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Relationships between white blood cell count and insulin resistance, glucose effectiveness, and first- and second-phase insulin secretion in young adults

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Abstract

The Increasing prevalence of type 2 diabetes mellitus (T2DM) has been observed in younger adults. Insulin resistance [IR], decreased first-, second-phase insulin secretion, and glucose effectiveness (GE) (IR, first phase insulin secretion [FPIS], second phase insulin secretion [SPIS], and GE), denoted as diabetes factors (DF), are core for developing T2DM. A body of evidence has shown that inflammation contributes to the development of diabetes. In the present study, our goals were first, evaluate the relationships between white blood cell (WBC) count and, second, examine the relative tightness between the 4 DFs to WBC count. Thus, the pathophysiology of T2DM in Chinese young men could be more understood.

21112 non-obese males between 18 to 27 years old were recruited (mean age: 24.3±0.017), including 1745 subjects with metabolic syndrome. DFs were calculated by the published equations by our groups as follows:

$$IR = \log(1.439 + 0.018 \times sex - 0.003 \times age + 0.029 \times body\ mass\ index\ [BMI] - 0.001 \times systolic\ blood\ pressure\ [SBP] + 0.006 \times diastolic\ blood\ pressure + 0.049 \times triglycerides\ [TG] - 0.046 \times high\text{-}density\ lipoprotein\ cholesterol\ [HDLC] - 0.0116 \times fasting\ plasma\ glucose\ [FPG]) \times 10^{3.3331}$$

$$FPIS = 10\ [1.477 - 0.119 \times FPG + 0.079 \times BMI - 0.523 \times HDLC]^{2}$$

$$SPIS = 10\ [-2.4 - 0.088 \times FPG + 0.072 \times BMI]$$

$$GE = (29.196 - 0.103 \times age - 2.722 \times TG - 0.592 \times FPG) \times 10^{-33}$$

The association between DFs and WBC count was analyzed using a simple correlation. The r-values of the simple correlation are regarded as the tightness of the relationships.

Higher WBC, FPIS, SPIS, IR, age, BMI, blood pressure, FPG, TG, Cholesterol, low-density lipoprotein cholesterol and lower HDL-C and GE were observed in subjects with metabolic syndrome. A similar trend was seen across the quartiles of WBC levels. Among the 4 DFs, GE has the highest r-value ($r = -0.093$, $P < .001$), followed by IR ($r = 0.067$, $P < .001$), SPIS ($r = 0.029$, $P < .001$) and FPIS ($r = 0.027$, $P < .001$).

Elevated WBC count is significantly associated with all the 4 DFs and the relative order of the tightness, from the highest to the lowest, are GE, IR, SPIS, and FPIS in Chinese young men.

Abbreviations: BMI = body mass index, DFs = diabetes factors, FFA = free fatty acid levels, FPG = fasting plasma glucose, FPIS = first phase insulin secretion, GE = glucose effectiveness, HDL-C = high-density lipoprotein cholesterol, IR = insulin resistance, LDL-

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C = low-density lipoprotein cholesterol, MetS = metabolic syndrome, SBP = systolic blood pressure, SPIS = second phase insulin secretion, T2D = type 2 diabetes, TG = triglycerides, WBC = white blood cell, WC = waist circumference.

Keywords: first-phase and second-phase insulin secretion, glucose effectiveness, insulin resistance, type 2 diabetes, white blood cell

1. Introduction

The prevalence of type 2 diabetes mellitus (T2DM) has dramatically increased in the past 2 decades in Taiwan, as well as many other countries worldwide.[4] This trend is found not only among the middle-aged but also the younger adults and adolescents. A nationwide longitudinal study in Taiwan showed a significantly increased incidence in young adults aged 20–40 years whose relative incidence was 1.31 (95% confidence interval (CI): 1.20–1.42) for men and 1.04 (95% CI: 1.01–1.08) for women respectively.[5] Thus, the early detection and management of T2DM among this population become critical issues for public health.

The pathophysiology of T2DM is complicated and has been studied intensively in the past.[6] Insulin resistance (IR) is the most well-known perturbation which might be inherited from parents. Other than this, 3 factors are also important. The first 1 is the defect in insulin secretion. However, it should be noted that there are 2 phases of insulin secretion, namely, the first- and second-phase insulin secretion (first phase insulin secretion [FPIS], second phase insulin secretion [SPIS], accordingly). From the physiological aspect, FPIS is the insulin response to glucose load within 10 minutes. The insulin stored in the beta-cell granules is secreted during this period.[7] Evidence has shown that the FPIS has long decreased even before the occurrence of clinically overt diabetes.[8,9] On the other hand, the SPIS indicates the newly produced insulin after the first phase.[10] Lastly, glucose effectiveness (GE), the ability of glucose to regulate its uptake and production, is often overlooked. In some studies, GE was found to be responsible for most of the glucose disappearance in T2DM.[11,12] Surprisingly, these 3 aforementioned factors (FPIS, SPIS, and GE) are important but less studied in the past. In the present study, for convenience, we denote these 4 factors as 'diabetes factor' (DF).

The positive correlation between inflammation and IR has been noted for a long time. A vast amount of studies has demonstrated that an elevated level of proinflammatory cytokines and acute-phase protein results in a higher incidence of IR.[13–15] Among these markers, white blood cell (WBC) count is the most common test widely available in different levels of medical facilities and has also been proven to be related to IR.[16–18] This relationship could be explained by the negative pleiotropic effects of inflammation on different adipose tissues, glucose metabolism and hyperlipidaemia.[19–21]

Other than IR, there are limited data related to the association between insulin secretion and inflammation. Some of the recent studies showed that insulin secretion defects and β-cell death might be caused by inflammation such as glucotoxicity, endoplasmic reticulum stress and the amyloid deposits in the islets.[22–24] However, the results are still inconsistent.[13,14,25] Finally, concerning GE, there is only 1 study that reveals the activation of innate immunity could induce decreased GE.[26]

In the present study, we enrolled 21,112 Taiwanese males aged 18 to 27 years without taking any medications known for diabetes, hypertension, or hyperlipidemia. Our goal is to try to identify the relationships between WBC and the 4 DFs. Thus, we can understand the pathophysiology of diabetes in these young men.

2. Materials and methods

2.1. Subjects

We enrolled 21,112 Chinese young men between 18 to 27 years old (mean age: 24.3 ± 0.017) from MJ Health Screening Center, Cardinal Tien hospital, and Tri-Service general hospital in Taiwan (between 2010–2015). MJ Health Screening Center is a local chain clinic that provides regular health checkups for its members. Cardinal Tien hospital is a local district hospital and Tri-Service general hospital is a medical center. To diminish the selection bias, we obtained data from these 3 different levels of health facilities. All the data was collected anonymously during the routine health checkup after informed consent was obtained from the participants. The clinic and hospitals provided data after the study protocol was approved by the institutional review board.

We excluded participants who were obese (body mass index [BMI] $\geqq 25\,kg/m^2$) and those who were taking any medication known to affect blood pressure, glucose levels, or lipid profiles during the study period. All participants were divided into 2 groups, with or without metabolic syndrome (MetS), based on the criteria of the World Health Organization.[27] To evaluate the effects of WBC count, we further divided the participants into 4 groups according to the quartiles of WBC levels.

2.2. Materials and protocols

Senior nursing staff obtained participants' records including

(1) medical history covering current medications and a thorough questionnaire;
(2) a physical examination that measured body weight, height, waist circumference (WC) and blood pressure;
(3) laboratory tests including FPG, lipid profiles and WBC count.

BMI was calculated as the subject's body weight (kg) divided by the square of the subject's height (m). WC was measured at the level of the natural waist, which was identified as the level at the hollow molding of the trunk when the trunk was laterally concave. Both SBP and diastolic blood pressure were measured by standard mercury sphygmomanometers on the right arm of subjects while they were seated.

After the subjects had fasted for 10 hours, we drew blood from the antecubital vein, and plasma was then separated within 1 hour and stored at -30°C. FPG was measured using the glucose oxidase method (YSI 203 Glucose Analyzer, Yellow Springs Instruments, Yellow Springs). Total cholesterol and triglycerides (TG) levels were analyzed using the dry multilayer analytical slide method and the Fuji Dri-Chem 3000 analyzer (Fuji Photo Film,

Tokyo, Japan). As for the measurement of serum high-density lipoprotein cholesterol (HDLC) and low-density lipoprotein cholesterol (LDLC), we applied an enzymatic cholesterol assay following dextran sulfate precipitation.

To quantify the DFs, we used the equations developed by our groups and listed them below (International Units). To demonstrate the reliability of our equations, we have given a short statement here. When performing these studies, we used approximately 70% of the participants to build the equation and used the remaining 30% for external validation. Thus, the accuracy of the equations could be tested.

(1) IR: We enrolled 327 subjects and measured the IR using the insulin suppression test. The *r*-value between the measured and calculated GE was 0.581 ($P < .001$). It was published in the 'Journal of Diabetes Investigation' in 2013.
IR = log (1.439 + 0.018 × sex - 0.003 × age + 0.029 × BMI - 0.001 × SBP + 0.006 × diastolic blood pressure + 0.049 × TG - 0.046 × HDLC - 0.0116 × FPG) × 10 $^{3.333}$[1]

(2) FPIS: We enrolled186 subjects and measured the FPIS by frequent sampled intravenous glucose tolerance tests. The r-value between the measured and calculated GE was 0.671 ($P < .000$). It was published in the 'International Journal of Endocrinology' in 2015.
FPIS = 10 [1.477 - 0.119 × FPG + 0.079 × BMI - 0.523 × HDLC] [2]

(3) SPIS: We enrolled 82 participants and measured the SPIS using a modified low dose glucose infusion test. The r-value between the measured and calculated GE was 0.65 ($P = .002$). It was published in the journal 'Metabolic Syndrome and Related Disorders' in 2016.
SPIS = 10 [-2.4 - 0.088 × FPG + 0.072 × BMI]

(4) GE: We enrolled 227 participants and measured the GE by frequent sampled intravenous glucose tolerance tests. The r-value between the measured and calculated GE was 0.43 ($P = .001$). It was published in the journal 'Metabolic Syndrome and Related Disorders' in 2016.
GE = (29.196 - 0.103 × age - 2.722 × TG - 0.592 × FPG) × 10 $^{-3}$[3]

2.3. Statistical analyses

We performed statistical analyses using SPSS 19.0 (IBM, Inc., Armonk, NY). Data were shown as means ± standard deviations. All data were tested for normal distribution by using the Kolmogorov–Smirnov test and for homogeneity of variances by using the Levene test. Data were log-transformed before analysis if not normally distributed. Furthermore, we performed t–tests to evaluate the differences between groups with and without metabolic syndrome. One-way analysis of variance was applied to analyze the differences between the mean values of the 4 groups, which was arranged from the highest to the lowest level of WBC count. For between-group comparisons, we used the post-hoc Bonferroni method. At the same time, ordinal logistic regression was also applied to further quantify the effects of higher WBC quantiles on the DFs.

To analyze the independent associations between WBC count and the other 4 valuables of interest (IR, FPIS, SPIS, GE), we used simple correlations. The slopes obtained represent the rate changes of the 4 factors in response to the increasing WBC count. Among the 4 factors, GE showed an inverse correlation with WBC count. To make it easy to compare with the others, we plotted a reciprocal line of GE from the fourth quadrant to the first quadrant. These lines will be presented graphically in the results.

Table 1

Clinical characteristics of subjects with or without MetS.

	MetS (−)	MetS (+)	*P*
n	19367	1745	
Age (yr)	24.3 ± 2.5	24.6 ± 2.5	<.001
Body mass index (kg/m^2)	22.9 ± 3.0	28.8 ± 4.5	<.001
Waist circumference	77.3 ± 7.7	92.1 ± 10.5	<.001
Systolic blood pressure (mm Hg)	118.9 ± 12.3	132.8 ± 12.1	<.001
Diastolic blood pressure (mm Hg)	68.4 ± 8.7	76.9 ± 9.9	<.001
Fasting plasma glucose (mg/dL)	93.6 ± 6.7	100.5 ± 10.8	<.001
Triglyceride (mg/dL)	88.0 ± 43.3	173.7 ± 74.0	<.001
HDL-C (mg/dL)	51.4 ± 11.4	39.5 ± 8.2	<.001
Cholesterol (mg/dL)	175.2 ± 31.2	192.1 ± 35.9	<.001
LDL-C (mg/dL)	106.2 ± 28.5	117.9 ± 32.3	<.001
FPIS (μU/min)	125.3 ± 145.5	513.3 ± 589.6	<.001
SPIS (pmol/mmol)	0.072 ± 0.060	0.203 ± 0.207	<.001
IR ($10^{-4} \cdot min^{-1} \cdot pmol^{-1} \cdot L^{-1}$)	3.688 ± 0.021	3.735 ± 0.026	<.001
GE ($10^{-2} \cdot dL \cdot min^{-1} \cdot kg^{-1}$)	0.021 ± 0.001	0.018 ± 0.002	<.001
Hemoglobin (10^3/μ L)	15.3 ± 1.2	14.3 ± 1.4	<.001
White blood cell count (10^3/μ L)	6.6 ± 1.6	6.7 ± 1.8	<.001
Platelate count (10^3/μL)	247.3 ± 52.0	266.6 ± 57.9	<.001

FPIS = first phase insulin secretion, HDL-C = high-density lipoprotein cholesterol, IR = insulin resistance, GE = glucose effectiveness, LDL-C = Low-density lipoprotein cholesterol, Log-γ-GT = Log γ-Glutamyl transpeptidase, MetS (+) = with metabolic syndrome, MetS(−) = Without metabolic syndrome, SPIS = second phase insulin secretion.
Data are shown mean ± SD.

3. Results

3.1. Clinical characteristics of participants with or without metabolic syndrome

Among the 21,112 young men, we found metabolic syndrome in 1,745 participants. The clinical characteristics and the 4 DFs are shown in Table 1. We observed higher WBC, FPIS, SPIS, IR, age, BMI, WC, BP, FPG, TG, cholesterol, LDL and lower HDL-C, and GE in subjects with metabolic syndrome.

3.2. Components of metabolic syndrome according to quartiles of WBC count

Among the 4 groups divided by quartiles of WBC, similar trends as those shown in Table 1 were also observed (Table 2), that is, higher FPIS, SPIS, IR, age, BMI, WC, BP, FPG, TG, total cholesterol, LDL and lower HDL-C and GE were significantly associated with higher WBC count.

3.3. The changes of DFs in quartiles of WBC count

The changes of DFs in the quartiles of WBC showed a similar trend seen in the ANOVA. Other than the IR between WBC quartile 2 to quartile 4, all the other comparisons were all significant (Table 3).

3.4. Relationship between WBC count and 4 DFs

Table 3 shows the results of simple correlations between WBC count and 4 DFs. All 4 factors correlate with WBC count

Table 2

Components of MetS according to graded WBC count.

	White blood cell 1	White blood cell 2	White blood cell 3	White blood cell 4	Total	*P*
n	5333	5338	5254	5187	21112	
Age (yr)	24.2 ± 2.6^{34}	24.3 ± 2.5^{4}	24.4 ± 2.5^{1}	24.5 ± 2.4^{12}	24.3±2.5	<.001
Body mass index (kg/m^2)	23.3 ± 3.5^{4}	23.2 ± 3.5^{4}	23.4±3.6	23.7 ± 3.6^{12}	23.4±3.5	<.001
Waist circumference	78.2 ± 8.9^{4}	78.1 ± 8.9^{4}	78.6±9.0	79.2 ± 9.1^{12}	78.5±9.0	<.001
Systolic blood pressure (mm Hg)	119.8±12.9	119.9±12.6	120.0±12.8	120.5±13.0	120.0±12.8	.029
Diastolic blood pressure (mm Hg)	68.7 ± 9.1^{4}	68.9 ± 9.1^{4}	69.2±9.1	69.7 ± 9.2^{12}	69.1±9.1	<.001
Fasting plasma glucose (mg/dL)	93.6 ± 7.0^{34}	94.0 ± 7.4^{4}	94.4 ± 7.4^{1}	94.7 ± 7.8^{12}	94.2±7.4	<.001
Triglyceride (mg/dL)	90.1 ± 52.0^{34}	92.7 ± 50.9^{4}	95.8 ± 50.4^{4}	101.9 ± 54.8^{12}	95.1±52.2	<.001
HDL-C (mg/dL)	51.0 ± 11.8^{4}	50.8 ± 11.7^{4}	50.3±11.4	49.6 ± 11.5^{12}	50.4±11.6	<.001
Cholesterol (mg/dL)	174.9 ± 31.7^{4}	175.4 ± 31.9^{4}	177.2±31.8	179.1 ± 32.3^{12}	176.6±31.9	<.001
LDL-C (mg/dl)	105.9 ± 28.7^{4}	106.1 ± 28.7^{4}	107.7±29.1	109.1 ± 29.3^{12}	107.2±29.0	<.001
FPIS (μU/min)	152.5±230.4	152.4±242.8	156.2±244.7	168.2±256.6	157.3±243.8	.002
SPIS (pmol/mmol)	0.081±0.081	0.081±0.087	0.083±0.093	0.087±0.098	0.083±0.090	.001
IR ($10^{-4}\cdot min^{-1}\cdot pmol^{-1}\cdot L^{-1}$)	3.690 ± 0.025^{4}	3.691 ± 0.025^{4}	3.692 ± 0.025^{4}	3.695 ± 0.026^{123}	3.692±0.025	<.001
GE ($10^{-2}\cdot dL\cdot min^{-1}\cdot kg^{-1}$)	0.021 ± 0.002^{34}	0.021 ± 0.002^{4}	0.021 ± 0.002^{4}	0.020 ± 0.002^{123}	0.021±0.002	<.001
Hemoglobin (10^3/μ L)	15.063 ± 1.2880^{234}	15.230 ± 1.2749^{14}	15.308 ± 1.2488^{14}	15.408 ± 1.3443^{123}	15.251±1.2953	<.001
White blood cell count (10^3/μ L)	4.807 ± 0.488^{234}	5.912 ± 0.255^{134}	6.860 ± 0.313^{124}	8.770 ± 1.410^{123}	6.571±1.641	<.001
Platelate count (10^3/μL)	231.8 ± 47.4^{234}	243.2 ± 49.1^{134}	252.5 ± 50.7^{124}	268.2 ± 57.0^{123}	248.9±52.8	<.001

FPIS = first phase insulin secretion, GE = glucose effectiveness, HDL-C = high-density lipoprotein cholesterol, IR = insulin resistance, LDL-C = Low-density lipoprotein cholesterol, Logγ-GT = Log γ-Glutamyl transpeptidase, MetS (+) = with metabolic syndrome, MetS(−) = Without metabolic syndrome, SPIS = second phase insulin secretion, WBC = white blood cell.
Data are shown mean ± SD.

significantly. GE has the highest *r*-value and thus is most tightly related to WBC ($r=-0.093$, $P<.001$), followed by IR ($r=0.067$, $P<.001$), SPIS ($r=0.029$, $P<.001$) and FPIS ($r=0.027$, $P<.001$). The graphic illustration of these relationships is shown in Figure 1. There is a significant difference between the slope of IR and FPIS, but not between GE and IR, FPIS, and SPIS.

4. Discussion

In the present study, we not only demonstrated that there are significant associations between WBC count and DFs but also we evaluated their relative tightness in our cohort of Chinese young men between 18 to 26 years-old. We found that GE has the highest r -value which indicates the tightest correlation, followed by IR, SPIS, and FPIS. It should be pointed out that only the slopes of IR and FPIS are significantly different (separated), but not the other slopes. To the best of our knowledge, the present study is the only 1 in this field and could provide new information to understand the pathophysiology of diabetes.

Early in 2002, WBC was first found to be related to FPG, which is 1 of the MetS factors.[28] Later, in 2009, Ble further showed that some features of the MetS are related to WBC.[29] Our group also published a 4-year-longitudinal study showing that the same influence of higher WBC on MetS could also be noted in the elderly.[30] This relationship could be explained by the negative pleiotropic effects of inflammation on different adipose tissues, glucose metabolism, and hyperlipidemia.[19–21]

Table 3

Relationship between WBC count and 4 DFs.

	r	*P*
First Phase Insulin Secretion	0.027	<.001
Second Phase Insulin Secretion	0.029	<.001
Insulin resistance	0.067	<.001
Glucose effectiveness	−0.093	<.001

DFs = diabetes factors, WBC = white blood cell.

The positive correlation between WBC count and IR in the present study is not novel. Many previous studies repeatedly showed similar findings in different ethnic groups. For instance, Facchini et al[18] examined the relationships between WBC count and several risk factors of coronary heart disease including plasma glucose, insulin levels during, and oral glucose tolerance test. They pointed out that IR remained positively correlated with WBC count after adjusted for confounding variables ($r=0.49$, $P<.01$). Another study done by Vozarova et al[31] in Pima Indians with the age of 27 ± 6 years also showed the concordant results ($r=-0.24$, $P<.0001$). The underlying pathophysiology behind this relationship might relate to the roles of chronic inflammation. The proinflammatory cytokines activate the intracellular pathways such as Jun N-terminal kinase and inhibitor of kappa B kinase beta/nuclear factor kappa B which, in turn, inhibit insulin receptor signaling cascade and promoting IR.[32]

Other than IR, we also found that both FPIS and SPIS are positively related to WBC count separately ($r=0.027$, $r=0.029$, $P<.001$, respectively). Our findings are interesting but controversial. To our knowledge, there is only 1 study is in line with our results. Targher et al[13,16] demonstrated that WBC count correlated consistently with 2-hour post-load insulin ($r=0.33$, $P<.01$) in ninety 38-year-old healthy men with normal glucose tolerance. Here, the 2-h post-load insulin is regarded as the SPIS. Other studies done by Temelkova-Kurktschiev et al[13] or Festa et al[14] all showed a non-significant relationship. To explain this discrepancy, the role of obesity might be the key. There was a vast amount of studies that showed that obese subjects have higher insulin secretions.[33–35] At the same time, obesity is also known to relate to low-grade inflammation mainly in peripheral tissues.[36] This further activates inflammatory response via signaling cascades including inositol-requiring enzyme 1, protein kinase R-like endoplasmic reticulum kinase, and activating transcription factor 6.[37,38] All of this body of evidence explains the central role of obesity in affecting inflammation. Being 1 of the inflammatory markers, WBC is also confirmed to be related to

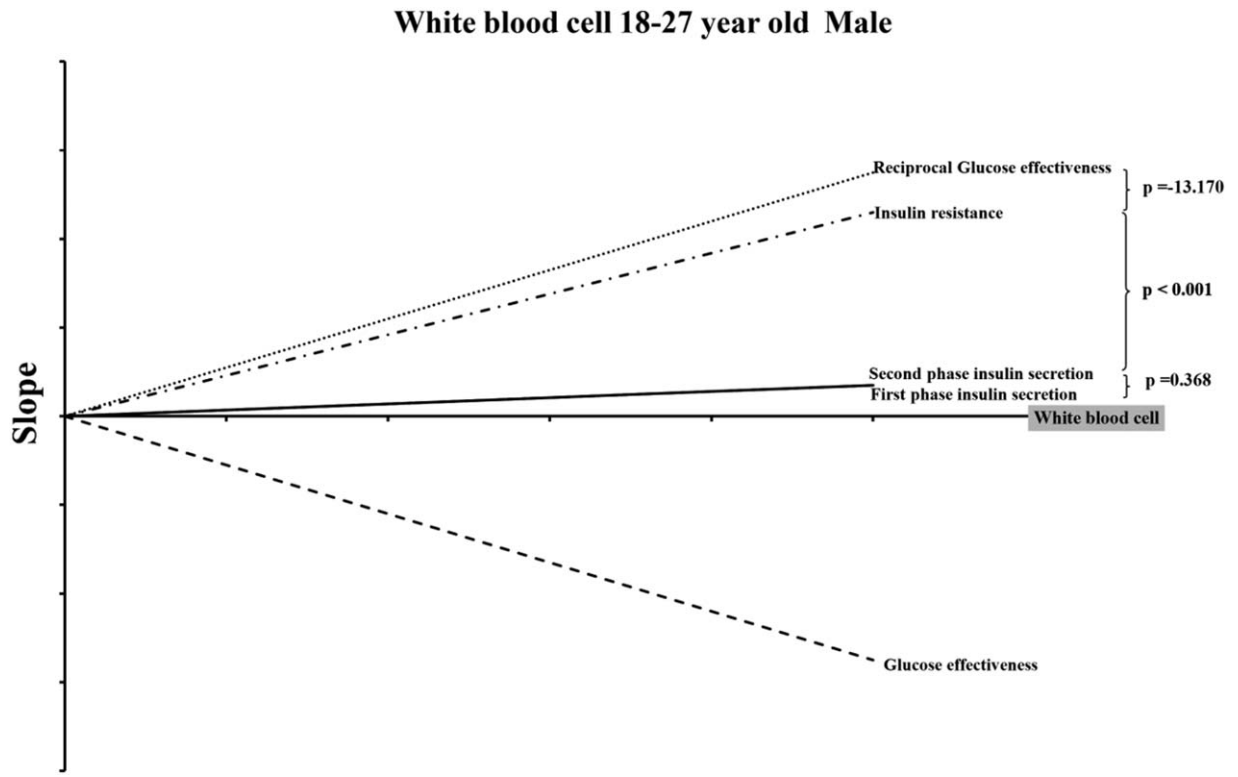

Figure 1. Slope of the relationship between 4 DFs and WBC count. DFs = diabetes factors, WBC = white blood cell.

obesity. From this body of evidence, it is clear that higher insulin secretion is associated with inflammation and obesity is the key to this scenario.

As mentioned in the introduction, GE is also an important factor in regulating glucose homeostasis. In our study, GE had the tightest correlation with WBC count among the 4 DFs ($r = -0.093$, $P < .001$). Since no other study ever investigated this area, we proposed a hypothesis to explain our result. Here, the role of free fatty acid (FFA) might be the key factor to connect GE and WBC count through 3 steps. First, undoubtedly, higher WBC count indicates underlying subclinical inflammation. Second, it has also been reported that inflammation is associated with elevated FFA levels directly and indirectly. FFA promotes the release of proinflammatory cytokines by activating NF-κB pathway.[39] At the same time, as discussed above, inflammation could also cause IR which further results in lipolysis and triglyceride storage, giving rise to an increasing amount of FFA.[40] The final step of the puzzle is completed by Tonelli et al[41] whose study showed that the higher FFA level leads to decreased GE by stimulating gluconeogenesis and altering the gene expression of hepatic enzymes which senses the elevated glucose concentration. This hypothesis is also supported by Ferguson et al[26] Their study showed that after the treatment of an experimental endotoxin (lipopolysaccharide), the activated innate immunity eventually leads to a decrease in GE.

There are limitations to this study. First, since it is a cross-sectional study, it is difficult to identify the causality according to the observed relationships. Nevertheless, the values of this observational study were to provide possible related factors and give directions for future research. Secondly, the measurements of the 4 DFs are not the gold standards and 1 might criticize their accuracy. However, they were all validated in published papers and these drawbacks should be compensated by our large number of participants.

5. Conclusion

In conclusion, we found that in a large cohort of Chinese men aged between 18 and 27, elevated WBC count is significantly associated with all the 4 DFs. The relative tightness of these correlations, from the highest to lowest, are GE, IR, FPIS, and SPIS, all of them are positively related except for GE. Our data not only help to expand the knowledge of T2DM pathophysiology but also provide new hints for future prevention and treatment.

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Author contributions

All authors were involved in the conception and design. Data collection were performed by Jiunn-Diann Lin, Chung-Ze Wu, Chieh-Hua Lu, Chang-Hsun Hsieh and Dee Pei. Statistical analysis was done by Yao-Jen Liang. Ting-Ya Kuo was involved in the interpretation of the data, drafting of the paper and revising it critically for intellectual content. Dee Pei and Yen-Lin Chen were involved in the final approval of the version to be published. All authors agree to be accountable for all aspects of the work.

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