blood cells in peripheral blood had changed significantly, not only in cell count but also in cell function. In Japan, centuries of tradition have shown that alternative therapies like hot spring hydrotherapy, acupuncture, and herbal medicine enhance the QOL of empirically healthy individuals. Evidence has been accumulating that this may be the result of immune system regulation. The scientific basis, however, has not yet been established.

In our investigation, we measured the number of leukocyte subsets, granulocyte and lymphocytes regulated before and after hot spring hydrotherapy. Moreover, every individual exposes to the risk of immunodeficiency in daily life with both internal and external stimuli. In this report, we tried to report the suitable menu for regulating the immune system modulate and discuss how we can compare and select each menu by evidence-based manner more than VAS [16-18]. The major purpose of this script is to concern with the following issues. One is to confirm the effect of hot spring hydrotherapy within a short time is crucial or not. In other words, the reputa-

tional results are possible or not. The regulation was constitution/condition dependent manner and regulate the ideal value to each individual from the condition before the menu. The second was that this regulatory bias was coincides with other factors influenced by pituitary nervous system, such as emotional hormone and the hormone receptor positive lymphocyte. The third one was that reputational effect can express by the linear function and could compare each other with the value of slope. We tried to discuss more, the possibility of the exhibition and comparison between each menu and established medicine in East and West.

The results showed that these subsets could reflect the number and function of immuno-competent cells. For example, in an individual with a low granulocyte number, the number increased after treatment, while it decreased in another individual with a higher cell number [19,20]. Our results led us to believe that leukocyte subsets could be an interesting indicator for the evaluation of alternative therapies. Many systems are in place to evaluate Western therapies that aim at healing the symptoms of an illness. However, when the purpose of a therapy is to enhance the QOL of healthy people, such as some alternative medical therapies, it is not widely-accepted. Evaluation system has been established. To fill this lack, we would like to propose the number and functions of leukocyte subsets as indicators for the evaluation of alternative therapies.

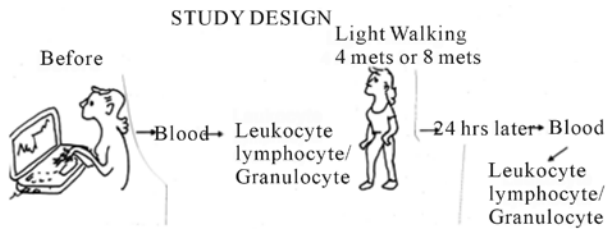
## 2. SUBJECTS AND METHODS

### 2.1. Subjects

We selected 14 healthy volunteers (mean age,  $41 \pm 15.2$  years, ranging 19 - 60 years old in both sexualities) and informed consented according to The Ethics Committee of Kanazawa Medical University. The contents of sexuality were 56.25% of the lady and 43.75% for gentleman. They were the students of Medical University and the staff for the school of medicine. None of them was a specialist for athletic field. The group was set up into two groups according to the intense of walking, 4 mets and 8 mets.

### 2.2. Walking Exercise by 4 Mets

These subjects participated in this study after giving their informed consent. We conducted the walking exercise at the country side of Japan. The course of exercise located at 40 meter in high and 1015 mb of atmospheric pressure. We sampled peripheral blood from the 14 volunteers before and after exercise, at the same time on each day, in consideration of circadian rhythm of leukocyte. The exercise start at ten o'clock for about one hour, corresponding 4 metabolic rates; mets [21-24]. Time interval of blood sampling between before and after exer-



**Figure 1.** Experimental design of this report: We sampled peripheral blood from the 16 volunteers before and after walking exercise, at the same time on each day, in accordance with the consideration of circadian rhythm of leukocyte. We sampled peripheral blood from the 14 volunteers before and after exercise, at the same time on each day, in consideration of circadian rhythm of leukocyte. These subjects participated in this study after giving them informed consent.

cise was approximately 24 hours. Measurements of the total leukocyte were assessed for differential leukocyte counts and granulocyte and lymphocyte ratio in the peripheral blood

We ordered to the laboratory of Ishikawa Prefecture Preventive Medicine Association for precise counts for the total and differential leukocyte counts in the peripheral blood from the subjects. The total and differential leukocyte counts were measured by the automated hematology analyzer XE-2100 (Sysmex, Inc., Kobe, Japan).

### 2.3. Walking Exercise by 8 Mets

After two weeks of cooling down for all volunteers, we set up again for the same exercise except for the intense of walking was 8 km/hour for the same kilo-meter of the same course (8 mets). We prepared peripheral blood from the same 14 volunteers before and after the exercise, at the same time on each day, in consideration of circadian rhythm of leukocyte. We conducted the walking exercise at the same course in country side of Japan (**Figures 1 and 2**).

### 2.4. Assessment of Lymphocyte Subsets by FCM

For the purpose of estimating CD positive cell, the whole blood obtained from the subjects by blood collection tube containing an anticoagulant EDTA-2K. 100  $\mu$ l of whole blood were added the antibody. After washing with PBS, the suspensions were mixed phycoerythrin (PE)-conjugated streptavidin (Beckman Coulter Inc. France) and fluorescence monoclonal antibody: peridinin chlorophyll protein-cyanin 5.5 (PerCP-Cy5.5)-conjugated CD2, fluorescein isothiocyanate (FITC)-conjugated CD4, FITC-conjugated CD8, FITC-conjugated CD16, FITC-conjugated CD19, FITC-conjugated CD57 (each Becton Dickinson Co. U.S.A.), allophycocyanin (APC)-conjugated CD8, and APC-conjugated CD57



**Figure 2.** We conducted the walking exercise at the country side of Japan. The course of exercise located at 40 meter in high and 1015 mb of atmospheric pressure. The exercise start at ten o'clock for about one hour, corresponding 4 metabolic rates; 4mets. Time interval of blood sampling between before and after exercise was approximately 24 hours.

(each Beckman Coulter). The negative controls were added PE-conjugated streptavidin and the isotype control antibodies to the CD antibodies. After incubation for 30 minutes at 4°C, these samples were hemolyzed using a 10-times dilution FACS Lysing Solution (Becton Dickinson). After washing with PBS, the cell suspensions were fixed using a 10-times dilution CellFIX (Becton Dickinson) and analyzed by flow cytometer FACS Caliber (Becton Dickinson) [18-20].

### 2.5. Statistical Analysis

The statistical comparisons between two groups (before and after walking) for the test of significant difference were performed using paired t-test and wilcoxon signed-ranks test. Further, the test of the correlation were performed a spearman's correlation coefficient by rank test. Data are expressed as means  $\pm$  standard error of mean (SE). A *P* value < 0.05 was considered to be statistically significant. The Kendall tau Rank Correlation and the two-sided p-value. The ordinary scatter plot and the scatter plot between ranks of X axis & Y axis are also shown.

## 3. RESULTS

### 3.1. Twenty-Four Hours Change of Total Leukocyte Counts after 4 Mets on the Basis of Each Individual

Each volunteer was prepared their blood before start for exercise and after informed consented to the experimental purpose by written Ethics of the Committee in Kanazawa Medical University. After warming up for 5 minutes by Radio exercise by NHK. We suggested to take a walk for one hour for 4 km of the course (4 mets).

We tried to exhibit the effect of peripheral total leukocyte number by individual level of change and plot in the x-axis as in each value before the exercise. As was in **Figure 3**, The relative value (%) was calculated before and after the exercise and plotted in the figure of the x-axis according to the value before exercise. As a result of the exhibition, there found three groups, separated, up-regulated individuals and down-regulated one and was no change. The correlation of change was expressed as a linear function and significant reverse correlation,  $-0.6893$  indicating ideal value of correlative index  $-0.5$  (**Figure 3**). The data obtained from 4mets exercise was brought ideal regulation. However, 8mets could not bring such a effect significantly. About the leukocyte subset, the ideal regulation of lymphocyte and monocyte were found by 4 mets, but not in granulocyte.

### 3.2. Twenty-Four Hours Change of Total Leukocyte Counts after 8 Mets

After 2-weeks of cooling down, we set up the same mode of trial at the same course and by the same volunteers. None of volunteer was dropped out with any serious problem. In this time, we set up 8 mets impacts of exercise, asking to walk out by half an hour for the same course of 4 kilometers (8 mets). Preparing 5-min. warming up, we tried to express the effect of peripheral total leukocyte number by individual level of change and plot in the x-axis as in each value before the exercise as is in 3.1. (**Figure 3**). As shown in lower panel in **Figure 3**, The correlation was exhibited on a linear function but good correlation was retreated from the results by the case by 8 mets. The slant value was  $-0.3617$ . The data obtained from 8mets exercise was lesser regulatory effect

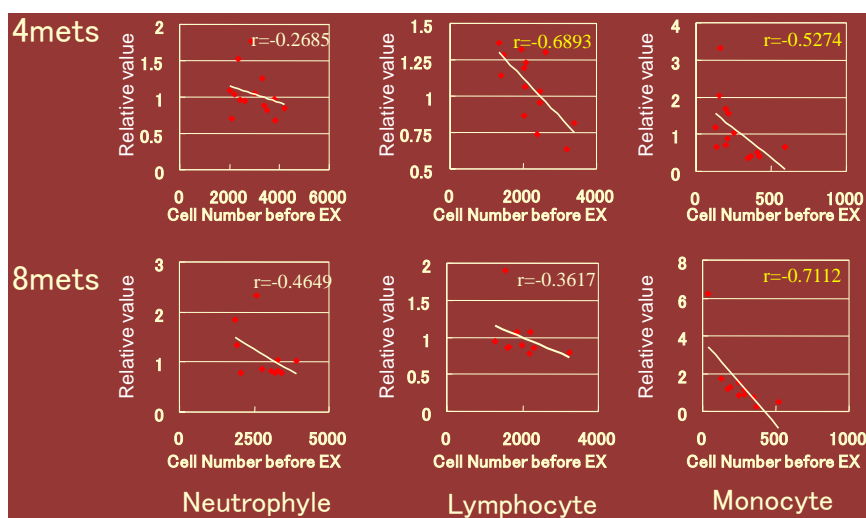
than that compared by 4 mets of exercise. About the leukocyte subset, the regulation by lymphocytes were more remarkable. However, only of the monocyte exhibited significant change by both impact 4 and 8 mets.

### 3.3. Lymphocyte Subsets Regulation by 4 Mets

We had been observed the reputational effect of exercise could be evidenced within a short period. But the possibility still remain that the leukocyte change was happen to emerge for the dairy life as accidental factor, such as stress and so on. In order to avoid such possibility, then we tried to show the change of the peripheral leukocyte number was the result of another network system of the inner system such as peripheral leukocyte, endocrine and brain system. We tried to access the effect CD positive lymphocyte. They were CD2, CD CD4, CD8, CD16, CD19, CD57. Within those CD positive cells, CD2, CD4, CD8, CD19 cells were regulated significantly as in number of each CD marker positive cells by 4 mets of exercise.

### 3.4. Twenty-Four Hours Change of Lymphocyte Subsets by 8 Mets

After 2 weeks of cooling down, we set up the same mode of trial at the same course and by the same volunteers. None of volunteer was dropped out with any serious problem. In this time, we set up 8 mets impacts of exercise, asking to walk out by half an hour for the same course of 4 kilometers (8 mets). Preparing 5-min warming up, we tried to express the effect on lymphocyte subsets as CD positive number. They were CD2, CD 4, CD 8,



**Figure 3.** We tried to express the effect of peripheral total leukocyte number by individual level of change and plot in the x-axis as in each value before the exercise. The relative value (%) of post walking was calculated with before and after and plotted in the x-axis. The statistically significant value of slope were indicated in yellow.



CD 16, CD 19, CD 57. As shown in **Figure 4**, the regulatory effect was disappeared in the all the subset in 8 mets of exercise.

### 3.5. Simple Sum up & Made Mean, Get No Meaning after 24 Hours Change of Lymphocyte Subsets by Both 4 & 8 Mets

As shown in **Figures 3 and 5**, we can compared and got the result that of 4mets of exercise was more effective to regulate lymphocyte and lymphocyte subset by individual change by plotting the linear function. In **Figures 4 and 6**, we tried to exhibited the same data with grouped and tried to make mean. From the results, there was no significant change between the value before and after the exercise both with 4 and 8 mets.

## 4. DISCUSSION

### 4.1. The Tailored Assessment Was Provided by Linear Slant

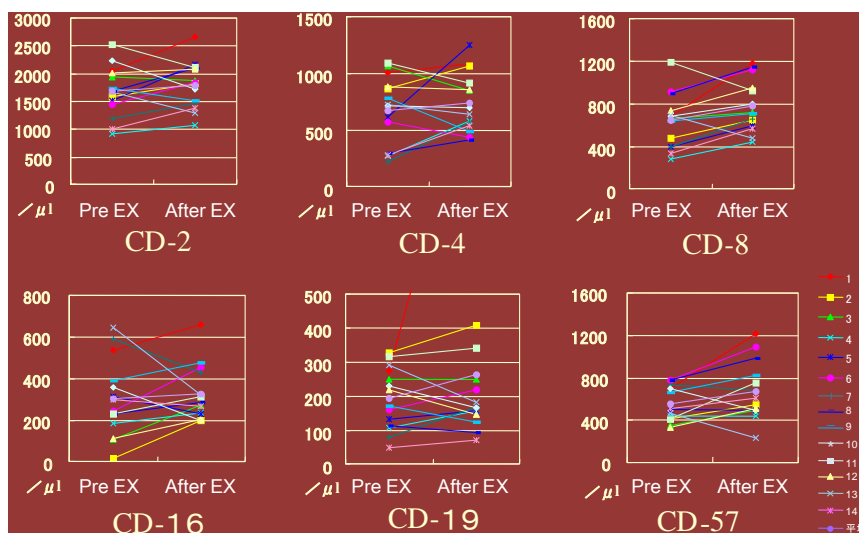
Our results showed that within 24 hours after light exercise, the white blood cells in peripheral blood had regulated significantly, not only in leukocyte subset but also lymphocyte subset. The results showed that these subsets could reflect the number and function of immuno-competent cells [13-19]. For example, in an individual with a low granulocyte number, the number increased after treatment, while it decreased in another individual with a higher cell number. Our results led us to believe that leukocyte subsets could be an interesting indicator for the evaluation of alternative therapies [15-20] Many systems are in place to evaluate Western

therapies that aim at healing the symptoms of an illness[25-27]. We hope that our work will attract more attention to the mechanisms of which each CAM menu regulates the human immune system. Abo reported that according to the lymphocyte subset content, lymphocyte rich type showed over 40% on the other hand granulocyte rich type show over 60% of granulocyte [28]. Each type exhibited different character even in the same age, sexuality and different age. Within the same age and the sexuality, even in gentleman can sorts out as G-rich type (granulocyte 60%), and L-rich type (lymphocyte 40%). On the other hand, as a stand point of sexuality difference, the lady belongs to L-rich type but the gentleman belongs G-rich type. According to the age-related change, G-rich type of young man change to L-rich type according to getting older [29,30].

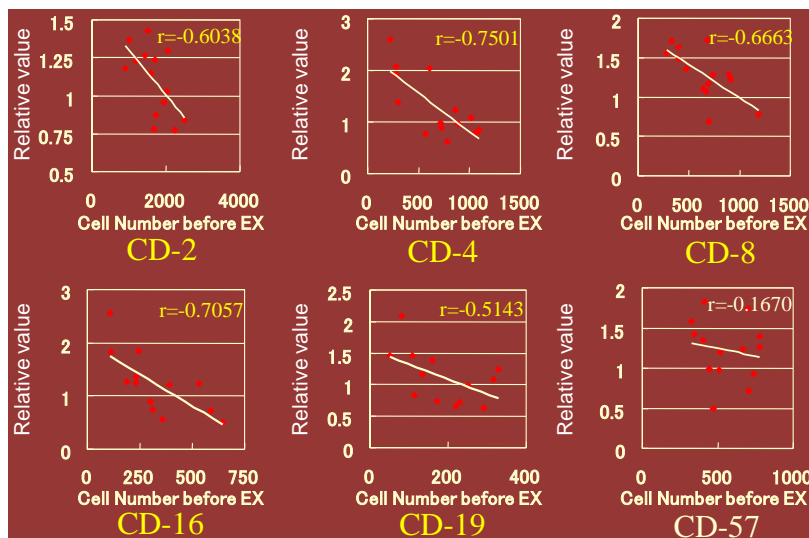
We have been trying to regulate the immune responsiveness through much mature by fragile daily condition from circumstance stress and so on. The main menu were, acupuncture, hot-spring hydrotherapy, light exercise etc. In this article, we would like to show the regulatory mechanism of the light exercise walking as a tailored scale. The circumstance, except for cases of contraindication, has been medically useful approved to be effective in many stress-related disorders and the improvement of dysfunction of the biological rhythm disturbance as well as chronic disease. The mechanism of effects has been reported in many studies, but many things are still unclear.

### 4.2. The Grouped Comparison by Conventional Assessment

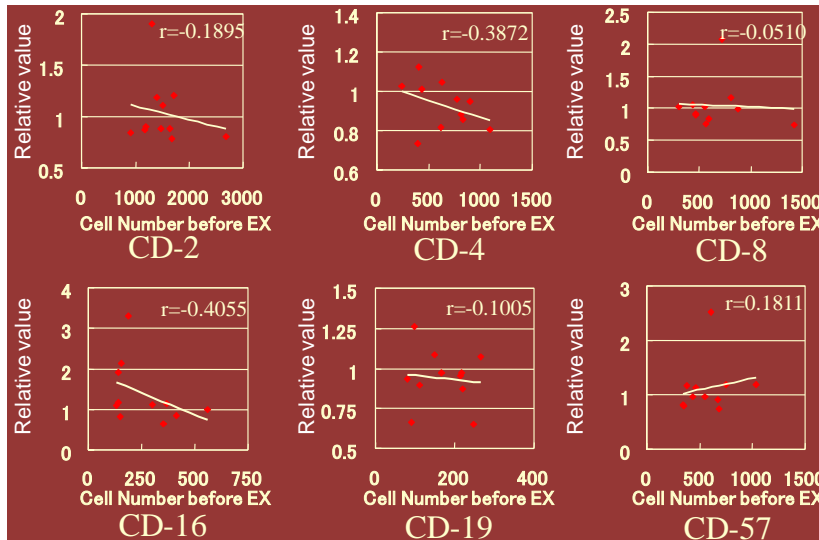
In order to assess correctly to the changes after the menu



**Figure 4.** The procedure and the value were the same obtained by the exercise in 4 mets. For the purpose of estimating CD positive cell, the whole blood obtained from the subjects by blood collection tube containing an anticoagulant EDTA-2K. Each data from volunteer were shown by different color in the figure, then summed up and made mean.



**Figure 5.** For the purpose of estimating CD positive cell, the whole blood obtained from the subjects by blood collection tube containing anticoagulant EDTA-2K. 100  $\mu$ l of whole blood were added the antibody. After washing with PBS, the suspensions were mixed phycoerythrin (PE)-conjugated streptavidin: peridinin chlorophyll protein-cyanin 5.5 (PerCP-Cy5.5)-conjugated CD2, CD4, CD8, CD16, CD19, CD57. We tried to express the effect of peripheral total leukocyte number by individual level of change and plot in the x-axis as in each value before the exercise. The relative value was calculated before and after the exercise and plotted in the figure of the x-axis for the value before exercise. The statistically significant value of slope were indicated in yellow.

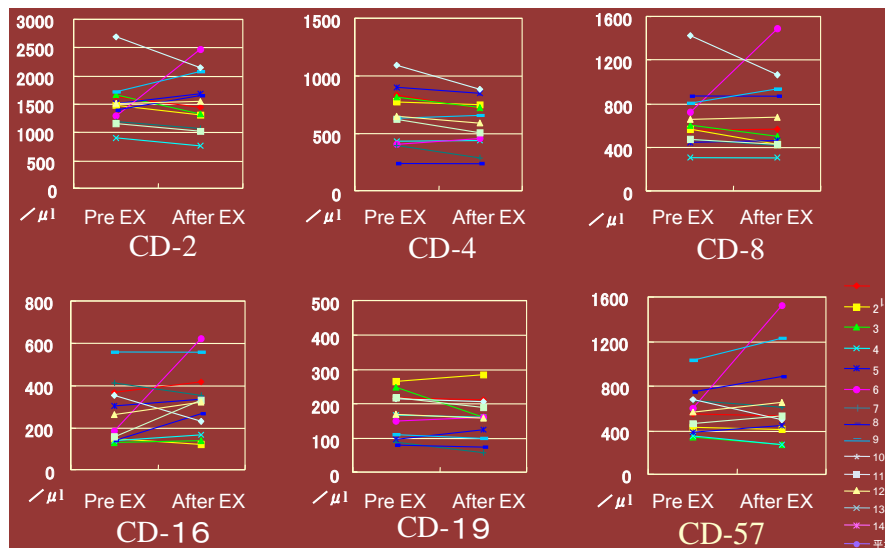


**Figure 6.** For the purpose of estimating CD positive cell, the whole blood obtained from the subjects by blood collection tube containing an anticoagulant EDTA-2K. The contents was the same in **Figure 5** except that the data were from the volunteers from 8 mets of walking. The statistically significant value of slope were indicated in yellow.

menu, it is important to conscious of circadian rhythm. Abo also reported that it was possible to sort the constitution, granulocyte-rich individual and lymphocyte-rich one with the peripheral leukocyte [31,32].

Each population of subset is depends on a circadian

rhythm. Within a same individual, granulocyte increase in the daytime, on the other hand, lymphocyte increased in the night time in a cycle 24 hrs. So we have to compare the effect of each menu for the peripheral leukocyte on the same time before and after the menu.



**Figure 7.** The procedure and the value were the same obtained by the exercise in 8 mets. For the purpose of estimating CD positive cell, the whole blood obtained from the subjects by blood collection tube containing an anticoagulant EDTA-2K. Each data from volunteer were shown by different color in the figure, then summed up and made mean.

With our report simple comparison of grouped value did not exhibit the valuable change in each individual. So this style of presentation was suggestive to the patient for immunodeficiency such as in DM.

It was reported that the leukocyte subset, granulocyte and lymphocyte regulated by various factors. One major point is that they are regulated by autonomous nervous system, resulting in circadian rhythm [30-33]. Therefore, in order to access the effect within a short time, it is necessary to consider this factor to adjust the time to collect the sample. For example, efficacy and impact of walking exercise has been widely recognized. However, the majority of walkers did not have a scientific background to know the best exercise menu for the one' QOL; quality of life. The purpose of this study was to demonstrate the best menu of walking that regulates the peripheral white blood cells in number and function as a marker of QOL expression.

However, almost all the judgments of efficacy are VAS (visual analog scale). Moreover, simple processing by grouped value and make mean fadeout the precise regulation according to each constitution. Therefore, we hope our tailored scale can suggest assessing every CAM therapies in the world for competition (Figures 4 and 7).

## 5. CONCLUSIONS

We have measured the number of leukocyte subsets, granulocyte and lymphocyte ratio after different impacts of light excursive. The conclusions were as following

1) The quantitative regulation of leukocyte subset could be assessed by linear slant, namely tailored scale for walking.

2) Light exercise by 4met walking proved to regulate the total number of leukocyte subsets.

3) Light exercise by 4met provided more moderate regulation of lymphocyte than that of 8mets.

4) Major lymphocyte subsets were regulated moderately by 4mets of exercise.

5) CD2, CD4, CD8, CD16 cells intensely regulated by 4mets but not 8mets.

6) These regulatory changes were not exhibited by conventional grouped processing.

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## ABBREVIATIONS

CAM: Complementary and alternative medicine, beside the western medicine, there are many traditional medicine and/or health promoting menu all over the world

CD: Cluster of differentiation. Each lymphocyte has name that expressed CD number, for example CD2, CD4, etc.

DM: Diabetes mellitus

FCM: Flow Cytometry

G-rich type: The individual that exhibit over 60% of granulocyte in peripheral blood, finding many in young gentleman

L-rich type: The individual that exhibit over 40% of lymphocyte in peripheral blood, finding lot in ladies and senile

METS: Metabolic Rate(s)

NHK: Nippon Hohsoh Kyokai, nationally established broad casted agent in Japan

QOL: Quality of life

VAS: Visual analog scale