

# Insulin Resistance-Induced Platelet Hyperactivity and a Potential Biomarker Role of Platelet Parameters: A Narrative Review

Amanuel Kelem <sup>1</sup>, Tiruneh Adane <sup>2</sup>, Elias Shiferaw <sup>2</sup>

<sup>1</sup>Department of Medical Laboratory Sciences, Asrat Woldeyes Health Science Campus, Debre Berhan University, Debre Berhan, Ethiopia;

<sup>2</sup>Department of Hematology and Immunohematology, School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia

Correspondence: Amanuel Kelem, Department of Medical Laboratory Sciences, Asrat Woldeyes Health Science Campus, Debre Berhan University, Debre Berhan, Ethiopia, Tel +251-9-11712112, Email amanuelkelem@gmail.com

**Background:** Insulin has an inhibitory effect on platelets; however, this is compromised in circumstances of Insulin Resistance (IR), leading to platelet hyperactivity. Platelet parameters such as mean platelet volume, platelet count, and platelet distribution width are simple and accessible potential biomarkers for the early diagnosis and prognosis of IR. Therefore, the aim of this review is to provide insight on the current status of knowledge regarding IR-induced platelet hyperactivation and the potential biomarker role of platelet parameters.

**Methods:** This narrative review included articles published in the English language. Searches were carried out at the electronic databases PubMed and Google Scholar. The search strategy was done by combining key words and related database-specific subject terms (Mesh terms) with the appropriate Boolean operators.

**Conclusion:** Increasing insulin sensitivity in insulin resistant patients would possibly cause substantial reduction in platelet activation, which in turn reduce complications related with platelet hyperactivation. The standard methods to measure IR are not frequently employed in clinical practice due to their expensiveness and complexity. Thus, early detection of IR using a simple and more widely available biomarkers such as mean platelet volume, platelet count and platelet distribution width would be beneficial. Particularly in developing countries where resource scarcity is a major constraint of the health sector, utilizing such easy and affordable biomarkers may have a crucial role.

**Keywords:** insulin resistance, platelet hyperactivity, platelet parameter

## Introduction

For the first time, Hajek et al showed that platelets have insulin receptors, with a density of about 500 receptors/cell.<sup>1</sup> Since the discovery of insulin receptors in human platelets, insulin signaling has been regarded as a key regulator of its function.<sup>2</sup> The density of functional insulin receptors on platelet membrane is comparable to that found in other target cells of insulin action.<sup>3</sup> Through a functioning insulin receptor found on the platelet surface, insulin directly regulates the actions of platelets.<sup>4</sup>

Insulin has an antiaggregating impact via reducing the responses to Adenosine diphosphate (ADP), thrombin, catecholamines, platelet-activating factor, collagen, sodium arachidonate, and angiotensin II.<sup>5</sup> Furthermore, insulin inhibits the transitory calcium increase in cytosol induced by angiotensin II and reduces intraplatelet calcium concentrations.<sup>6</sup> In vivo studies revealed that insulin infusion under euglycemic conditions impairs primary haemostasis under high shear rate conditions and uniformly inhibits platelet aggregation in response to multiple agonists. It also reduces the sensitivity of circulating platelets to agonists and platelet deposition to collagen.<sup>5</sup>

The effects of insulin depend, at least in part, on the activation of an endothelial-type constitutive nitric oxide synthase (eNOS).<sup>7,8</sup> Insulin causes a rapid rise in intraplatelet concentrations of both cyclic guanosine monophosphate

(cGMP) and cyclic adenosine monophosphate (cAMP) through increased nitric oxide (NO) production and intensifies the actions of mediators that activate adenylate cyclase. The NO-mediated stimulation of soluble guanylate cyclase activity immediately causes a rise in cGMP, but an increase in cAMP depends on cGMP production since cGMP inhibits a cAMP phosphodiesterase, thus increasing cAMP concentrations.<sup>7</sup> In addition, insulin amplifies the actions of substances including prostaglandin I<sub>2</sub> (PGI<sub>2</sub>), adenosine, and catecholamines that can activate adenylate cyclase through receptor-dependent and receptor-independent mechanisms to increase cAMP and inhibit platelet aggregation.<sup>5</sup>

In vitro tests employing platelets from healthy people showed that insulin binding to its receptor results in magnesium translocation into the platelet and is connected to decreased thrombin-induced platelet aggregation and lower synthesis of proaggregatory thromboxane (TX) B<sub>2</sub>.<sup>9</sup> The binding of insulin to its receptor located on platelets activates insulin receptor substrates (IRS-1) via tyrosine phosphorylation and mediates its interaction with the G<sub>i</sub> protein alpha subunit (G<sub>i</sub>α-subunit).<sup>7,10</sup> This results in reduced G<sub>i</sub> activity, which then affects tonic cAMP suppression, raises intraplatelet cAMP levels, inhibits P2Y<sub>12</sub> signaling, and resulting into decreased platelet activity.<sup>11</sup> Furthermore, it has been established that insulin inhibits tissue factor (TF) and modulates the concentrations of Plasminogen activator inhibitor-1 (PAI-1) to prevent platelet aggregation.<sup>12</sup>

In general, insulin infusion under euglycemic settings results in decreased sensitivity to multiple agonists and deposition to collagen, impaired primary hemostasis under high shear stress,<sup>13</sup> and decreased Thromboxane A<sub>2</sub> (TXA<sub>2</sub>) metabolite production.<sup>14</sup> Insulin causes a quick rise in the cyclic nucleotides cGMP and cAMP through an increase in NO, which has an inhibitory effect on platelet aggregation.<sup>7</sup> These findings imply that insulin reduces platelet reactivity.

There is a paucity of literatures which can give a comprehensive (all in one) information about the effect of IR on platelet hyperactivity as well as the potential biomarker role platelet parameters. Therefore, the aim of this review is to provide insight on the current status of knowledge regarding IR-induced platelet hyperactivation and the potential biomarker role of platelet parameters in IR.

## Methods

This narrative review included articles published in the English language. Searches were carried out at the electronic databases PubMed and Google Scholar. The search strategy was done by combining key words and related database-specific subject terms (Mesh terms) with the appropriate Boolean operators. The keywords used for the search strategy include: Insulin, insulin resistance, platelet (thrombocyte), platelet function, platelet hyperactivity, and platelet parameters. The software EndNote version X7 was used to manage references. An attempt has been made to comprehensively compile available updated information.

## Insulin Resistance and Platelet Hyperactivation

The discovery that human platelets contain insulin receptors that influence platelet activity prompted the theory that platelets might be impacted by Insulin Resistance (IR), resulting in a loss of the physiological effect that insulin has on platelet function.<sup>15</sup> In insulin-resistant conditions such as diabetes mellitus (DM) and Metabolic Syndrome (MetS), changes in a number of platelet functional measures have been described. It has been observed that platelets in people with insulin-resistant diseases, such as DM, are hyperactive to subthreshold stimuli and undergo rapid consumption, leading to accelerated thrombopoiesis of more reactive platelets.<sup>16</sup>

The inhibitory effects of insulin on platelets are compromised in circumstances of IR, such as central obesity, type 2 diabetes mellitus with obesity and hypertension.<sup>5</sup> Reduced sensitivity of platelets to physiological and pharmacological antiaggregating agents is a hallmark of platelet hyperactivation in IR.<sup>3</sup> It has been proposed that a key factor influencing platelet hyperactivity in IR is a loss of insulin-mediated platelet inhibition.<sup>17</sup> Effects of abnormal adipokine content on platelets are one of the mechanisms contributing to altered insulin activities on platelets.<sup>18</sup> Specifically, the adipokines resistin, leptin, PAI-1, and retinol binding protein 4 (RBP4) cause IR in megakaryocytes by suppressing insulin receptor substrates (IRS-1) expression, which has a detrimental effect on insulin signaling in platelets.<sup>19</sup>

The platelets of insulin-resistant individuals exhibit multi-step aberrations at the level of the NO/cGMP/cGMP-dependent protein kinase (PKG) and PGI<sub>2</sub>/cAMP/cAMP-dependent protein kinase (PKA) pathways. Particularly,

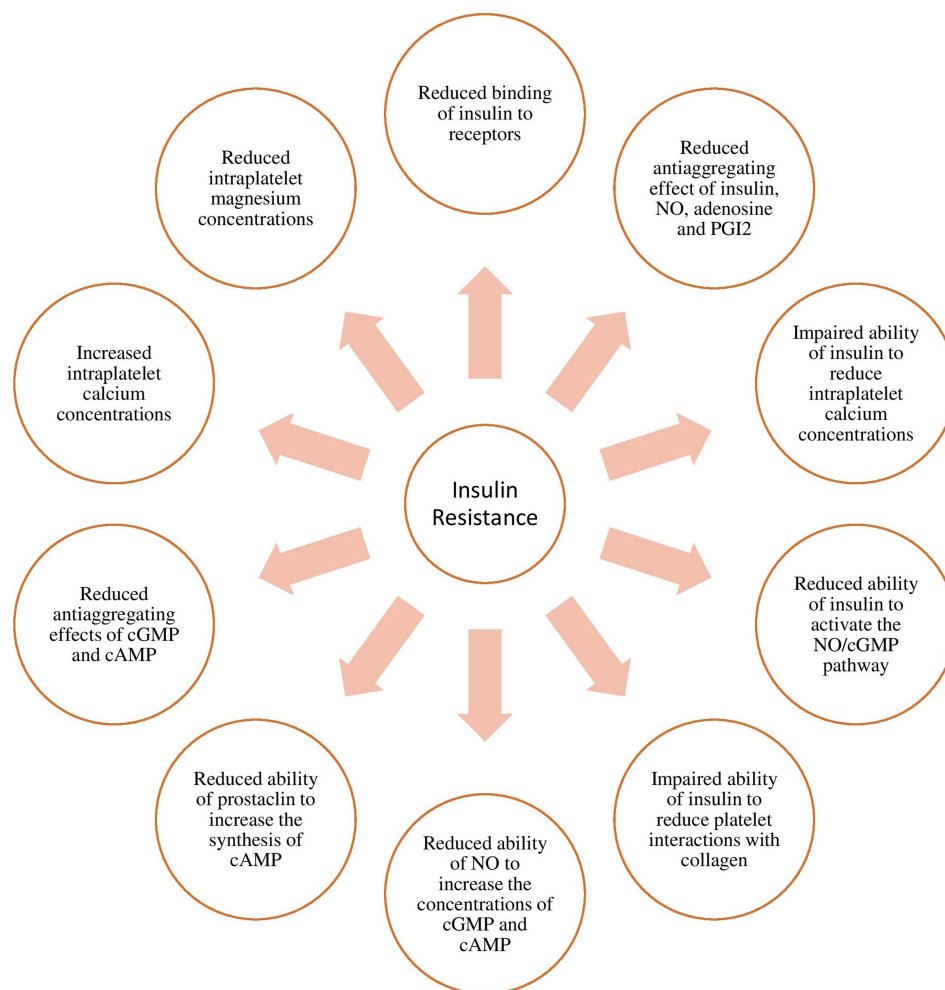
platelets have decreased NO and PGI<sub>2</sub> abilities to enhance cGMP and cAMP synthesis, respectively, and resistance to cGMP and cAMP themselves to activate their particular kinases cGMP-dependent protein kinase (PKG) and cAMP-dependent protein kinase (PKA).<sup>19,20</sup> Given that the cyclic nucleotides predominantly affect platelets by lowering intracellular Ca<sup>2+</sup>, the presence of alterations in Ca<sup>2+</sup> fluxes handling is implicated in IR. In fact, increased cytosolic Ca<sup>2+</sup> levels have been observed in insulin-resistant conditions.<sup>21</sup> Therefore, platelets from people with IR exhibit decreased response to the antiaggregating insulin, NO, and PGI<sub>2</sub>, in addition to increased pro-aggregatory stimuli (Figure 1).<sup>22</sup>

## Mechanisms of Platelet Hyperactivation in Insulin Resistance

Several mechanisms could be involved in the hyperactivation of platelet in insulin resistance.

### Hyperglycemia

Hyperglycemia is the primary indicator of IR and one of the factors that predicts platelet activation. Platelet hyperactivity has been linked to chronic hyperglycemia, as seen by increased fibrinogen binding, improved aggregation, and TX generation. Enhanced TX dependent platelet activation coexists with platelet hyperactivity in IR.<sup>23</sup> Aldose reductase, the first enzyme in the polyol pathway, is essential to the process by which platelets transduce glucose levels into enhanced TX production. In IR situations like type 2 diabetes mellitus, aldose-reductase activity is dramatically increased in vascular cells and is suggested to be a factor in vascular problems through escalating oxidative and osmotic stress.<sup>24</sup>



**Figure 1** Summary of Platelet Alterations in Insulin Resistant States.

Glucose flux via the aldose reductase enzyme results in oxidative stress (OxS), which in turn encourages the production of Reactive oxygen species (ROS). This is accomplished through a variety of processes, such as the depletion of nicotinamide adenine dinucleotide phosphate (NADPH), the reduction of glutathione (GSH) levels, and the increase of advanced glycation end products (AGEs).<sup>25</sup> In addition, ROS play a crucial role in functioning as a second messenger in thrombin- or collagen-activated platelets, causing changes in intraplatelet Ca<sup>2+</sup>, and signaling during agonist-induced platelet aggregation. The p38 $\alpha$  mitogen-activated protein kinase (MAPK)/cytosolic phospholipase A2 signaling, which stimulates arachidonic acid (AA) release and TXA2 synthesis, is strengthened by the increased OxS induced by hyperglycemia, leading to platelet activation.<sup>26</sup>

Hyperglycemia have also been associated with platelets mitochondrial dysfunction and damage, dissipation of the mitochondrial membrane potential, release of cytochrome c, and activation of caspase-3, and a subset of platelets may experience apoptosis.<sup>27</sup> Through the creation of a new prothrombotic interface for fibrin and other blood cells deposition, increased platelet death can result in the development of Platelet-derived microparticles (PMPs) that contain thrombotic mediators. High glucose exposure to platelets also affects platelet membrane components, and modifications to the fluidity of membrane proteins due to glycation or acetylation contribute to the enhanced intraplatelet Ca<sup>2+</sup> mobilization.<sup>26</sup> High amounts of cytosolic Ca<sup>2+</sup> have a significant impact on the procoagulant condition of platelet aggregates by causing phosphatidylserine to externalize and speeding up the membrane-dependent events that cause blood coagulation.<sup>28</sup>

### Dyslipidemia

There is proof that dyslipidemia increases the hyperactivation of platelets. Low-density lipoprotein (LDL) causes a rise in cytosolic Ca<sup>2+</sup>, the production of inositol trisphosphate (IP3), and the stimulation of protein kinase C (PKC) via interacting to G-protein coupled receptor on platelets. The pro-thrombotic characteristics of LDL, however, appear to be more closely linked to its oxidation.<sup>29</sup> In fact, oxidized-LDL can interact with receptors on platelets, such as the CD36 which entails the stimulation of both MAPK c-Jun N-terminal kinase (JNK) 2 and its upstream stimulator Mitogen-activated protein kinase kinase 4 (MKK4). Low-density lipoprotein stimulates the platelet AA signaling cascade at the molecular level, resulting in an increase in TXA2 synthesis. The fact that lipid-lowering medications have anti-thrombotic effects provides evidence for the relationship between hyperlipidemia in IR conditions and platelet hyperactivation.<sup>30</sup>

### Dysregulation of Calcium Signaling

The disruption of intracellular Ca<sup>2+</sup> homeostasis is one of the features of platelets from individuals with IR and several processes have been suggested as the cause of this anomaly. Reduced platelet Ca<sup>2+</sup>-ATPase activity in individuals with IR is one of the explanations.<sup>30</sup> Additionally, it is known that the Na<sup>+</sup>/Ca<sup>2+</sup> exchanger activity in the platelets from these individuals has been profoundly changed.<sup>31</sup> The higher rate of passive Ca<sup>2+</sup> leakage from the intracellular reserves is another factor influencing the increased resting cytosolic calcium in platelets. It is more than predicted that a disruption in calcium homeostasis will have a variety of effects on platelet function given that calcium regulates the majority of intracellular signaling in platelets.<sup>30</sup>

The activation of calpains is one of the effects of the elevated Ca<sup>2+</sup> in platelets. Even if calpain-mediated proteolysis contributes to normal platelet activation, IR has been linked to excessive calpain activation in platelets<sup>32</sup> which results in noticeable alterations in the platelet proteome.<sup>33</sup> The integrin-linked kinase and septin-5 were discovered to be novel calpain substrates in the platelets of DM patients, and it was demonstrated that their cleavage was responsible for the improved platelet adhesion and spreading as well as the increased  $\alpha$ -granule secretion, respectively.<sup>30</sup> Calpain also had the ability to cleave the chemokine RANTES (Regulated upon Activation, Normal T cell Expressed, and Secreted) into a form with increased chemotactic activity. The discovery that treating insulin resistant mice with calpain inhibitor conserved the platelet proteome and restored platelet hyperactivation supported the hypothesis that calpain is relevant *in vivo* for causing the platelet hyperactivity.<sup>30,33</sup>

### Oxidative Stress

Reactive oxygen species have a role in regulating platelet adhesion and aggregation, particularly at the vasculature.<sup>34</sup> However, cells and tissues proceed to OxS when ROS formation is excessive and not compartmentalized, exceeding

endogenous antioxidant capacity. This is thought to be an early sign in the pathophysiology of the majority of disorders linked to IR.<sup>2</sup> Oxidative stress levels have been observed to be higher in IR patients attributable to an imbalance between the generation of ROS and the antioxidant defenses.<sup>4</sup>

High levels of ROS affect platelet function through a variety of pathways, including isoprostane production, altered calcium mobilization, overexpression of membrane glycoproteins (GPs), and reduced NO bioavailability. The enzyme NADPH oxidase (Nox) is a significant generator of platelet ROS. Nox2 is expressed by platelets, and it has been demonstrated that its enhanced activity is associated with platelet activation, the generation of isoprostane, and/or the suppression of NO.<sup>26</sup> Patients with IR, such as diabetics, have simultaneous increases in Nox2 stimulation, platelet recruitment, and isoprostane levels.<sup>35</sup>

Patients with IR have increased oxidation processes, which considerably aid in the formation of isoprostanes from AA via a non-enzymatic process of lipid peroxidation that is mediated by oxygen-free radicals on cell membranes.<sup>36</sup> Isoprostanes is thought to be an indicator of OxS and may improve platelet sensitivity to agonists. Additionally, OxS raises intraplatelet calcium concentration by activating platelets and promoting the platelet aggregation response.<sup>4</sup> Increased OxS can cause platelet hyperactivity in three main ways, generally speaking. First and foremost, by producing more isoprostane. Second, by reducing endothelial nitric oxide synthase (eNOS) activity, which reduces NO generation and the last strategy involves boosting platelet receptor signaling.<sup>37</sup>

### Endothelial Dysfunction

When IR is present, the Phosphatidylinositol 3-kinase (PI3K) pathway is compromised, which reduces NO synthesis, and the MAPK pathway is stimulated, which increases endothelin 1(ET-1) synthesis. This promotes endothelial dysfunction, which in turn leads to platelet activation. Additionally, IR promotes vascular smooth muscle cell (VSMC) proliferation and increased release of free fatty acids (FFAs) in adipose tissue, which in turn enhances OxS and activates Protein Kinase C (PKC).<sup>4</sup> The increased release of FFAs brought on by IR also contributes to the emergence of pro-atherogenic lipid profiles and dyslipidemia. Ultimately, elevated blood levels of PAI-1, ET-1, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-6, and C-reactive protein (CRP), suggest a link between IR with low-grade inflammation and endothelial dysfunction.<sup>4,38</sup> Given that endothelial cells play a crucial role in preventing platelet adhesion and maintaining normal platelet function, the presence of endothelial dysfunction would favor platelet adhesion and activation.<sup>26</sup>

## Platelet Parameters and Other Biomarkers in Insulin Resistance

Hematological analyzers may provide a range of platelet parameters that can be used to quickly identify when the structure of the platelets has changed, aiding in the early diagnosis of the prothrombotic condition of the platelets. Platelet parameters can serve as an alert for diagnosing the initiation or progression of diseases.<sup>39</sup> Platelet size, enzymatic activity, and prothrombotic propensity are all correlated with platelet parameters including Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW).<sup>40</sup>

A growing body of research also points to the potential use of hematologic parameters including platelet parameters in identifying persons who are at risk for developing IR.<sup>41</sup> These parameters may be measured by automated hematology analyzers during regular hemograms at a relatively modest cost.<sup>42</sup> There are very few accepted methods to measure IR. The homeostasis model assessment of insulin resistance (HOMA-IR), while useful for this, is not frequently employed in clinical practice. Thus, early detection of IR utilizing a simple and more widely available biomarker for the disease would be beneficial.<sup>41</sup>

### Insulin Resistance and Mean Platelet Volume

Mean platelet volume is a commonly used marker of platelet size and activity that could be measured easily and inexpensively.<sup>43</sup> Automatic measurement of platelet cell size is used to determine MPV, which is obtained by dividing plateletcrit by the total number of platelets.<sup>44</sup> Larger platelets have higher enzymatic and metabolic activity with increased prothrombotic potential than smaller ones.<sup>43</sup> Larger platelets, in particular, exhibit greater granule content, higher TXA2 levels, and enhanced expression of adhesion proteins such as GpIb and GpIIB/IIIA.<sup>45</sup> Consequently, MPV could be an indicator of platelet activation.<sup>44</sup> Additionally, elevated MPV is a clear indicator of thrombocyte production showing a greater

platelet turnover rate and more young platelets being produced.<sup>46</sup> It has also been proposed that MPV also directly related with platelet aggregation function since the platelet aggregation function weakens when the level of MPV decreases.<sup>47</sup>

Mean platelet volume could be a useful independent factor that can provide insight into the pathophysiology, risk stratification, and optimal management of different diseases. Mean platelet volume increases over the upper limit of the normal range in a number of situations, including when the incidence of young platelets is increased owing to rapid platelet production or destruction.<sup>44</sup> In situations closely linked to IR, such as MetS, obesity, DM, and hypertension, the MPV value has been observed to rise, and a link between MPV and cardiovascular diseases has also been indicated.<sup>46</sup> Such findings may aid in the early identification of a disease and the prompt commencement of effective therapy.<sup>44</sup>

Prothrombotic propensity is caused by metabolic illnesses such dyslipidemia, obesity, high blood pressure, and glucose levels, at least in part due of platelet hyperactivation brought on by IR. Studies have found a high correlation between these clinical characteristics and platelet activation, as measured by MPV.<sup>43</sup> Metabolic syndrome and DM, IR as a primary underlying cause in both cases, were also associated with high MPV scores.<sup>44</sup> Additional data support the idea that IR plays a key role in the connection between MPV and MetS.<sup>48</sup>

Insulin resistance and MPV have been shown to be positively associated.<sup>43</sup> As measured by MPV, larger platelets have more adhesion receptors and denser granules, generate more serotonin and thromboglobulin, and have a higher prothrombotic potential.<sup>49</sup> Although the mechanism underlying higher MPV in insulin resistance states such as MetS is not fully understood, the following explanation may help. An indication of IR is an increase in adipose tissue, which releases a number of adipokines and cytokines, including leptin, adiponectin, IL, and NO, which induce megakaryocytes to produce larger platelets.<sup>50</sup>

Additionally, insulin resistant individuals have extensively damaged vascular endothelium cells, and endothelial dysfunction may potentially cause the same mechanism.<sup>43</sup> Furthermore, MPV tends to be greater in those with higher HOMA-IR index.<sup>51</sup> However, some researches did not discover a statistically significant difference in MPV levels in insulin resistant conditions,<sup>52</sup> indicating that the relationship between MPV and IR needs more investigation.

Obesity, the primary factor causing IR, and MPV have repeatedly been linked,<sup>50</sup> This is further corroborated by the result that weight loss in obese patients is likewise linked to a drop in MPV.<sup>43</sup> Increased plasma osmotic pressure from hyperglycemia, an aspect of IR, causes platelet osmotic swelling and higher MPV.<sup>53</sup> Reduced MPV in diabetic individuals who had better glycemic control is another evidence of this. This shows that in patients with IR, improved glycemic management may be beneficial in reducing platelet activity as evidenced by lower MPV.<sup>43</sup> Consequently, it could be advantageous to employ MPV as both a risk marker and a therapeutic goal.<sup>54</sup>

## Insulin Resistance and Platelet Count

The platelet count (PC) is a cheap and simple-to-read test that is frequently requested in clinical settings.<sup>41</sup> Extreme PC readings have reportedly been linked to cardiovascular illnesses.<sup>31</sup> Higher PC have been linked to IR in adult populations, even when they are within the normal range. According to studies, PC is positively correlated with IR and may be used as a diagnostic tool to help identify people who are insulin resistant. Additionally, it has been suggested that individuals with increased PC take the likelihood of IR into account.<sup>41</sup> Another piece of evidence for the direct correlation between IR and PC might be the tight relationship between HOMA-IR and PC.<sup>55</sup>

Several possible mechanisms could underlie the significant association between PC and IR. Obesity is a condition marked by excess adipose tissue and frequently accompanied by IR.<sup>56</sup> Omental adipose tissue secretes excessive amounts of numerous adipokines, including thrombopoietin, a megakaryocytic growth factor.<sup>57</sup> Given that excessive thrombopoietin production is linked to both platelet activation and hyperinsulinemia/IR, the connection between PC and IR may be reinforced by thrombopoietin.<sup>41</sup>

Increasing adipose tissue can also release a variety of cytokines and adipokines including leptin, TNF- $\alpha$ , adiponectin, and IL.<sup>47</sup> For instance, IL-6 enhances the liver's production of thrombopoietin (TPO), which in turn stimulates the proliferation of megakaryocyte progenitors and raises PC.<sup>58</sup> These pro-inflammatory cytokines also promote a persistent low grade inflammatory state, which elevates PC. Additionally, PLT lifespan is shorter in subjects with IR, resulting in increased PC.<sup>47</sup> Following bariatric surgery, insulin resistant obese individuals showed a reduction in PC,<sup>31</sup> suggesting a possible link between PC and IR in these patients.

The relationship between elevated PC and IR may also be influenced by OxS, the other underlying cause of IR. Insulin resistance has been demonstrated to be linked to increased OxS and activated platelet function.<sup>59</sup> Higher level of oxidative indicators like glutathione and thiobarbituric acid compounds are linked to higher PC in animal models.<sup>60</sup> Additionally, studies have shown that diets with increased antioxidant levels lowered platelet numbers, which might be justified by a reduction in OxS.<sup>61</sup>

## Insulin Resistance and Platelet Distribution Width

The platelet distribution width is a morphometric indicator that depicts the size distribution of the peripheral platelet population.<sup>62</sup> Platelet distribution width is regarded as a marker of platelet function and activation<sup>63</sup> and it can measure the variation in platelet size.<sup>39</sup> Higher PDW readings show a wider range of platelet sizes, which may be the result of higher platelet activation, destruction, and consumption.<sup>63</sup> Platelet distribution width illustrates the diversity of platelet morphology, which includes the existence of larger, reticulated platelets. Despite the paucity of data on PDW, researches have linked or predicted cardiovascular problems with PDW.<sup>62</sup>

Increased PDW has been linked to endothelial dysfunction-related diseases, including IR.<sup>62</sup> Platelets' signaling pathways are dysregulated in insulin-resistant diseases like DM, which makes it more likely for them to activate and aggregate in response to a particular stimulus. When platelets become active, their discoid form changes to a spherical one, modifying the PDW. Poor glycemic control, a sign of IR, has also been connected to PDW.<sup>39</sup>

Hypercoagulability is another potential pathophysiological cause for increased platelet size variability or high PDW in IR. On the one hand, increased platelet consumption and destruction during thrombosis lead to a decline in PC, while on the other, stimulation of thrombopoiesis results in an increase in the release of younger, larger platelets from the bone marrow into the blood circulation.<sup>63</sup> Increases in PDW are also connected to hyperleptinemia and hypoadiponectinemia in IR.<sup>26</sup>

## Other Biomarkers of Platelet Hyperactivation in Insulin Resistance

### Soluble P-Selectin

The platelet is the principal cellular source of P-selectin, a cellular adhesion molecule with procoagulant activity and the capacity to activate leukocyte integrins. Blood levels of the soluble form of P-selectin are consistent with platelet activation.<sup>26</sup> P-selectin, which is normally stored in the alpha granules of platelets, can move to the plasma membrane during an inflammatory response where it can interact with ligands to form leukocyte-platelet aggregates that encourage the adherence and infiltration of inflammatory cells. P-selectin plasma levels have been shown to be higher in insulin-resistant patients, which has been linked to platelet activation.<sup>64</sup>

### CD40 Ligand

Additionally, activated platelets produce the trimeric transmembrane protein Soluble CD40 ligand (sCD40L), which shares structural similarities with the TNF- $\alpha$  superfamily.<sup>65</sup> More than 95% of the sCD40L in the blood emanates from platelets; it is highly stored in the cytoplasm of unstimulated platelets, expressed on the platelet surface, and then cleaves into the soluble trimeric fragment and is released shortly after platelet activation. Measuring sCD40L is regarded as a biomarker of cardiovascular events, including IR.<sup>26</sup>

Studies have shown that IR-related metabolic diseases and adipose tissue inflammation are ameliorated by genetically- or antibody-mediated inhibition of CD40L signaling.<sup>66</sup> Numerous proinflammatory and prothrombotic factors, such as IL-1, IL-6, IL-8, IL-12, TNF- $\alpha$ , Monocyte chemoattractant protein-1 (MCP-1), and matrix metalloproteinases (MMPs) are expressed as a result of CD40/CD40L interaction, accelerating the adhesion of monocytes to the vascular endothelium, which also promotes endothelial injury caused by ROS and the rupture of atheromatous plaques.<sup>26</sup> As a result, sCD40 plasma levels are regarded as reliable indicators of *in vivo* platelet activation in IR.<sup>67</sup>

### Platelet-Derived Microparticles

The PMPs are tiny, membrane-bound microparticles with a diameter of less than 0.1 micron that contain bioactive proteins and genetic material (ie, mRNAs and microRNAs). Elevated levels of circulating PMPs, which are produced when platelets are activated by different agonists, are linked to the majority of cardiovascular risk factors, including IR.<sup>68</sup>

## Conclusion and Recommendations

Patients with insulin resistance have evidence of platelet hyperactivity. This results from a combination of factors including the effects of hyperglycemia, hyperlipidemia, endothelial dysfunction and oxidative stress. Thus, increasing insulin sensitivity in insulin resistant patients would possibly cause substantial reduction in platelet activation, which in turn reduce complications related with platelet hyperactivation. The standard methods to measure insulin resistance are not frequently employed in clinical practice due to their expensiveness and complexity. Thus, early detection of insulin resistance using a simple and more widely available biomarkers such as mean platelet volume, platelet count and platelet distribution width would be beneficial. Particularly in developing countries where resource scarcity is the major constraint of the health sector, utilizing such easy and affordable biomarkers may have a crucial role.

Therefore, we recommend physicians and other healthcare providers to consider the possibility of platelet function abnormality into account when choosing a diagnostic and treatment strategy for insulin resistant patients, with an emphasis on platelet hyperactivation and its associated complications. Additionally, in light of the current evidences, using platelet parameters as a possible diagnostic and prognostic biomarker of insulin resistance in the routine clinical practice may play an important role.

There is a paucity of literatures on the role of novel platelet parameters such as immature platelet fraction (IPF), high immature platelet fraction (H-IPF), platelet large cell ratio (P-LCR), platelet-X (PLT-X), and plateletricit (PCT) in insulin resistance. Therefore, researchers are recommended to investigate the association between these novel platelet parameters with insulin resistance.

## Abbreviations

AA, Arachidonic acid; cAMP, Cyclic Adenosine Monophosphate; CD40L, CD40 ligand; cGMP, Cyclic Guanosine Monophosphate; DM, Diabetes Mellitus; GP, Glycoprotein; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; IL, Interleukin; IR, Insulin Resistance; MetS, Metabolic Syndrome; MPV, Mean Platelet Volume; NO, Nitric Oxide; Nox, NADPH oxidase; OxS, Oxidative Stress; PAI-1, Plasminogen Activator Inhibitor-1; PC, Platelet Count; PDW, Platelet Distribution Width; PGI<sub>2</sub>, Prostaglandin I<sub>2</sub>; PMP, Platelet-derived microparticle; ROS, Reactive Oxygen Species; sCD40L, Soluble CD40 ligand; TNF- $\alpha$ , Tumour necrosis factor  $\alpha$ ; TX, Thromboxane; TXA<sub>2</sub>, Thromboxane A<sub>2</sub>.

## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

## Funding

The authors received no specific funding for this work.

## Disclosure

The authors declare that they have no competing interests in this work.

## References

1. Hajek AS, Joist JH, Baker RK, Jarett L, Daughaday W. Demonstration and partial characterization of insulin receptors in human platelets. *J Clin Invest.* 1979;63(5):1060–1065.
2. Gaspar RS, Trostchansky A, Paes AMDA. Potential role of protein disulfide isomerase in metabolic syndrome-derived platelet hyperactivity. *Oxid Med Cell Longev.* 2016;2016:2423547. doi:10.1155/2016/2423547
3. Falcon C, Pfliegler G, Deckmyn H, Vermynen J. The platelet insulin receptor: detection, partial characterization, and search for a function. *Biochem Biophys Res Commun.* 1988;157(3):1190–1196. doi:10.1016/S0006-291X(88)81000-3
4. Kaur R, Kaur M, Singh J. Endothelial dysfunction and platelet hyperactivity in type 2 diabetes mellitus: molecular insights and therapeutic strategies. *Cardiovasc Diabetol.* 2018;17(1):121. doi:10.1186/s12933-018-0763-3



5. Anfossi G, Russo I, Trovati M. Platelet dysfunction in central obesity. Nutrition, metabolism, and cardiovascular diseases. *NMCD*. 2009;19(6):440–449. doi:10.1016/j.numeed.2009.01.006
6. Touyz RM, Schiffrin EL. Blunted inhibition by insulin of agonist-stimulated calcium, pH and aggregatory responses in platelets from hypertensive patients. *J Hypertens*. 1994;12(11):1255–1263. doi:10.1097/00004872-199411000-00008
7. Trovati M, Anfossi G, Massucco P, et al. Insulin stimulates nitric oxide synthesis in human platelets and, through nitric oxide, increases platelet concentrations of both guanosine-3', 5'-cyclic monophosphate and adenosine-3', 5'-cyclic monophosphate. *Diabetes*. 1997;46(5):742–749. doi:10.2337/diab.46.5.742
8. Trovati M, Paola M, Mattiello L, Piretto V, Mularoni E, Anfossi G. The insulin-induced increase of guanosine-3', 5'-cyclic monophosphate in human platelets is mediated by nitric oxide. *Diabetes*. 1996;45(6):768–770. doi:10.2337/diab.45.6.768
9. Kakourous N, Rade JJ, Kourliouros A, Resar JR. Platelet function in patients with diabetes mellitus: from a theoretical to a practical perspective. *Int J Endocrinol*. 2011;2011:742719. doi:10.1155/2011/742719
10. Ferreira IA, Eybrechts KL, Mocking AI, Kroner C, Akkerman J-WN. IRS-1 mediates inhibition of Ca<sup>2+</sup> mobilization by insulin via the inhibitory G-protein Gi. *J Biol Chem*. 2004;279(5):3254–3264. doi:10.1074/jbc.M305474200
11. Hunter R, Hers I. Insulin/IGF-1 hybrid receptor expression on human platelets: consequences for the effect of insulin on platelet function. *J Thromb Haemost*. 2009;7(12):2123–2130. doi:10.1111/j.1538-7836.2009.03637.x
12. Paneni F, Beckman JA, Creager MA, Cosentino F. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part I. *Eur Heart J*. 2013;34(31):2436–2443. doi:10.1093/eurheartj/ehf149
13. Westerbacka J, Yki-Järvinen H, Turpeinen A, et al. Inhibition of platelet-collagen interaction: an in vivo action of insulin abolished by insulin resistance in obesity. *Arterioscler Thromb Vasc Biol*. 2002;22(1):167–172. doi:10.1161/hq0102.101546
14. Mayfield RK, Halushka PV, Wohltmann HJ, et al. Platelet function during continuous insulin infusion treatment in insulin-dependent diabetic patients. *Diabetes*. 1985;34(11):1127–1133. doi:10.2337/diab.34.11.1127
15. Santilli F, Vazzana N, Liani R, Guagnano MT, Davi G. Platelet activation in obesity and metabolic syndrome. *Obes Rev*. 2012;13(1):27–42. doi:10.1111/j.1467-789X.2011.00930.x
16. Rodriguez BAT, Johnson AD. Platelet measurements and type 2 diabetes: investigations in two population-based cohorts. *Front Cardiovasc Med*. 2020;7. doi:10.3389/fcvm.2020.00118
17. Morange PE, Alessi MC. Thrombosis in central obesity and metabolic syndrome: mechanisms and epidemiology. *Thromb Haemost*. 2013;110(4):669–680. doi:10.1160/TH13-01-0075
18. Gerrits AJ, Gitz E, Koekman CA, Visseren FL, van Haeften TW, Akkerman JWN. Induction of insulin resistance by the adipokines resistin, leptin, plasminogen activator inhibitor-1 and retinol binding protein 4 in human megakaryocytes. *Haematologica*. 2012;97(8):1149. doi:10.3324/haematol.2011.054916
19. Anfossi G, Russo I, Massucco P, et al. Impaired synthesis and action of antiaggregating cyclic nucleotides in platelets from obese subjects: possible role in platelet hyperactivation in obesity. *Eur J Clin Invest*. 2004;34(7):482–489. doi:10.1111/j.1365-2362.2004.01370.x
20. Russo I, Del Mese P, Doronzo G, et al. Platelet resistance to the antiaggregatory cyclic nucleotides in central obesity involves reduced phosphorylation of vasodilator-stimulated phosphoprotein. *Clin Chem*. 2007;53(6):1053–1060. doi:10.1373/clinchem.2006.076208
21. Resnick LM. Cellular ions in hypertension, insulin resistance, obesity, and diabetes: a unifying theme. *J Am Soc Nephrol*. 1992;3(4):S78. doi:10.1681/ASN.V34s78
22. Anfossi G, Russo I, Trovati M. Platelet resistance to the anti-aggregating agents in the insulin resistant states. *Curr Diabetes Rev*. 2006;2(4):409–430. doi:10.2174/1573399810602040409
23. Santilli F, Simeone P, Liani R, Davi G. Platelets and diabetes mellitus. *Prostaglandins Other Lipid Mediat*. 2015;120:28–39.
24. Ferroni P, Basili S, Falco A, Davi G. Platelet activation in type 2 diabetes mellitus. *J Thromb Haemost*. 2004;2(8):1282–1291. doi:10.1111/j.1538-7836.2004.00836.x
25. Chung SS, Ho EC, Lam KS, Chung SK. Contribution of polyol pathway to diabetes-induced oxidative stress. *J Am Soc Nephrol*. 2003;14(suppl 3):S233–S6. doi:10.1097/01.ASN.0000077408.15865.06
26. Barale C, Russo I. Influence of cardiometabolic risk factors on platelet function. *Int J Mol Sci*. 2020;21(2):623. doi:10.3390/ijms21020623
27. Tang WH, Stitham J, Jin Y, et al. Aldose reductase-mediated phosphorylation of p53 leads to mitochondrial dysfunction and damage in diabetic platelets. *Circulation*. 2014;129(15):1598–1609. doi:10.1161/CIRCULATIONAHA.113.005224
28. Obydenny SI, Sveshnikova AN, Ataulkhanov FI, Pantelev MA. Dynamics of calcium spiking, mitochondrial collapse and phosphatidylserine exposure in platelet subpopulations during activation. *J Thromb Haemost*. 2016;14(9):1867–1881. doi:10.1111/jth.13395
29. Pedreño J, Hurt-Camejo E, Wiklund O, Badimón L, Masana L. Low-density lipoprotein (LDL) binds to a G-protein coupled receptor in human platelets: evidence that the proaggregatory effect induced by LDL is modulated by down-regulation of binding sites and desensitization of its mediated signaling. *Atherosclerosis*. 2001;155(1):99–112. doi:10.1016/S0021-9150(00)00545-1
30. Randriamboavonjy V. Mechanisms involved in diabetes-associated platelet hyperactivation. In: *The Non-Thrombotic Role of Platelets in Health and Disease*. IntechOpen; 2015.
31. Fang KC, Cheng YL, Su CW, et al. Higher platelet counts are associated with metabolic syndrome independent of fatty liver diagnosis. *J Chin Med Assoc*. 2017;80(3):125–132. doi:10.1016/j.jcma.2016.07.003
32. Randriamboavonjy V, Pistrosch F, Bölek B, et al. Platelet sarcoplasmic endoplasmic reticulum Ca<sup>2+</sup>-ATPase and  $\mu$ -calpain activity are altered in type 2 diabetes mellitus and restored by rosiglitazone. *Circulation*. 2008;117(1):52–60. doi:10.1161/CIRCULATIONAHA.107.719807
33. Randriamboavonjy V, Isaak J, Elgheznawy A, et al. Calpain inhibition stabilizes the platelet proteome and reactivity in diabetes. *Am J Hematol*. 2012;120(2):415–423.
34. Darley-Usmar V, Halliwell B. Blood radicals: reactive nitrogen species, reactive oxygen species, transition metal ions, and the vascular system. *Pharm Res*. 1996;13(5):649–662. doi:10.1023/A:1016079012214
35. Cangemi R, Pignatelli P, Carnevale R, et al. Platelet isoprostane overproduction in diabetic patients treated with aspirin. *Diabetes*. 2012;61(6):1626–1632. doi:10.2337/db11-1243
36. Lawson JA, FitzGerald GA, Rokach J. Isoprostanes: formation, analysis and use as indices of lipid peroxidation in vivo. *J Biol Chem*. 1999;274(35):24441–24444. doi:10.1074/jbc.274.35.24441

37. Singh I, Mok M, Christensen A-M, Turner AH, Hawley JA. The effects of polyphenols in olive leaves on platelet function. *Nutr Metab Cardiovasc Dis.* 2008;18(2):127–132. doi:10.1016/j.numecd.2006.09.001
38. Inoguchi T, Li P, Umeda F, et al. High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C--dependent activation of NAD (P) H oxidase in cultured vascular cells. *Diabetes.* 2000;49(11):1939–1945. doi:10.2337/diabetes.49.11.1939
39. E-GA M, Abdallah N, Eldars W. Mean platelet volume and platelet distribution width correlate with microvascular complications in Egyptian people with type 2 diabetes mellitus. *Curr Diabetes Rev.* 2021;17(8):8–15.
40. Bhanukumar M, Ramaswamy PK, Peddi NK, Menon VB. Mean platelet volume and platelet distribution width as markers of vascular thrombosis in type 2 diabetes mellitus. *J Postgrad Med.* 2016;50(3):127–131. doi:10.5005/jp-journals-10028-1204
41. Park JM, Lee JW, Shim JY, Lee YJ. Relationship between platelet count and insulin resistance in Korean adolescents: a nationwide population-based study. *Metab Syndr Relat Disord.* 2018;16(9):470–476. doi:10.1089/met.2018.0016
42. Joshi A, Jaison J. Study of platelet parameters-mean platelet volume (mpv) and platelet distribution width (PDW) in type 2 diabetes mellitus. *Ann Path Lab Med.* 2020;2020:6.
43. Ding Q, Wang F, Guo X, Liang M. The relationship between mean platelet volume and metabolic syndrome in patients with type 2 diabetes mellitus: a retrospective study. *Medicine.* 2021;100(13):e25303. doi:10.1097/MD.00000000000025303
44. Braester A, Shturman A, Raviv B, Dorosinsky L, Rosental E, Atar S. What a family doctor should know about incidental finding of high mean platelet volume, metabolic syndrome, and pre-diabetes. *Isr Med Assoc J.* 2021;23(11):699–702.
45. Nardin M, Verdoia M, Barbieri L, De Luca G. Impact of metabolic syndrome on mean platelet volume and its relationship with coronary artery disease. *Platelets.* 2019;30(5):615–623. doi:10.1080/09537104.2018.1499885
46. Baldane S, Ipekci SH, Kebapcilar A. Relationship between insulin resistance and mean platelet volume in gestational diabetes mellitus. *J Lab Physicians.* 2015;7(2):112–115. doi:10.4103/0974-2727.163134
47. Zhao F, Yan Z, Meng Z. Relationship between mean platelet volume and metabolic syndrome in Chinese patients. *Sci Rep.* 2018;8(1):14574. doi:10.1038/s41598-018-32751-1
48. Sansanayudh N, Muntham D, Yamwong S, Sritara P, Akrawichien T, Thakkinstian A. The association between mean platelet volume and cardiovascular risk factors. *Eur J Intern Med.* 2016;30:37–42. doi:10.1016/j.ejim.2015.11.028
49. Noris P, Melazzini F, Balduini CL. New roles for mean platelet volume measurement in the clinical practice? *Platelets.* 2016;27(7):607–612. doi:10.1080/09537104.2016.1224828
50. Vizioli L, Muscari S, Muscari A. The relationship of mean platelet volume with the risk and prognosis of cardiovascular diseases. *Int J Clin Pract.* 2009;63(10):1509–1515. doi:10.1111/j.1742-1241.2009.02070.x
51. Kebapcilar L, Kebapcilar AG, Ilhan TT, et al. Is the mean platelet volume a predictive marker of a low apgar score and insulin resistance in gestational diabetes mellitus? A retrospective case-control study. *Oxid Med Cell Longev.* 2016;10(10):Oc06–oc10.
52. Yang X-J, Zhang L-Y, Q-H M, et al. Platelet parameters in Chinese older adults with metabolic syndrome. *Endocr Connect.* 2020;9(7):696–704. doi:10.1530/EC-20-0209
53. Shah B, Sha D, Xie D, Mohler ER, Berger JS. The relationship between diabetes, metabolic syndrome, and platelet activity as measured by mean platelet volume: the national health and nutrition examination survey, 1999–2004. *Diabetes Care.* 2012;35(5):1074–1078. doi:10.2337/dc11-1724
54. Furman-Niedziejko A, Rostoff P, Rychlak R, et al. Relationship between abdominal obesity, platelet blood count and mean platelet volume in patients with metabolic syndrome. *Folia Med Cracov.* 2014;54(2):55–64.
55. Lim HJ, Seo MS, Shim JY, Kim KE, Shin YH, Lee YJ. The association between platelet count and metabolic syndrome in children and adolescents. *Platelets.* 2015;26(8):758–763. doi:10.3109/09537104.2014.995613
56. Kim SH, Reaven G. Obesity and insulin resistance: an ongoing saga. *Diabetes.* 2010;59(9):2105–2106. doi:10.2337/db10-0766
57. Maury E, Brichard SM, Pataký Z, Carpentier A, Golay A, Bobbioni-Harsch E. Effect of obesity on growth-related oncogene factor- $\alpha$ , thrombopoietin, and tissue inhibitor metalloproteinase-1 serum levels. *Obesity.* 2010;18(8):1503–1509. doi:10.1038/oby.2009.464
58. Chen YL, Hung YJ, He CT, et al. Platelet count can predict metabolic syndrome in older women. *Platelets.* 2015;26(1):31–37. doi:10.3109/09537104.2014.880415
59. Karamouzis I, Pervanidou P, Berardelli R, et al. Enhanced oxidative stress and platelet activation combined with reduced antioxidant capacity in obese prepubertal and adolescent girls with full or partial metabolic syndrome. *Horm Metab Res.* 2011;43(09):607–613. doi:10.1055/s-0031-1284355
60. Karbinger MS, Sierra L, Minahk C, Fonio MC, de Bruno MP, Jerez S. The role of oxidative stress in alterations of hematological parameters and inflammatory markers induced by early hypercholesterolemia. *Life Sci.* 2013;93(15):503–508. doi:10.1016/j.lfs.2013.08.003
61. Bonaccio M, Di Castelnuovo A, De Curtis A, et al. Adherence to the Mediterranean diet is associated with lower platelet and leukocyte counts: results from the Moli-sani study. *Am J Hematol.* 2014;123(19):3037–3044.
62. Zaccardi F, Rocca B, Pitocco D, Tanese L, Rizzi A, Ghirlanda G. Platelet mean volume, distribution width, and count in type 2 diabetes, impaired fasting glucose, and metabolic syndrome: a meta-analysis. *Diabetes Metab Res Rev.* 2015;31(4):402–410. doi:10.1002/dmrr.2625
63. Tzur I, Barchel D, Izhakian S, et al. Platelet distribution width: a novel prognostic marker in an internal medicine ward. *J Community Hosp Intern Med.* 2019;9(6):464–470. doi:10.1080/20009666.2019.1688095
64. Patel MS, Miranda-Nieves D, Chen J, Haller CA, Chaikof EL. Targeting P-selectin glycoprotein ligand-1/P-selectin interactions as a novel therapy for metabolic syndrome. *Transl Res.* 2017;183:1–13. doi:10.1016/j.trsl.2016.11.007
65. André P, Nannizzi-Alaimo L, Prasad SK, Phillips DR. Platelet-derived CD40L: the switch-hitting player of cardiovascular disease. *Am Heart Assoc.* 2002;2022:896–899.
66. Poggi M, Engel D, Christ A, et al. CD40L deficiency ameliorates adipose tissue inflammation and metabolic manifestations of obesity in mice. *Arterioscler Thromb Vasc Biol.* 2011;31(10):2251–2260. doi:10.1161/ATVBAHA.111.231357
67. Russo I, Traversa M, Bonomo K, et al. In central obesity, weight loss restores platelet sensitivity to nitric oxide and prostacyclin. *Obesity.* 2010;18(4):788–797. doi:10.1038/oby.2009.302
68. Zahran AM, Sayed SK, Abd El Hafeez HA, Khalifa WA, Mohamed NA, Hetta HF. Circulating microparticle subpopulation in metabolic syndrome: relation to oxidative stress and coagulation markers. *Diabetes Metab Syndr Obes.* 2019;12:485. doi:10.2147/DMSO.S191750

Diabetes, Metabolic Syndrome and Obesity

Dovepress

### Publish your work in this journal

Diabetes, Metabolic Syndrome and Obesity is an international, peer-reviewed open-access journal committed to the rapid publication of the latest laboratory and clinical findings in the fields of diabetes, metabolic syndrome and obesity research. Original research, review, case reports, hypothesis formation, expert opinion and commentaries are all considered for publication. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/diabetes-metabolic-syndrome-and-obesity-journal>