

ORIGINAL RESEARCH

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Platelet function and insulin resistance in aged and middle-aged obese female patients

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Abstract

It is well known that obesity is associated with insulin resistance (IR), and IR may interact with platelet functions. Aging is also associated with IR and enhanced platelet aggregation (PA). Though platelet reactivity has been investigated in female individuals, to date there is insufficient data on PA in obese and elderly women for whom the physiological changes associated with aging may overlap the factors associated with obesity. Therefore we investigated PA and IR in obese aged and middle-aged female subjects. Thirty obese elderly women over 60 years of age and 30 middle-aged obese women under the age of 50 years with nonspecific complaints were enrolled in the study from internal medicine outpatient clinic. Anthropometric measurements, fasting blood glucose and insulin levels, and PA tests for collagen, epinephrine, and adenosine diphosphate were evaluated. Obesity was defined as body mass index (BMI) $>30 \text{ kg/m}^2$. Homeostasis model assessment of IR (HOMA-IR) index was calculated to estimate IR. PA tests were performed with a PA profiler. Mean age of the elderly and middle-aged women were 69.6 ± 9.5 years and 38.6 ± 10.5 years, respectively. Waist circumference and BMI were similar between two groups. Mean HOMA-IR index value and PA with epinephrine were higher in the elderly than the control group ($P=0.04$; $P=0.01$, respectively). There was a positive correlation between HOMA-IR and PA with epinephrine in the elderly. Insulin resistance and platelet function test for epinephrine increased with advancing age in obese women. Large-scaled studies are needed in this area.

Keywords: Insulin resistance, platelet aggregation, elderly, obesity

Introduction

Cardiovascular disease is the leading cause of death for older adults. Platelet functions, aging, menopause, and obesity are substantial factors for cardiovascular diseases for elderly women. Platelets have a dynamic functional repertoire which alters by aging and shown to be enhanced for platelet aggregation (PA) in the elderly [1-4]. The membranes of human platelets express complete insulin receptors. In insulin-sensitive subjects, hormones play an anti-aggregating role; however, in conditions of insulin resistance (IR), such as central obesity, a significant reduction of platelet sensitivity to anti-aggregating effects of insulin has been reported [5].

Central obesity and related IR both increase in the postmenopausal period with advancing age [5-9]. Aging is associated with an increase in the fat mass and decrease in the lean mass resulting in poor outcomes. Though IR tend to increase in obese individuals, it may also increase in non-obese elderly as well with regard to age related physiological changes in both cellular and system level such as changes in the hormone levels and body composition.

As IR is influenced multifactorial in the elderly [10] with the increased incidence of visceral obesity after menopause [8], there may be differences between the elderly and the middle-aged obese individuals.

Previously, platelet functions were evaluated in the insulin-resistant state [5], and in postmenopausal women with hormone replacement therapy (HRT) about the effect of estrogen replacement therapy on PA [11].

Recently, in several studies, it has been reported that platelets from diabetic patients are generally more reactive, and less responsive to antiplatelet therapy, and it has been suggested that the status of hyperreactivity of platelets in diabetes may be explained by several factors such as IR, poor glycemic control, and increased response to adenosine diphosphate (ADP), reactivity on contact with collagen, as well as increased levels of inflammatory status, fibrinogen levels, and increased production of epinephrine and thrombin receptor agonist peptide [1].

However, little is known regarding age-related alterations for PA in obese and elderly women. Therefore, the aim of the study was to evaluate platelet function tests in the elderly and middle-aged obese women with regard to IR, and anthropometric measurements.

Materials and Methods

Patient Population

Ninety-seven consecutive female patients over 60 years of age with non-specific complaints, between December 2012 and March 2013 were recruited in the study from the internal medicine outpatient clinic. Subjects who had underlying diseases such as cardiovascular diseases, self-reported infections, diabetes mellitus (DM), autoimmune diseases and subjects with body mass index (BMI) <30 kg/m², as well as smokers, were excluded from the study. Subjects using medications likely to influence the results during the last 4 weeks, such as acetylsalicylic acid, oral contraceptives or HRT were also excluded. Finally, thirty elderly obese women were enrolled in the study.

The control group consisted of forty consecutive women (ages >30 and <48 years) with non-specific complaints from the same department. Elderly women were in postmenopausal period, but control group had to have regular cycles (recent history of regular menstruation with 25–35 days per cycle). After applying the aforementioned exclusion criteria described above, individuals with hysterectomy or bilateral oophorectomy were also excluded. Finally, thirty middle-aged obese women were also enrolled in the study.

The study protocol was approved by the local Ethics Committee. All subjects gave written informed consent before the study.

Anthropometrics and Laboratory Assessments/Reagents

Clinical examinations and laboratory blood analyses were carried out in the same center. Body mass index was calculated using the weight (kg) divided by the height (m) squared. Waist circumference was measured at the midpoint between the lower border of the rib cage and the iliac crest. Blood samples were collected after a 12-hour fasting period.

Serum concentrations of glucose were determined by enzymatic procedures. Serum insulin levels were measured by chemiluminescence (IMMULITE 2000, Diagnostic Products Corporation, Los Angeles, CA, USA). Insulin resistance was estimated using the Homeostasis model assessment of IR (HOMA-IR) index, which was calculated by the following formula: Fasting insulin [μ IU/mL] $\times$ fasting glucose [mg/dL]/405, with higher values indicating a greater amount of IR [12]. Epinephrine, ADP soluble calf skin collagen, and other nonspecific reagents were purchased from Bio/Data Corporation (Horsham, PA, USA) used as platelet aggregating agents. Platelet function tests were performed with a PA profiler (Bio/Data Corporation, Horsham, PA, USA). Human platelet-rich plasma (PRP) was prepared according to a previously published method [13]. Venous blood samples were freshly obtained from patients. In order to obtain PRP, the blood samples were immediately mixed with 3.8% citrate (9:1 volume/volume) and then centrifuged at $150 \times g$ for 15 minutes at room temperature. PRP (the top layer) was removed using a plastic Pasteur pipette and transferred to a clean plastic centrifuge tube. The remaining red cells and buffy coat were centrifuged at $1500 \times g$ for 15 minutes to obtain autologous platelet-poor plasma. In vitro PA was monitored simultaneously using a Lumi-Aggregometer (Bio/Data Corporation, Horsham, PA, USA) according to the manufacturers' instructions [14]. Measurements were made for ADP, collagen, and epinephrine; the final concentrations were 10

μ mol, 2 μ g/L, and 10 μ mol, respectively. The aggregation responses were quantified as the maximum extent of aggregation, calculated by the maximum change in light transmission. This response was expressed as a percentage, considering the difference between light transmission for the platelet suspension and suspension buffer as a value of 100% (normal values were 60%–90% for collagen; 70%–90% for epinephrine; 70%–90% for ADP).

Statistical Analysis

For continuous variables, the differences between the elderly group and the control group were assessed using Student's t-test. Pearson's correlation coefficients were calculated to evaluate the correlations between continuous variables. Numerical variables were summarized as means $\pm$ standard deviations. A level of $P < 0.05$ was considered statistically significant. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS/Windows version 18.0, SPSS Inc., Chicago, IL, USA).

Results

Demographic and anthropometric characteristics of the elderly and control groups are shown in Table 1. The elderly group was between 61 and 81 years of age where the middle-aged group was between 31 and 47 years of age. No statistically significant differences with regard to BMI and waist circumference were observed between the elderly and control group ($P=0.70$ and $P=0.30$, respectively).

Table 1. Demographic and anthropometric characteristics of the elderly and control groups

Parameter	Elderly group (n=30)	Control group (n=30)	P
Age, years (range)	69.6 $\pm$ 9.5 (61 – 81)	38.6 $\pm$ 10.5 (31 – 47)	0.001
Body Mass Index (kg/m ²)	31.3 $\pm$ 4.1	32.5 $\pm$ 6.3	0.70
Waist Circumference (cm)	117.0 $\pm$ 16.1	114.1 $\pm$ 19.8	0.30

Data are expressed as mean $\pm$ standard deviation

Biochemical parameters and platelet function tests for the two groups are presented in Table 2. Although the mean level of fasting insulin was found to be higher than the control group, this difference was not statistically significant ($P=0.80$). Mean HOMA-IR index value was higher in the elderly group than the control group ($P=0.04$). Platelet counts were similar between the groups ($P=0.45$). Epinephrine induced aggregation of platelet was higher in the elderly than the control group ($P=0.01$), where platelet function tests for ADP and collagen were similar between the two groups ($P=0.9$ and $P=0.7$, respectively).

There was a strong positive correlation between HOMA-IR and platelet function test for epinephrine ($r=0.980$, $P=0.01$) in the elderly. The correlations between body weight and BMI as well as the associations between HOMA-IR, insulin, and fasting blood glucose are not discussed for both groups and the sample as a whole. Moreover, the associations between the aggregating reagents are not mentioned, as these associations are beyond the scope of this article. No other correlations were present in the elderly and the control group.

Table 2. Biochemical parameters and platelet aggregation tests in the elderly and control groups

Parameter	Elderly group (n=30)	Control group (n=30)	P
Fasting Glucose (mg/dL)	95.9 ± 16.1	89.1 ± 22.1	0.70
Fasting Insulin (μU/mL)	10.9 ± 3.1	8.9 ± 2.5	0.80
HOMA-IR	2.80 ± 0.01	2.01 ± 0.3	0.04
Platelet (x10 ³ /mm ³)	415.7 ± 51.3	399.1 ± 42.9	0.45
ADP (%)	68.5 ± 15.7	65.9 ± 19.0	0.90
Epinephrine (%)	79.2 ± 16.7	65.9 ± 22.9	0.01
Collagen (%)	69.5 ± 17.1	66.1 ± 11.6	0.70

Data are expressed as mean ± standard deviation. HOMA-IR, Homeostasis model assessment-insulin resistance; ADP, Adenosine diphosphate

Discussion

Aging is generally associated with changes in platelet function and IR [3-6,15,16]. Since visceral obesity increases after menopause and relates with IR, and also platelet functions alter in the insulin-resistant state [5] and during HRT [11], we aimed to investigate aforementioned variables in older and adult groups in order to reveal age related changes in IR, PA, and anthropometric measurements with obesity. We report that IR and epinephrine induced PA was higher in elderly obese women than the middle-aged group, in this study.

In the present study, we investigated IR in elderly female subjects who are in postmenopausal period and in middle-aged women with no menstrual cycle deterioration. Though both groups were obese and there was no statistically significant difference for BMI between the two groups; IR in the elderly group was significantly higher than the control group. This data is consistent with the study of Gupte et al., that aging was associated with an increase in IR [15]. Recently, it has been suggested that the aging process was characterized by a progressive increase in fat mass and more central distribution of adipose tissue [16]. However, we failed to demonstrate such an association. Though mean waist circumference in the elderly group was higher than the control group, it was not statistically significant which may be due to the limited number of participants. Besides, body composition analyses were not available for our study group. Moreover, IR also increases in menopause [17]. It has been reported that estrogens improve glucose homeostasis in experimental and clinical studies [18]. However, through which mechanisms estrogen exactly influences IR remains unclear [19]. It may be speculated that higher IR in the elderly women group may be in part due to menopausal changes in the estrogens and other related hormones.

The association of increasing age and platelet reactivity was investigated in several studies, though the longstanding view is that platelet reactivity increases with age, the results are not very clear [1-4,20-23]. Platelet aggregation in PRP was found to be increased with increasing age, relative to young individuals, in response to the agonists ADP, epinephrine, collagen, and arachidonic acid, in a previous study [20]. However, in this study by Johnson et al., the finding of enhanced platelet sensitivity to aggregating stimuli based on a group defined as ‘high-risk age group’ with a mean age of 45.7, and a younger group (mean age=24.2) [20]. In the present study, we found an increased response to one agonist rather than a uniform increase in platelet reactivity; the PA for epinephrine was

significantly higher in the elderly than the control group. The age range of the two study groups are very different from each other, this may have caused the difference in the results. The results from the study by Emery et al. are consistent with our findings of similarity of PA responses to ADP and collagen in the elderly and control group; in which they showed that there were not any significant age-related differences of PA in citrated whole blood in response to the agonists’ collagen, arachidonic acid, and ADP [21]. However, they did not study platelet responses to epinephrine [21]. In contrast with this study, Kasjanovova and Balaz reported that platelets of individuals aged 60 years of age demonstrated greater aggregation in response to ADP and collagen than younger individuals’ platelets [22]. Likewise, Chao et al. reported that collagen-induced PA increased with aging in non-smoker men where the range of age was 40 to 69 years [23]. However, both studies covered a restricted age range from middle age to 65/69 years old, incomparable to our range of age in the elderly group. In 2009, Gilstad et al. focused on individuals aged 45 to 92 years with stable angina where age was negatively correlated with PA, integrin alphaIIb beta3 activation, and P-selectin exposure, suggesting that there may be differences for the changes of PA with aging [24]. In a recent study of 533 consecutive stented patients with chest pain, testing platelet function to predict hypo responsiveness to clopidogrel, reported that non-insulin-dependent DM, African American race, gender and age predicted hyporesponsiveness [1]. The significantly higher response to epinephrine in our older age group may be, in part, due to postmenopausal changes in the hormones. However, the results of the studies about the effect of estrogens, gender and HRT on adhesion and aggregation of platelets are sparse and conflicting. While Otahbachi et al. reported that females scored higher with epinephrine in comparison with male subjects [25], Bar J et al. reported that the use of HRT was associated with a decrease in the aggregation of platelets induced by adrenaline [11]. However, in another study, it has been reported that HRT may increase platelet activation [26]. Additionally, our findings are not consistent with the results of the subgroup of Johnson et al. where they reported that there was no significant difference in platelet responses to either ADP or adrenaline between the two groups of premenopausal women (mean age 45.9) and postmenopausal women (mean age 57.0) [20]. However, the age ranges of the two study groups were different from each other as described before. On the other hand, platelets are sites of insulin action and considered to be subject to variation of insulin sensitivity. Insulin is considered to reduce platelet responses to the agonists ADP, thrombin, adrenaline, platelet-activating factor, collagen and sodium arachidonate [27]. Insulin down-regulates the number of α_2 adrenergic receptors on platelets in the presence of (-)-epinephrine by 50-60% when compared with the control [28]. However, the anti-aggregator ability of insulin is reduced in obese insulin-resistant subjects [29]. We found a strong positive correlation between HOMA-IR and platelet function test for epinephrine in the obese elderly group, it could be speculated that higher IR in the elderly group may have functioned against aforementioned down-regulation mechanisms.

Recently, a lower-than-expected efficacy of antiplatelet drugs in the prevention of cardiovascular events has been defined as drug “resistance”, firstly described for acetylsalicylic acid treatment, has also been suggested for pharmacologically different antiplatelet drugs such as P2Y12 inhibitors. The “resistance” for clopidogrel

has been suggested to be associated with DM, BMI, age, gender, smoking, and genetic polymorphisms [30,31] which also may suggest enhanced PA in the elderly in association with glucose metabolism disorders, gender, and body composition. However, in a systematic review and meta-analyses of randomized clinical trials on efficacy and safety of P2Y12 inhibitors according to diabetes, age, gender, BMI and body weight; it has been reported that across a wide spectrum of vascular diseases, clopidogrel does not seem to have a different efficacy profile according to diabetes, age, or gender [30]. Additionally, most of the studies reporting “resistance” to antiplatelet medication have relied on ex vivo measurements of platelet functions [31]. However, such tests have high within-subject variability, and the relation between ex vivo and in vivo platelet activation is unclear which could explain the varying associations across studies between baseline ex vivo “resistance” and cardiovascular outcomes [30,31].

The limitations of this study were limited number of the participants, lack of body composition analyses, and biochemical analyses regarding hormone levels.

Conclusion

Increasing age was associated with higher PA for epinephrine and IR shown as the HOMA-IR value for women, in this study. The reason for the increased response to epinephrine and relationship regarding IR remain uncertain in the elderly obese women. Thus, underlying mechanisms in the age-related pattern of PA tests in the obese elderly women should be further investigated in large scaled studies investigating gender differences, especially in the very old age group.

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Competing interests

The authors declare that they have no competing interest

Financial Disclosure

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Ethical approval

The study protocol was approved by the local Ethics Committee. All subjects gave written informed consent before the study.

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