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Platelet number is positively and independently associated with glycated hemoglobin in non-diabetic overweight and obese subjects

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KEYWORDS
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Abstract *Background and aims:* A significant increase in platelet count may be a risk factor for atherosclerotic cardiovascular disease. This study investigates the association between platelet number and glucose metabolism, evaluated by glycated hemoglobin (HbA1c) levels, in a apparently healthy population represented by overweight and obese subjects with normal glucose and HbA1c levels.
Methods and results: As many as 240 subjects, 177 women and 63 men, aged 18–70 years, were enrolled. Body mass index (BMI), waist circumference (WC), systolic and diastolic blood pressure levels, platelet count and fasting blood glucose, insulin, insulin resistance, HbA1c, uric acid, triglyceride, total cholesterol, high and low density lipoprotein cholesterol concentrations were evaluated. Concerning the univariate correlation analyses between platelets number and all other variables, platelet count was significantly (and positively) correlated only with HbA1c ($P < 0.05$) and female sex ($P < 0.01$). HbA1c ($P < 0.05$), female sex ($P < 0.001$), and diastolic blood pressure ($P < 0.01$), positively, and age ($P < 0.05$) and systolic blood pressure ($P < 0.05$), negatively, were significantly and independently associated to platelet count in a final multiple regression analysis.
Conclusion: This is the first study showing a strong positive and independent relationship between HbA1c and platelet number in non-diabetic overweight and obese subjects.

Abbreviations: HbA1c, glycated hemoglobin; BMI, body mass index; WC, waist circumference; HbA, hemoglobin A; CVD, cardio vascular diseases; TC, cholesterol; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; TG, triglycerides; HOMA-IR, homeostasis Model Assessment of insulin resistance.

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Introduction

Platelets are involved in the pathogenesis of atherothrombotic diseases, and their number is regarded as a marker of inflammation [1] as well as an indicator of vascular repair activity and cardiovascular risk [2]. Accordingly, platelets have an important role in vascular culture together with CD34-positive cells [3], inducing the differentiation of human CD34-positive cells into foam cells [4], which are a well-known contributing factor in the development of atherosclerotic lesions and have been observed in atherosclerotic lesions in humans [5]. In addition, platelet-rich plasma promotes angiogenesis [6].

Obesity is a global epidemic, and central obesity in particular is an important risk factor for major cardiovascular events, due to the atherosclerotic alterations of coronary, cerebral and lower limb arterial vessels [7]. Abdominal obesity is significantly associated with increased platelet counts [8], and obese subjects show higher aggregation and reactivity, lower membrane fluidity and reduced sensitivity to antiplatelet drugs [9–11]. The possible mechanisms responsible for platelet dysfunction in obesity can be summed up as follows: a) lower sensitivity to insulin and other molecules acting via intracellular cyclic nucleotides (nitrates and prostacyclin in particular); b) altered intracellular ionic milieu with elevated cytosolic Ca^{2+}; and c) higher oxidative stress, which stimulates isoprostane production from arachidonic acid [11]. Obesity is also characterized by higher circulating levels of leptin, that promotes platelet aggregation at plasma concentrations corresponding to those commonly assessed in obese patients (>50 ng/ml), whereas this effect cannot be found out at usual concentrations measured in non-obese individuals (<10 ng/ml) [12].

Independently of obesity, Jesri et al. showed that patients affected by metabolic syndrome have higher platelet counts than subjects without variables characterizing metabolic syndrome (hypertension, dyslipidemia, and impaired glucose metabolism) [13]. Diabetes per se is associated to an increase of platelet number [14].

HbA1c is glycated hemoglobin in which glucose is attached to the N-terminal valine residue of each b-chain of hemoglobin A (HbA). The extent of hemoglobin glycation is influenced by the concentration of glucose in the blood and, since the life span of erythrocytes is around 120 days, HbA1c reflects the average glucose concentration over the preceding 8–12 weeks [15]. HbA1c has been recommended by the American Diabetes Association since 1988 for routine monitoring of patients with diabetes [16]. Interestingly, an International Expert Committee Report has recently suggested to consider a HbA1c value $\geq$ 48 mmol/l (6.5%) as diagnostic for diabetes [17]. This suggestion has been accepted by the American Diabetes Association (ADA) [18], the World Health Organization (WHO) [19], and the International Diabetes Federation (IDF) [20], but this criterion is valid only if the blood HbA1c determination is performed according to the standardized International Federation of Clinical Chemistry (IFCC) method. This recommendation was motivated by improvements in the measurement of HbA1c and by the certain advantages of its measurement over that of glucose, such as the convenience of not requiring the patient to fast and the reduced intra-individual variability compared with fasting or glucose measurements after loading [17–20]. A correlation between HbA1c levels and platelet number has been recently found in a population of non-diabetic and prediabetic patients affected by coronary artery disease [21].

No study has previously examined whether HbA1c is a determinant of platelet number in non-diabetic healthy obese patients without a personal history of CVD. Thus, the primary goal of this study was to examine whether platelet number is related to HbA1c independently of factors well known to influence the platelet count, such as age, sex, obesity and abdominal fat accumulation, and cardiovascular risk factors typical of metabolic syndrome such as blood pressure and fasting blood glucose, lipids, insulin, insulin resistance and uric acid. To this purpose, a population of apparently healthy subjects, represented by overweight and obese individuals with normal glucose and HbA1c levels, was investigated.

Methods

Subjects

Subjects were recruited consecutively from January 2010 to March 2018 at the Outpatient Clinic of Nutrition of the Medical Oncology Unit, Department of Biomedical Sciences and Human Oncology, University of Bari, School of Medicine, Policlinico, Bari, Italy. The subjects were referred to the Outpatient Clinic with the aim of improving the quality of their diet and/or to loose body weight. They were enrolled at the first visit whether they did not take any kind of drugs (including oral contraceptives or drugs for osteoporosis). Exclusion criteria concerned subjects suffering from endocrine diseases (diabetes mellitus, hypo or hyperthyroidism, hypopituitarism *etc.*), malignancies, unstable hypertension, chronic inflammatory diseases, renal and liver failure, angina pectoris, myocardial infarction, heart failure, genetic heart diseases, minor and major stroke, and inherited thrombocytopenias.

The group of participants was made up of 240 subjects, 177 women and 63 men, aged between 18 and 70 years, all with BMI >25.0. The higher prevalence of female sex in our Outpatient Clinic, with women almost three times more represented than men, does not correspond to the difference by gender of the prevalence of overweight (39.8% men, 24.4% women) and obesity (8.5% men, 9.4% women) in the Italian adult population, as described by Gallus et al. [21]. It is well known that women are more interested than men in controlling their body weight and their body shape and, therefore, it is not strange that almost 3 of 4 patients coming to our Outpatient Clinic were women, spontaneously.

Subjects were examined by means of medical history, routine tests and electrocardiogram (ECG). None was following a rigid low-calorie diet or carrying out a high level of physical activity, while each of them kept on doing their common lifestyle.

A written informed consent was obtained from each subject. The study was carried out in accordance with the principles of the Declaration of Helsinki as revised in 2001.

Clinical and biochemical assessment

Body weight was rounded to the nearest kg. Height was determined to the nearest cm. BMI was calculated as weight (kg) divided by the square of height (m). The waist circumference was measured at the narrowest part of the abdomen, or in the area between the 10th rib and the iliac crest (minimum circumference). The blood pressure of ambulatory patients was determined in a sitting position after at least 10-min rest and at least three different times, using a mercury manometer with appropriately sized cuff. Mean arterial pressure (MAP) was calculated in all patients. MAP was obtained by doubling the diastolic blood pressure, adding the sum to the systolic blood pressure, and then dividing the result by 3.

Blood samples were drawn between 08:00 and 09:00 a.m. after an overnight fast. Serum insulin concentrations were measured by radioimmunoassay (Behring, Scoppito, Italy). Plasma glucose was determined using the glucose oxidase method (Sclavus, Siena, Italy), while the concentrations of plasma lipids (triglycerides, total cholesterol, HDL cholesterol) were quantified by automatic colorimetric method (Hitachi; Boehringer Mannheim, Mannheim, Germany). LDL cholesterol was calculated using the Friedewald equation [22].

Blood glucose, insulin, total cholesterol (TC), high (HDL), low (LDL) density lipoprotein cholesterol and triglycerides (TG) were detected after overnight fasting in all subjects. The Homeostasis Model Assessment of insulin resistance (HOMA-IR) [23] was obtained from fasting insulin and fasting glucose.

Platelet count was determined by a Coulter Hematology analyzer (Beckman–Coulter, Brea, CA). Blood samples were collected in the morning and analyzed within 3 h after venipuncture. EDTA served as an anticoagulant.

Statistical analysis

Laverage value, Cook's Distance and Jackknife residual was used to identify the outliers. The sample characteristics were expressed as mean ± standard deviation or median (interquartile range [IQR]) values for continuous data, and number (%) for categorical data. Shapiro-Walk test was used to determine whether the variables showed a normal distribution and the variables not normally distributed was transformed. It was evaluated the linear relation between platelet count and each parameter. Variables with p-value lower than 0.35 in the univariable analysis were included in a multivariable general linear model (GLM); the Akaike's information criterion (AIC), adjusted R-square statistic and the significance level of the F-statistic were the criteria used to estimate the final model. Normality of residuals assumption for the GLM model was checked both in univariable and multivariable analysis. The estimated relationship with the multivariable model between HbA1c (back-transformed in its original unit of measurement) and platelet count is plotted. All tests of statistical significance were two-tailed, and p-values less than 0.05 were considered statistically significant. Statistical analysis was performed by software SAS (version 9.4 for PC).

Results

Table 1 sums up the general, anthropometric, hormonal, metabolic and cardiovascular parameters of the population enrolled in the study.

Table 2 shows the univariate relationship between platelet number and other investigated variables, and the final multiple regression model. Concerning the linear correlation analyses between platelets count and all other variables of the population, platelet count was significantly (and positively) correlated only with HbA1c and female sex. Gender, age, BMI, waist circumference, systolic and diastolic blood pressure, and fasting glucose, insulin, HOMA-IR, triglycerides, cholesterol, LDL cholesterol, HDL cholesterol, and uric acid did not show a significant correlation with platelet count. Variables with p-value lower than 0.35 in the univariable analysis (HbA1c, Age, BMI, triglycerides, uric acid, insulin and sex) were included in initial multivariable general linear model.

The final multiple regression model confirmed a positive and independent association between platelet number and both HA1c and female sex; moreover, platelet count maintained a significant independent relationship with age (negative).

Figure 1 shows the predicted values of platelet count by HbA1c and sex.

Discussion

The aim of the present study was to investigate the relationship between platelet number and HbA1c in a population represented by overweight and obese subjects

Table 1 General, anthropometric, metabolic, and cardiovascular parameters (n = 240 subjects).

Characteristics	Median (IQR)
Platelet count (n x 10^9/l)	252,5 (217,5–291)
HbA1c (%)	5,4 (5,1–5,6)
Age (years)	42 (31–50)
Body mass index (Kg/m^2)	32,3 (29–36,75)
Waist circumference (cm)	106 (98–117)
Mean arterial pressure (mmHg)	90.1 ± 11.3[a]
Fasting blood glucose (mg/dl)	88 (82–96)
Triglycerides (mg/dl)	89 (62–122,5)
HDL-cholesterol (mg/dl)	50 (41–60,5)
LDL-cholesterol (mg/dl)	121,9 ± 34,7[a]
Cholesterol (mg/dl)	191 (162–216)
Uric acid (mg/dl)	4,3 (3,5–5,3)
Insulin (μUI/ml)	11,4 (8–17,15)
HOMA-IR	2445 (1,66–3,95)
Sex (woman)	177 (73.7%)[b]

[a] Mean ± st.dev.
[b] n (%).

Table 2 Univariate relation and final multiple regression model between platelet count (logarithmic transformation) and other investigated variables.

Characteristics	Univariate relation		Multivariable relation	
	Estimated (St.err)	*P*	Estimated (St.err)	*P*
HbA1c (%)	0.0730 (0.0310)	**0.0191**	0.0831 (0.0319)	**0.0097**
Age (years)	−0.0016 (0.0011)	0.1662	−0.0024 (0.0012)	**0.0401**
Body mass index (Kg/m^2)	0.0043 (0.0025)	0.0878		
Waist circumference (cm)	0.0007 (0.0010)	0.4720		
Mean arterial pressure (mmHg)	0.0002 (0.0013)	0.8550		
Fasting blood glucose (mg/dl)	0.0009 (0.0012)	0.4632		
Triglycerides (mg/dl)[a]	0.0270 (0.0293)	0.3463	0.0538 (0.0329)	0.1034
HDL-cholesterol (mg/dl)[a]	0.0162 (0.0533)	0.7617		
LDL-cholesterol (mg/dl)	0.0002 (0.0004)	0.6156		
Cholesterol (mg/dl)[a]	0.0597 (0.0692)	0.3893		
Uric acid (mg/dl)[a]	−0.0713 (0.0442)	0.1076		
Insulin (μUI/ml)[a]	0.0440 (0.0234)	0.0611	0.0264 (0.0248)	0.2886
HOMA-IR[a]	0.0400 (0.0219)	0.0683		
Sex (woman)	0.0898 (0.0316)	**0.0048**	0.1219 (0.0336)	**0.0003**

Variables included in the stepwise regression method: HbA1C, Age, BMI, Triglycerides, Uric acid, Insulin, Sex.
The multivariable model is statistically significant (Fisher-statistic = 5.15, p = 0.0002) and adjusted R-square is 0.099.
[a] Logarithmic transformation.

without manifested CV disease. We describe for the first time a strong positive and independent relationship between HbA1c and platelet number.

We investigated subjects with HbA1c levels under 5.7%, that are absolutely normal concerning glucose control. Since platelets is either a marker of inflammation [1] or an indicator of vascular repair activity and risk for cardiovascular disease (CVD) [2], our findings suggest that, when overweight and obese subjects are under study, early alterations of glucose metabolism are responsible for a higher cardiovascular risk, even when otherwise normal subjects with normal glucose and HbA1c levels are investigated. These results are in line with the findings of our previous study, performed in apparently healthy overweight and obese subjects, showing that even a glycemic threshold of 90 mg/dl promotes early signs of atherosclerosis, such as the thickening of carotid intima-media [24]. In synthesis, what this study seems to suggest is that cardiovascular risk starts to increase in obese subjects for levels of glucose control (fasting glucose and/or HbA1c) that do not change the cardiovascular risk in normal weight subjects.

The platelet count was also positively and independently associated with female sex, but this information is well known [25]. Moreover, we found an independent positive association between platelet count and diastolic blood pressure. This finding is in line with a recent study performed in Beijing, showing that blood platelet count

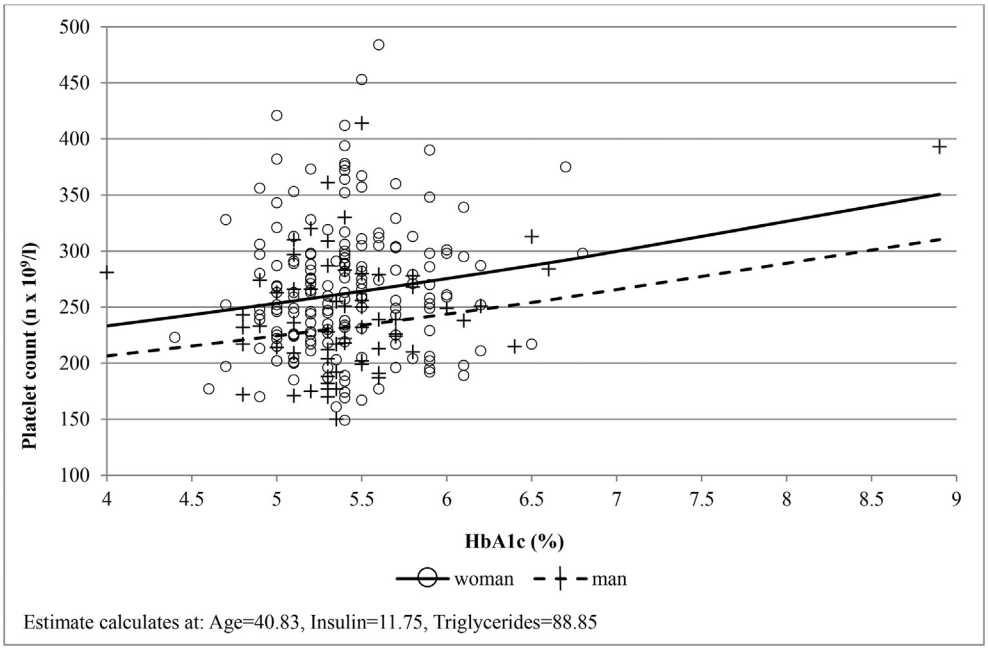

Figure 1 Predicted values of platelet count by HbA1c and sex (other variables are evaluated at their mean value).

was related to diastolic blood pressure, at least in males [26]. Finally, we found an independent negative relationship between platelet number and systolic blood pressure. We do not have a clear-cut explanation for opposite associations of systolic and diastolic blood pressure with platelet count, but is seems evident that blood pressure changes may influence the platelet number by modifying the megakaryocyte production or the spleen platelet catabolism. Interestingly, Pathansali et al. showed that platelet changes in hypertension may be secondary to changes in megakaryocytes (MKs), and that anti-hypertensive treatment can alter the function of MKs and platelets [27]. Moreover, it was recently shown that the platelet to lymphocyte ratio, with higher number of platelets, was higher in non-dipper than in dipper hypertensive subjects [28]. However, it may well be that the opposite pattern of relationship of platelet count with systolic and diastolic blood pressure may not have any biological meaning and might occur by chance.

In our opinion, a strong aspect of this study is that the data were taken only in individuals without medications and, therefore, we did not have any interference of drugs on the statistical associations between platelet number and other factors. By contrast, a limitation of the study is that we examined only the number of platelets, and not the volume or the function of these blood components. Moreover, we cannot exclude that higher prevalence of women in the study on the basis of "their willingness to control their body weight and shape" could be a selection bias affecting the conclusions.

Conclusion

HbA1c was a strong and independent determinant of platelet number in a healthy population, represented by overweight and obese subjects otherwise showing a normal range of glucose and HbA1c levels. These results suggest that, in obesity, a very early impairment of glucose metabolism in otherwise absolutely normal people may be a risk factor for an increase in the platelet number, that is per se a cardiovascular risk factor.

Contributorship statement

DG has substantially contributed to the conception of the study; DG, GVA and TV to the design of the study, interpretation of data and drafting the paper; DG, GVA, TV, BN, RT and LL to the acquisition of data; DG, GVA, TV, BN,RT, LL and GE to revising critically for the important intellectual content.

All Authors have read and approved the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to accuracy or integrity of any part of paper are appropriately investigated and resolved.

Conflicts of interest

None.

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