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Postprandial activation of platelets as a possible mechanism for the development of atherosclerosis

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Abstract

Platelets, apart from being cellular mediators of thrombosis that occurs at the late stages of atherosclerosis, they are actively involved in the early phases of the disease. A chronic, subclinical activation of platelets may play a crucial role in the development of the atherosclerotic plaque. Postprandial dysmetabolism could play the role of the daily platelet stimulant since it induces a pro-inflammatory and pro-oxidative environment in the endothelium which favors platelet activation. The effects of alimentary or oral glucose load hyperglycemia/hyperinsulinemia on platelet functions are contradictory and depend on the functional marker that is determined and the clinical profile of the volunteers. In general, the homeostatic mechanisms of healthy people prevent the postprandial activation of platelets while the platelets of metabolic syndrome or diabetic patients are more susceptible to acute hyperglycemic increments compared to healthy ones. A similar pattern is observed for postprandial hyperlipidemia. Oxidative stress, impairment of insulin's antiplatelet actions, activation of platelet by triglyceride-rich lipoproteins remnants, reduced bioavailability of NO and alterations of the calcium and magnesium homeostasis are some of the mechanism that explain the postprandial modulation of platelets. Nutritional and pharmacological interventions, aiming to attenuate postprandial platelet responses, may have a beneficial role on atherosclerosis development.

Key words: atherosclerosis; hyperglycemia; hyperlipidemia;oxidative stress; platelets; postprandial state

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1. Introduction

Western dietary patterns are characterized by the daily intake of high-energy meals, rich in refined carbohydrates and fats. The consumption of such meals is followed by an acute, transient, overwhelming flow of high-energy substrates (glucose and fatty acid) into cells. The insufficient metabolic clearance of these substrates (postprandial dysmetabolism), which is more pronounced under conditions of insulin resistance, leads to the production of reactive oxygen and nitrogen species (RONS), advanced glycation end products (AGEs) and triglyceride-rich lipoprotein (TRL) remnants.[1] Zilversmith was the first researcher who clearly linked the postprandial state with atherosclerosis in 1979.[2] Since then several studies have demonstrated an association of postprandial dysmetabolism with endothelial dysfunction, oxidative stress, inflammation and thrombosis.[3] The mechanistic studies have been confirmed in large cohort studies which demonstrated the positive correlation of postprandial hyperglycemia and hyperlipidemia with cardiovascular disease (CVD) risk.[4] Taking into account that the biochemical perturbations observed in the postprandial state favors platelet dysfunction and that platelet activation can trigger atherogenetic mechanisms it is reasonable for someone to expect that postprandial modulation of platelet functions may serve as an additional explanatory mechanism for the cardiovascular consequences of postprandial dysmetabolism. Whether this notion is confirmed by the scientific data so far is described in the following paragraphs.

2. Platelet activation and atherosclerosis

It is now widely accepted that platelets, apart from being cellular mediators of thrombosis that occurs at the late stages of atherosclerosis, they are actively involved in the early phases of the disease. A chronic, subclinical activation of platelets plays a crucial role in the initiation and progression of atherosclerosis since activated platelets can interact with endothelial cells even if the endothelial integrity is intact. Actually, the adhesion of platelets to a non-injured, but activated endothelium, is similar to the adhesion observed under injured conditions.[5] A chronic infusion of activated platelets to dyslipidemic mice accelerated atherogenesis[6] while, in animal models of atherosclerosis, the administration of ADP antagonists resulted to a 47% decrease of the atheromatous plaque and improvement of plaque stability.[7] Moreover, the presence of adhered platelets to endothelium precedes the development of atherosclerotic lesions in both hypercholesterolemic rabbits[8] and apoE-knock out mice.[9] The firm adhesion of platelets to the inflamed endothelium is mediated by platelet aIIbβ3 and GP1bα and endothelial ICAM-1 and αvβ3 integrins.[10] Adhered platelets are further activated due to the secretion of platelet activating mediators by activated endothelial cells, such as thrombin, ADP and platelet activating factor (PAF). Moreover, PAF, embedded in the outer leaflet of the endothelial membrane, can further activate platelets in a juxtracrine fashion inducing by this way the upregulation of cytokine and chemokine expression by vascular cells which in turn activate leukocytes and endothelial cells in an inflammation supporting loop. The secretion of chemokines, as a result of platelet-endothelial interactions, recruits monocytes which bind to platelets through P-selectin/GPIb-IX-V and PSGL-1/CD11b/CD18 interactions. The platelet-monocyte aggregates further enhance the secretion of ROS, PDGF, chemokines and cytokines which drive the inflammatory process in endothelium and augments atherosclerosis development. Further details can be found in several excellent recent reviews on this topic.[11,12]

It is therefore obvious that a chronic, subclinical activation of platelets can be also a chronic stimulant for endothelial dysfunction and atherosclerosis. Whether postprandial hyperglycemia and hyperlipidemia might be a daily stimulant of platelets is presented in the following chapters.

3. Postprandial hyperglycemia, hyperlipidemia and atherosclerosis development

Several large cohort studies have demonstrated the close relationship between postprandial dysmetabolism and cardiovascular complications. In fact, most studies indicate that postprandial hyperglycemia and hyperlipidemia are as deleterious as fast-

ing hypeglycemia and dyslipidemia. The outcomes of the cohort studies are supported and merely explained by mechanistic studies providing several possible pathways that link postprandial hyperglycemia/hyperlipidemia with vascular inflammation and atherosclerosis.

3.1 Postprandial hyperglycemia and atherosclerosis

A meal of high glycemic load can induce acute, excessive increments of glucose and insulin lasting at least 2 hours after the meal. Several prospective and cross-sectional studies demonstrated associations between postprandial hyperglycemia or hyperinsulinemia and CVD incidence. A strong association of postprandial glycemia with coronary heart disease (CHD) incidence and mortality was found in the Honolulu study (6,400 diabetics, 12 years follow up).[13] Postprandial glucose levels between 157-185 mg/dL associated with a double risk for CHD mortality compared to postprandial glucose levels lower than 144 mg/dL. The postprandial levels of glucose after a 50g-oral glucose load (OGL) positively correlated with CHD mortality in diabetic patients of the Whitehall study after a follow-up of 33 years. This study demonstrated a linear correlation of post OGLT glucose levels when these levels exceeded 86 mg/dL.[14] The DECODE study (25,000 subjects, 10 years follow up) also showed that the relative risk for total mortality correlated with high postprandial glucose levels independently of fasting glucose levels.[15] Postprandial glucose levels had a better prognostic value for myocardial infarction (MI) compared to fasting glucose levels in the Diabetes Intervention Study (6,000 diabetics, 11 years follow up) while post-challenge glucose levels better correlate with the carotid intima media thickness and early atherosclerosis than fasting glucose and glycated hemoglobin.[16] A list of representative studies and meta-analyses demonstrating the strong relationship between postprandial glucose and CVD risk is presented in **Table 1**.

The biochemical mechanisms underlying the interconnection between postprandial hyperglycemia and atherosclerosis are all linked with the initiation of oxidative and inflammatory processes in the endothelium. High post-meal glucose increments can lead to the formation of advanced glycation products (AGEs) which bind to their receptors (RAGEs) in mononuclear and endothelial cells and activate MAP kinases, protein kinase C (PKC) and NF-kB which in turn upregulate the transcription of pro-inflammatory mediators (TNFα, IL-1, IL-6), adhesion molecules (vascular cell adhesion molecule 1), vasoconstrictors (angiotensin II, endothelin-1), plasminogen activator inhibitor (PAI-1) and growth factors (transforming growth factor and vascular endothelial growth factor). Moreover, AGEs inhibit NO synthase rendering by this way the vascular endothelium prothrombotic.[17]

Postprandial hyperglycemia is also accompanied by the production of RONS. Energy dense meals lead to a rapid increase of glucose, triacylglycerols (TAGs) and free fatty acids (FFA) in the circulation. The overwhelming flow of energy rich substrates to oxidative catabolism, mainly in muscle cells and adipocytes, soon saturate Krebs cycle and the respiratory chain resulting to incomplete reduction of O_2 to H_2O and leakage of superoxide anions (O_2^{-}.) in the intracellular compartments.[18] This is more prominent in cells which are not insulin dependent, such as the endothelial cells. The entrance of glucose into endothelial cells with GLUT-1 transporters is not regulated by insulin and it is determined by the extracellular glucose levels.[19] Therefore, high postprandial glucose increments can induce ROS production through the oxidative metabolism of glucose. Intracellular ROS degrade DNA, modify the structure and function of proteins and lead to the activation p38 and ER kinases which phosphorylate IRS1,2 thus inhibiting the GLUT-4 dependent transport of glucose into myocytes and adipocytes. This ultimately lead to insulin resistance, increased lipolysis and FFA levels in the circulation which further augment oxidative stress.[20] Hyperglycemia-induced intracellular ROS can also induce intracellular accumulation of glyceraldehydes-3-phosphate which activates DAG synthesis. DAG upregulates PKCβ and PKCδ activity.[21] Among other actions PKCs activates NF-kB and the transcriptional upregulation of IL-1, IL-6, TNFα, IL-18 and CRP.[22] Other transcriptional factors, such as

Table 1. **Representative studies and meta-analyses demonstrating the strong relationship between postprandial glucose and CVD risk**

Study	Volunteers	Follow up period	Results
Honolulu Heart Program[13]	6,400 healthy individuals	23 years	Postprandial glucose correlates with CHD risk
Whitehall study[14]	18,403 healthy individuals	33 years	Glucose intolerance (postload glucose 5.3–11.0 mmol/l) is associated with increased mortality risk from all causes
DECODE study[15]	25,000 healthy, T2DM, IGT and IFT	10 years	Elevated 2 h glucose concentrations are better predictors of mortality than fasting glucose
The Funagata Diabetes Study[69]	2,651 Healthy, diabetics type II, IGT and IFT		IGT but not IFG, was a risk factor for cardiovascular disease
DIS Study[16]	6,000 NIDDM	11 years	High postprandial glucose levels but not fasting glucose levels are associated with CHD disease and mortality
A meta regression analysis of published data from 20 studies[70]	95,783 non diabetics with cardiovascular events	12.4 years	Fasting and postprandial glucose level were strongly associated with the subsequent 12-year occurrence of a cardiovascular event

*CHD: Coronary Heart Disease; IGT: Impaired Glucose Tolerance; IFG: Impaired Fasting Glucose; DECODE: Diabetes Epidemiology Collaborative Analysis of Diagnostic Criteria in Europe; DIS study: Diabetes Intervention Study

AP-1 and EGR-1, are also activated by ROS which in turn upregulate the production of metalloproteases and PAI-1.[23]

3.2 Postprandial hyperlipidemia and atherosclerosis

Postprandial lipemia refers to the post meal increments of TAGs. It is a physiological phenomenon occurring at least 2-3 times per day. The dietary TAGs and the TAGs produced by hepatic de novo lipogenesis, are transferred in the form of chylomicrons and very low density lipoproteins (VLDL) respectively, to the peripheral tissues. Under physiological conditions the TAGs of the TAG-rich lipoproteins (TRLs) are hydrolyzed by lipoprotein lipase (LPL) disposing their fatty acids content in the peripheral tissues, either for storage or oxidation. Following hydrolysis by LPL chylomicron and VLDL remnants and cleared by the liver. Postprandial TAG increments normally last 6-8 hours in healthy subjects although elevated TAG levels can be found in the circulation 10-12 hours after fatty meals.[24] Genetic polymorphisms of the receptors and enzymes involved in the lipoprotein metabolism, metabolic conditions, such as obesity, insulin resistance, fasting dyslipidemia, along with the consumption of hypercaloric and hyperlipidemic meals usually results to insufficient removal of TRLs from the circulation, their partial hydrolysis by LPL and hepatic lipases and the accumulation of remnant-like particles. Small remnant particle are atherogenic due to their high cholesterol content and their small size which favors their infiltration into the subendothelial space where they are phagocytized by resident macrophages which are then transformed into foam cells. The postprandial dysregula-

tion of lipid metabolism can also initiate mechanism of oxidative stress and endothelial inflammation in similar ways with hyperglycemia. TRLs are more susceptible to oxidative modifications and augment lipid peroxidation under conditions of postprandial oxidative stress. Fat-rich meals (>60% fat) can activate leukocytes, the complement system, upregulate genes of inflammation and induce elevations of CRP, IL-6, TNFα and IL-18.[25]

It is now well established that high postprandial TAG rises can be an independent risk factor for CVDs.[26] Prospective studies have demonstrated the independent relationship between TRLs and coronary atherosclerosis. In the Physician's Health Study of 14,916 men aged 40 to 84 years and a follow up of 7 years non-fasting TAGs significantly predicted myocardial infarction (MI) risk.[27] Similar results were obtained for MI[28] in the Copenhagen study of 14,000 volunteers and 26 years follow up. Finally, very convincing evidence for the ability of non-fasting TAGs to better predict CVD risk was obtained from the Women's Health Study where healthy women were stratified into fasting (blood collection 8 and more hours since last meal) and non-fasting groups (meal within 8 hours prior to blood collection). After adjustment for classical risk factors fasting TAGs were not correlated with CVD events while non-fasting TAG levels were independently associated with CVD events. TAG levels measured 2-4 hours after the meal stronger predicted CVD events.[29]

4. Postprandial state and platelet activation

The above paragraphs clearly show that postprandial dysmetabolism induces a pro-inflammatory, pro-coagulant and pro-oxidative environment in the endothelium. Such an environment could favor the priming or even the activation of platelets which in turn could accelerate the endothelial damage and the development of atherosclerotic plaque. Both intracellular and extracellular changes could mediate the modulation of platelet functions under postprandial conditions.

4.1 Glucose and insulin have direct actions on platelets

The aggregation and degranulation of platelets are directly dependent on blood glucose levels. Glucose is the substrate for the anaerobic production of ATP whose hydrolysis covers the energy requirements of platelets. From a metabolic point of view platelets are less privileged than other cells since they contain fewer and smaller mitochondria (6-10 per cell). Under resting conditions platelets cover their energy requirements 60-70% by anaerobic glycolysis and the rest from the oxidative catabolism of fatty acids. A small amount of glycogen can be stored in the cytoplasm of platelets, however, under resting conditions is not used as an energy substrate and platelets' energy demands are dependent on the influx of glucose from the circulation. The main energy demanding process of the resting platelets is the polymerization-depolymerization of actin which is crucial for the maintenance of the structural integrity of platelets. On the other hand, when platelets are activated, their energy requirements are tripled to support actin polymerization, shape change, aggregation and degranulation. The increased energy requirements of activated platelets are derived from glycogenolysis and increased influx of extracellular glucose.[30]

GLUT-3 is the main isoform of the glucose transporters in platelets while GLUT-1 is found in minor quantities. Under resting conditions the 85% of the GLUT-3 is stored in the membranes of alpha-granules while the 15% in expressed on the plasma membrane.[31] Upon activation alpha-granules fuse with the plasma membrane increasing the number of GLUT-3 on the membrane and glucose transport into platelets is almost doubled.[32] GLUT-3 have a very strong affinity for glucose with a Km of 1.5 mM implying that this transporter is saturated with glucose even at very low concentrations. In most insulin dependent tissues the GLUT-3 expression on the surface of cells is triggered by insulin through the activation of protein kinase B.[33]

Recent evidence support that insulin is able to activate glucose influx into platelets by increasing the expression of GLUT-3 in platelet plasma membranes. However, this action is more complicated than it seems since GLUT-3 expression on the surface of platelets demands Ca^{2+} release from intracellular stores when at the same time insulin inhibits

this release. Ferreira et al. propose that the insulin-induced glucose influx is mediated by two platelet isoforms of PKB, namely PKBα and PKBβ. They also propose that the action of insulin depends on the extracellular concentration of glucose. Specifically, at low glucose concentrations (0.1 mM) both insulin and thrombin increased glucose entrance into platelets by increasing the affinity of GLUT-3 (thrombin and insulin lowered Km). However, under normal glucose levels not only the ability of insulin to increase glucose efflux is lost but insulin could actually inhibit glucose uptake from platelets. Therefore, it seems that glucose uptake from platelets depends on changes in the expression and affinity of GLUT-3 which are regulated by a complex interplay between extracellular glucose, insulin and PKBs.[33]

On the other hand several *in vitro* experiments demonstrated the ability of insulin to inhibit ADP, PAF-, thrombin-, epinephrine-induced platelet aggregation.[34,35] This can be achieved by two ways. Firstly, the binding of insulin to its receptor activates the phosphorylation of IRS-1. The phosphorylated IRS-1 can then bind to the Gai subunit of the Gi proteins and inhibit the deactivation of adenylate cyclase by Gi-coupled receptors (such as the receptors of PAF, the $P2Y_{12}$ receptor of ADP and the PAR-1 receptor of thrombin). By this way, insulin keeps the activity of adenylate cyclase and intracellular cAMP at high levels. cAMP inhibits the release of Ca^{2+} to the cytoplasm which is a pre-requisite for platelet activation. In addition, insulin activates guanylate cyclase and by this way increase intracellular cGMP levels. Similarly to cAMP, cGMP inhibits Ca^{2+} efflux from its intracellular stores and platelet aggregation. The activation of NO synthase and the subsequent increase of NO seem to mediate the upregulation of cyclic nucleotides by insulin. Treatment of human platelets with physiological concentrations of insulin resulted to increased intracellular levels of both cAMP and cGMP.[34–36] A second way by which insulin lowers intracellular Ca^{2+} levels and by this way platelet aggregation is through the activation of the Na^+/K^+-ATPase pump. Its activation results to the hyperpolarization of the extracellular membrane and the inhibition of Ca^{2+} release in the cytoplasm[35].

4.2 Acute postprandial or oral glucose load hyperglycemia and platelets

The effects of alimentary or OGL hyperglycemia/hyperinsulinemia on platelet functions are contradictory and depend on the functional marker that is determined and the clinical profile of the volunteers. In general, the platelets of metabolic syndrome or diabetic patients are more susceptible to acute hyperglycemic increments compared to healthy ones. Whether this is due to longer and higher hyperglycemic peaks, inability of insulin to exert antiplatelet actions or other reasons is not clear. Specifically, acute hyperglycemia can induce increases of platelet-monocyte aggregates[37], soluble P-selectin levels[38] and ADP-induced P-selectin expression in type II diabetic patients.[39] However, similar studies were unable to find significant elevations of ADP- and thrombin-induced P-selectin expression in diabetic patients[38] or sP-selectin secretion in volunteers with impaired glucose tolerance.[40] On the other hand, measures of platelet activity are not affected or even attenuated by acute hyperglycemia in healthy subjects. This response was observed after a high-carbohydrate, low-fat meal[41] and after meals of different glycemic index (GI) values.[42] Ueno et al. clearly showed that the post-OGTT platelet reactivity depends on the IR status of coronary artery disease patients on aspirin and clopidogrel treatment. While IGT was associated with increases of maximal ADP-induced platelet aggregation, a significant post-OGTT reduction of platelet aggregation was observed in non-IR patients.[43] Finally, in an elegant study, Spectre and coworkers demonstrated that in type II diabetic patients high postprandial insulin increments and pre-meal insulin injections may have adverse effects on platelet activation. Specifically, they compared postprandial platelet activation after pre-meal injections of placebo or insulin. Although pre-meal insulin reduced postprandial hyperglycemia it activated U46619-induced platelet P-selectin expression and fibrinogen binding. Postprandial platelet activation was positively correlated to postprandial hyperinsulinemia and inversely to hyperglycemia.[44]

Apart from the insulin resistance status the impact of other nutritional, clinical and pharmacological fac-

tors on platelet responses to acute hyperglycemia are incompletely understood. The glycemic index of the meal seems to be unable to differentiate the platelets response as shown by Ahuja and coworkers in a randomized cross-over study where the acute effects of a high GI-high carbohydrate, a low GI-high carbohydrate and a low GI moderately high in protein and fat meal were compared.[42] The milieu of bioactive compounds of the meals could strongly affect platelet responses to hyperglycemia. Several acute ingestion studies has proven the ability of different food extracts, polyunsaturated fatty acids but mainly polyphenolic compounds to inhibit platelet activity 1-4 hours after ingestion of the active ingredients.[45] However, only few studies investigated whether nutritional interventions, either long term or acute, could modulate platelet responses after standard meals or glucose loads. Ellis et al were unable to demonstrate a significant effect of a 6-weeks strawberry beverage supplementation on platelet aggregation after a high carbohydrate/fat load despite its ability to lower postprandial PAI-1 and IL-1β.[46] Recently, our group have shown that postprandial *ex vivo* ADP- and PAF-induced platelet aggregation was attenuated when boiled wild plants of Crete were included in a typical meal.[47]

There are only indirect evidences showing that the pharmacological treatment of postprandial hyperglycemia can attenuate markers of platelet activation in diabetics. Metformin and sulfonylureas, which are the first choices in oral diabetes treatment, have long been known for their ability to attenuate platelet activity in long term studies. Metformin exerts its antiplatelet activity mainly indirectly by optimizing glycemic control while sufonylureas have also direct actions on platelets mediated by the inhibition of cyclooxygenase and lipoxygenase pathways, inhibition of activated glycogen synthetase, activation of adenylate cyclase, stimulation of prostacyclin production and free radical scavenging activity.[48] As far as we know there is only one study testing the ability of either metformin of sulfonylureas to attenuate post-meal activation of platelets. Yngen et al. compared the effects of repaglinide and glibenclamide on platelet function and endothelial markers in patients with Type 2 diabetes before and after a meal. Both treatments could not inhibit postprandial platelet activation.[39] In contrast, a 3-months treatment of diabetics with acarbose was able to decrease the plasma levels of platelet microparticles (PMPs), sP-selectin and sL-selectin in diabetics and this effect was greater in the subgroup of patients with thrombotic tendency.[49] Similar results were obtained from a study which showed that miglitol therapy could also decrease plasma levels of sP-selectin, sE-selectin and sL-selectin in patients with type II diabetes.[50]

The mechanisms explaining the hyperglycemia-induced platelets modulation are incompletely understood however, oxidative stress seems to underlie the response of platelets to elevated glucose levels. When platelets are found in a hyperglycemic environment then increased amounts of glucose flow in the cells where it serves as a substrate for glycolysis. Respiratory chain is unable to cope with the increased amounts of reducing equivalents produced by the aerobic oxidation of glucose, thereby producing superoxide anions which triggers oxidative stress[51]. Increased lipid peroxides can activate platelets and sensitize them to the actions of their agonists. In accordance to this observation, diabetics contain elevated levels of isoprostanes which are correlated with platelet activation.[52] Postprandial oxidative stress can also augment platelet aggregation indirectly by reducing the bioavailabilty of NO, a well known antiplatelet mediator.[51]

In vitro studies have also shown that incubation of platelets in hyperglycemic buffers resulted to a dose-dependent increase of TXA_2 production from arachidonic acid.[53] In addition, hyperglycemia can induce the expression of the calcium channels, transient receptor potential channel canonical type 6 (TRPC6) on the plasma membrane of platelets of healthy subjects while the same receptors are expressed in increased numbers in diabetic patients compared to healthy ones. The increased expression of these channels on the surface of platelets is followed by an enhanced influx of calcium into cells.[54] *In vitro* studies also demonstrated the ability of AGEs, at concentrations that physiologically occur after a meal to increase surface activation markers (CD62 and CD36) along with the expression of AGEs receptor (RAGE).[55]

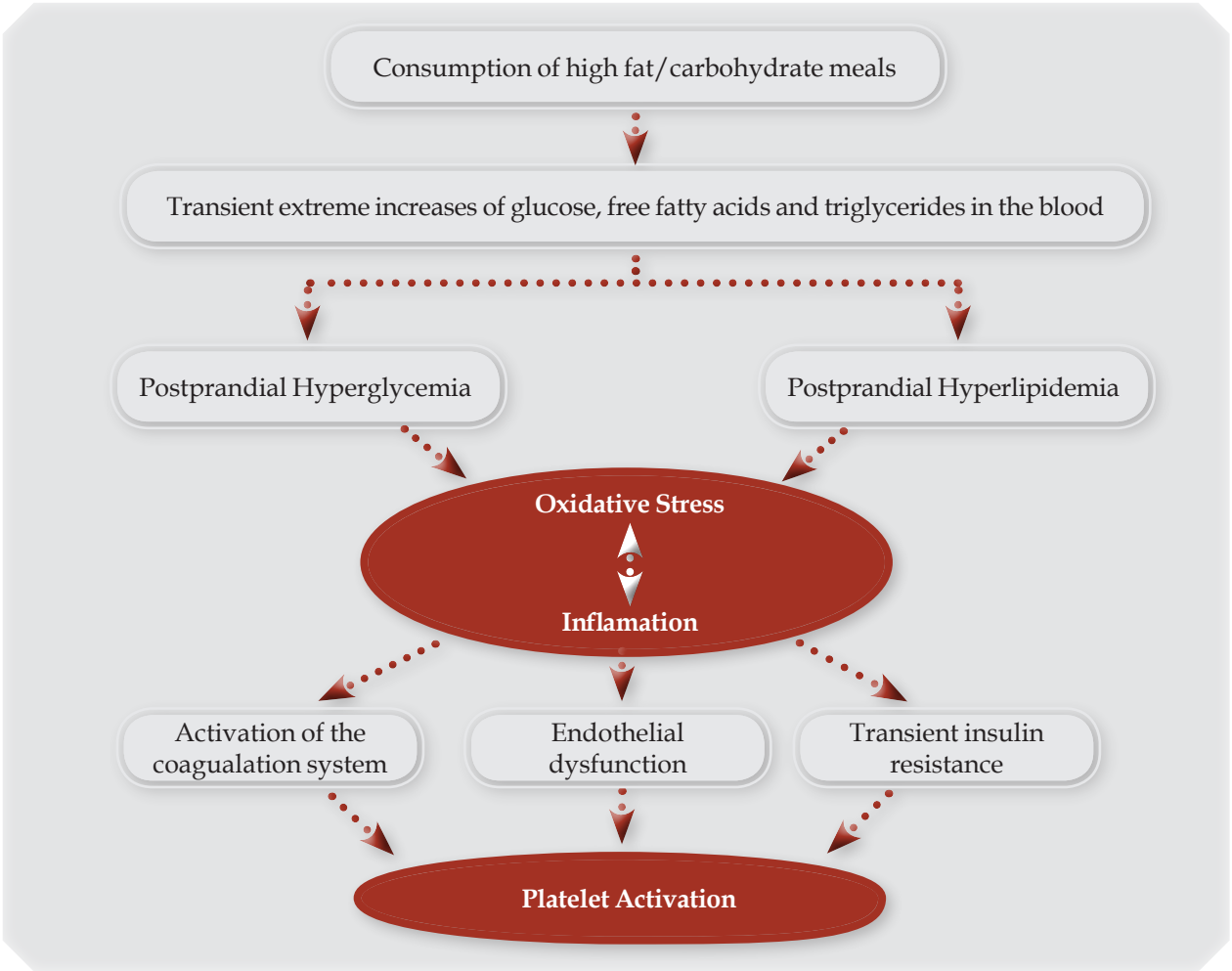

Figure 1. General mechanisms of platelet activation under postprandial dysmetabolism conditions

Even less studies, investigating the intracellular changes of the signal transduction pathways after acute hyperglycemic episodes, exist. Acute hyperglycemia can activate PKCα, PKCβ1, PKCβ2 in platelets of healthy volunteers *in vivo* while only PKCβ1 and PKCβ2 were activated in diabetics.[56] Finally, one OGTT study in obese normotensive, obese hypertensive and healthy controls demonstrated the ability of hyperglycemia to alter magnesium homeostasis in platelets by lowering intracellular magnesium levels only in the obese volunteers. The magnitude of the reduction inversely correlated with the magnitude of nor-epinephrine differences.[57]

4.3 Postprandial lipemia and platelets

The pattern of platelets responses after a fatty meal or an oral fat load test is similar to the one observed after hyperglycemia. Specifically, acute postprandial lipemia does not influence or even attenuates platelet activity during the postprandial period in healthy volunteers. This was observed when platelet activity was assessed as PF4 and beta-thromboglobulin production[58], *ex vivo* collagen-induced platelet activation[59], *ex vivo* ADP- and collagen-induced platelet aggregation[60] or LPS-induced CD40L and CD62P expression.[61] On the other hand postprandial lipemia seems to enhance P-selectin expression on the surface of platelets even in healthy volunteers. A 30-50% increase of P-selectin expression in stimulated and unstimulated platelets was observed after a fatty meal in normolipidemic volunteers.[58] The percentage of platelets expressing P-selectin and GPIIb-IIa on their

surface and the number of platelet-monocyte aggregates were significantly higher 3.5h after a moderate fat (40% meal) compared to fasting levels.[62] A significant increase of platelet-monocyte aggregates was also observed 2h after the consumption of a fatty meal in patients with a history of acute myocardial infarction.[63] Finally, subjects with and without carotid plaques demonstrated similar increases of plasma platelet microparticles after a fat tolerance test. Postprandial hypertriglyceridemia significantly correlated with percent elevations of PMPs.[64]

The attenuation of platelet aggregation under hyperlipidemic conditions, at least in normal volunteers, can be attributed to the antiplatelet activity of chylomicrons. Chylomicrons isolated from postmeal plasma could inhibit ADP-induced platelet aggregation and thrombin-induced platelet serotonin release.[65] On the other hand, chylomicron and VLDL remnant particles can stimulate whole blood platelet aggregation *in vitro*[66] indicating that patients with impaired TRL clearance and remnant hyperlipoproteinemia are prone to thrombotic complications and atherosclerotic risk.

As in the case of postprandial hyperglycemia, nutritional or supplementation studies investigating the ability of dietary factors to modulate platelet reactivity after oral fat loads or high-fat meals are scarce. Freese et al compared the postprandial effects of three mixed, high fat meals, differing in their fatty acid composition (rich in oleic acid, linoleic acid and saturated fatty acids, respectively), on collagen- and ADP-induced platelet aggregation in healthy, female subjects. Despite the fact that all meals decreased collagen-induced platelet aggregation the response of platelets to the meal was not differentiated according to the fatty acid content of the meal.[60] Esposito et al. have shown that a high fat meal can impair NO-mediated platelet inhibition in response to a L-arginine load while a isoenergetic fatty meal containing dietary antioxidants from vegetables could partially restore the NO-mediated platelet inhibition.[67] Finally, very recently Xanthopoulou et al. demonstrated the ability of wine, when it consumed along with a fatty meal to attenuate PAF-induced platelet aggregation in healthy males.[68]

5. Conclusions

The homeostatic mechanisms of healthy people prevent the postprandial activation of platelets. However, when postprandial dysmetabolism appears under condition of insulin resistance, metabolic syndrome, dyslipidemia and diabetes then postprandial hyperglycemia and hyperlipidemia may serve as a daily chronic stimulant of platelets, which in turn can augment endothelial inflammation and atherosclerosis **(Figure 1)**. Under such conditions nutritional and pharmacological interventions, aiming to attenuate postprandial platelet responses, may have a beneficial role on atherosclerosis development. However, the formulation of such interventions should be based on the biochemical mechanisms underlying the different responses of platelet to the postprandial state in healthy and insulin resistant people. Unfortunately, these mechanisms are currently unknown and much work should be done in the future to this direction.

Περίληψη

Η μεταγευματική ενεργοποίηση των αιμοπεταλίων ως πιθανός μηχανισμός για την ανάπτυξη αθηροσκλήρωσης

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Τα αιμοπετάλια, εκτός από κυτταρικοί μεσολαβητές της θρόμβωσης που λαμβάνει χώρα στα τελευταία στάδια της αθηροσκλήρωσης, εμπλέκονται ενεργά και στα πρώτα στάδια ανάπτυξης της νόσου. Μια χρόνια, υποκλινική ενεργοποίηση των αιμοπεταλίων συμμετέχει ενεργά στην ανάπτυξη της αθηροσκληρωτικής πλάκας. Ο μεταγευματικός δυσμεταβολισμός θα μπορούσε να παίξει το ρόλο της καθημερινής ενεργοποίησης των αιμοπεταλίων δεδομένου ότι προκαλεί ένα προ-φλεγμονώδες και προ-οξειδωτικό περιβάλλον στο ενδοθήλιο που ευνοεί την ενεργοποίηση των αιμοπεταλίων. Τα αποτελέσματα της επαγόμενης από ένα γεύμα ή από μία φόρτιση γλυκόζης υπεργλυκαιμία/υπερινσουλιναιμία στις λειτουργίες των αιμοπεταλίων είναι αντιφατικές και εξαρτώνται από το βιοδείκτη αιμοπεταλιακής ενεργοποίησης που έχει επιλεγεί να προσδιοριστεί και το κλινικό προφίλ των εθελοντών. Σε γενικές γραμμές, οι ομοιοστατικοί μηχανισμοί των υγιών ανθρώπων αποτρέπουν την μεταγευματική ενεργοποίηση των αιμοπεταλίων. Αντιθέτως, τα αιμοπετάλια ασθενών με μεταβολικό σύνδρομο και διαβήτη είναι πιο επιρρεπή σε οξεία υπεργλυκαιμικά και υπερλιπιδαιμικά επεισόδια. Το οξειδωτικό στρες, η διαταραχή της αντιαιμοπεταλιακής δράσης της ινσουλίνης, η ενεργοποίηση των αιμοπεταλίων από τα υπολείμματα των πλούσιων σε τριγλυκερίδια λιποπρωτεϊνών, η μειωμένη βιοδιαθεσιμότητα του ΝΟ και μεταβολές της ομοιοστασίας του ασβεστίου και του μαγνησίου είναι μερικοί από το μηχανισμούς που εξηγούν τη μεταγευματική επίδραση στα αιμοπετάλια. Διατροφικές και φαρμακολογικές παρεμβάσεις, με στόχο να μειωθεί η μεταγευματική απόκριση ενεργοποίηση των αιμοπεταλίων, θα μπορούσαν να παίξουν ευεργετικό ρόλο στην αποτροπή ανάπτυξης της αθηρωματικής πλάκας.

Λέξεις ευρετηρίου: αθηροσκλήρωση, αιμοπετάλια, μεταγευματική κατάσταση, οξειδωτικό στρες, υπεργλυκαιμία, υπερλιπιδαιμία

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References

1. Sies H, Stahl W, Sevanian A. Nutritional, dietary and postprandial oxidative stress. *J Nutr* 2005, 135:969-972
2. Zilversmit DB. Atherogenesis: A postprandial phenomenon. *Circulation* 1979, 60:473-485
3. Wallace JP, Johnson B, Padilla J, Mather K. Postprandial lipaemia, oxidative stress and endothelial function: A review. *Int J Clin Pract* 2010, 64:389-403
4. O'Keefe JH, Bell DSH. Postprandial hyperglycemia/hyperlipidemia (postprandial dysmetabolism) is a cardiovascular risk factor. *Am J Cardiol* 2007, 100:899-904
5. Frenette PS, Johnson RC, Hynes RO, Wagner DD. Platelets roll on stimulated endothelium *in vivo*: An interaction mediated by endothelial P-selectin. *Proc Natl Acad Sci U.S.A.* 1995, 92:7450-7454
6. Huo Y, Schober A, Forlow SB, Smith DF, Hyman MC, Jung S, et al. Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. *Nat Med* 2003, 9:61-67
7. Afek A, Kogan E, Maysel-Auslender S, Mor A, Regev E, Rubinstein A, et al. Clopidogrel attenuates atheroma formation and induces a stable plaque phenotype in apolipoprotein E knockout mice. *Microvasc Res* 2009, 77:364-369
8. Theilmeier G, Michiels C, Spaepen E, Vreys I, Collen D, Vermylen J, et al. Endothelial von Willebrand factor recruits platelets to atherosclerosis-prone sites in response to hypercholesterolemia. *Blood* 2002, 99:4486-4493
9. May AE, Kälsch T, Massberg S, Herouy Y, Schmidt R, Gawaz M. Engagement of glycoprotein IIb/IIIa (alpha(IIb)beta3) on platelets upregulates CD40L and triggers CD40L-dependent matrix degradation by endothelial cells. *Circulation* 2002, 106:2111-2117
10. Bombeli T, Schwartz BR, Harlan JM. Adhesion of activated platelets to endothelial cells: Evidence for a GPIIbIIIa-dependent bridging mechanism and novel roles for endothelial intercellular adhesion molecule 1 (ICAM-1), alphavbeta3 integrin, and GPIbalpha. *J Exp Med* 1998, 187:329-339
11. Demopoulos CA, Karantonis HC, Antonopoulou S. Platelet activating factor- a molecular link between atherosclerosis theories. *Eur J Lipid Sci Technol* 2003, 105:705-716
12. Nording HM, Seizer P, Langer HF. Platelets in inflammation and atherogenesis. *Front Immunol* 2015, 6:98
13. Rodriguez BL, Lau N, Burchfiel CM, Abbott RD, Sharp DS, Yano K, et al. Glucose intolerance and 23-year risk of coronary heart disease and total mortality: The Honolulu Heart Program. *Diabetes Care* 1999, 22:1262-1265
14. Brunner EJ, Shipley MJ, Witte DR, Fuller JH, Marmot MG. Relation between blood glucose and coronary mortality over 33 years in the Whitehall Study. *Diabetes Care* 2006, 29:26-31
15. Glucose tolerance and mortality: Comparison of WHO and American Diabetes Association diagnostic criteria. The DECODE study group. European Diabetes Epidemiology Group. Diabetes Epidemiology: Collaborative analysis Of Diagnostic criteria in Europe. *Lancet Lond Engl* 1999, 354:617-621
16. Hanefeld M, Fischer S, Julius U, Schulze J, Schwanebeck U, Schmechel H, et al. Risk factors for myocardial infarction and death in newly detected NIDDM: The Diabetes Intervention Study, 11-year follow-up. *Diabetologia* 1996, 39:1577-1583
17. Yan SF, Ramasamy R, Naka Y, Schmidt AM. Glycation, inflammation, and RAGE: A scaffold for the macrovascular complications of diabetes and beyond. *Circ Res* 2003, 93:1159-1169
18. Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol J-P, et al. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *JAMA* 2006, 295:1681-1687
19. Viñals F, Gross A, Testar X, Palacín M, Rösen P, Zorzano A. High glucose concentrations inhibit glucose phosphorylation, but not glucose transport, in human endothelial cells. *Biochim Biophys Acta* 1999, 1450:119-129

20. Dandona P, Ghanim H, Chaudhuri A, Dhindsa S, Kim SS. Macronutrient intake induces oxidative and inflammatory stress: Potential relevance to atherosclerosis and insulin resistance. *Exp Mol Med* 2010, 42:245-253
21. Du X, Matsumura T, Edelstein D, Rossetti L, Zsengellér Z, Szabó C, et al. Inhibition of GAPDH activity by poly (ADP-ribose) polymerase activates three major pathways of hyperglycemic damage in endothelial cells. *J Clin Invest* 2003, 112:1049-1057
22. Esposito K, Nappo F, Marfella R, Giugliano G, Giugliano F, Ciotola M, et al. Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: Role of oxidative stress. *Circulation* 2002, 106:2067-2072
23. Oszajca K, Bieniasz M, Brown G, Swiatkowska M, Bartkowiak J, Szemraj J. Effect of oxidative stress on the expression of t-PA, u-PA, u-PAR, and PAI-1 in endothelial cells. *Biochem Cell Biol Biochim Biol Cell* 2008, 86:477-486
24. Karpe F. Postprandial lipoprotein metabolism and atherosclerosis. *J Intern Med* 1999, 246:341-355
25. Alipour A, Elte JWF, van Zaanen HCT, Rietveld AP, Castro Cabezas M. Novel aspects of postprandial lipemia in relation to atherosclerosis. *Atheroscler Suppl* 2008, 9:39-44
26. Kolovou GD, Mikhailidis DP, Kovar J, Lairon D, Nordestgaard BG, Ooi TC, et al. Assessment and clinical relevance of non-fasting and postprandial triglycerides: an expert panel statement. *Curr Vasc Pharmacol* 2011, 9:258-270
27. Stampfer MJ, Krauss RM, Ma J, Blanche PJ, Holl LG, Sacks FM, et al. A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. *JAMA* 1996, 276:882-888
28. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA* 2007, 298:299-308
29. Bansal S, Buring JE, Rifai N, Mora S, Sacks FM, Ridker PM. Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. *JAMA* 2007, 298:309-316
30. Linden MD. Platelet physiology. *Methods Mol Biol Clifton NJ* 2013, 992:13-30
31. Craik JD, Stewart M, Cheeseman CI. GLUT-3 (brain-type) glucose transporter polypeptides in human blood platelets. *Thromb Res* 1995, 79:461-469
32. Heijnen HF, Oorschot V, Sixma JJ, Slot JW, James DE. Thrombin stimulates glucose transport in human platelets via the translocation of the glucose transporter GLUT-3 from alpha-granules to the cell surface. *J Cell Biol* 1997, 138:323-330
33. Ferreira IA, Mocking AIM, Urbanus RT, Varlack S, Wnuk M, Akkerman J-WN. Glucose uptake via glucose transporter 3 by human platelets is regulated by protein kinase B. *J Biol Chem* 2005, 280:32, 625-633
34. Randriamboavonjy V, Fleming I. Insulin, insulin resistance, and platelet signaling in diabetes. *Diabetes Care* 2009, 32:528-530
35. Trovati M, Anfossi G. Insulin, insulin resistance and platelet function: Similarities with insulin effects on cultured vascular smooth muscle cells. *Diabetologia* 1998, 41:609-622
36. Ferreira IA, Mocking AIM, Feijge MAH, Gorter G, van Haeften TW, Heemskerk JWM, et al. Platelet inhibition by insulin is absent in type 2 diabetes mellitus. *Arterioscler Thromb Vasc Biol* 2006, 26:417-422
37. Kaplar M, Kappelmayer J, Veszpremi A, Szabo K, Udvardy M. The possible association of *in vivo* leukocyte-platelet heterophilic aggregate formation and the development of diabetic angiopathy. *Platelets* 2001, 12:419-422
38. Yngen M, Ostenson CG, Li N, Hjemdahl P, Wallén NH. Acute hyperglycemia increases soluble P-selectin in male patients with mild diabetes mellitus. *Blood Coagul Fibrinolysis Int J Haemost Thromb* 2001, 12:109-116
39. Yngen M, Ostenson C-G, Hjemdahl P, Wallén NH. Meal-induced platelet activation in Type 2 diabetes mellitus: effects of treatment with repaglinide and glibenclamide. *Diabet Med J Br Diabet Assoc* 2006, 23:134-140
40. Koltai K, Feher G, Kesmarky G, Keszthelyi Z, Czopf L, Toth K. The effect of blood glucose levels on

hemorheological parameters, platelet activation and aggregation in oral glucose tolerance tests. *Clin Hemorheol Microcirc* 2006, 35:517-525

41. Ahuja KDK, Adams MJ, Robertson IK, Ball MJ. Acute effect of a high-carbohydrate low-fat meal on platelet aggregation. *Platelets* 2009, 20:606-609
42. Ahuja KDK, Thomas GA, Adams MJ, Ball MJ. Postprandial platelet aggregation: Effects of different meals and glycemic index. *Eur J Clin Nutr* 2012, 66:722-726
43. Ueno M, Fujita K, Yamamoto H, Ikeda T, Suga T, Yamaji K, et al. Impact of impaired glucose tolerance on clopidogrel response in patients with coronary artery disease. *J Thromb Thrombolysis* 2015, 40:174-181
44. Spectre G, Östenson C-G, Li N, Hjemdahl P. Postprandial platelet activation is related to postprandial plasma insulin rather than glucose in patients with type 2 diabetes. *Diabetes* 2012,61:2380-2384
45. Bachmair EM, Ostertag LM, Zhang X, de Roos B. Dietary manipulation of platelet function. *Pharmacol Ther* 2014, 144:97-113
46. Ellis CL, Edirisinghe I, Kappagoda T, Burton-Freeman B. Attenuation of meal-induced inflammatory and thrombotic responses in overweight men and women after 6-week daily strawberry (Fragaria) intake. A randomized placebo-controlled trial. *J Atheroscler Thromb* 2011, 18:318-327
47. Fragopoulou E, Detopoulou P, Nomikos T, Pliakis E, Panagiotakos DB, Antonopoulou S. Mediterranean wild plants reduce postprandial platelet aggregation in patients with metabolic syndrome. *Metabolism* 2012, 61:325-334
48. Papazafiropoulou A, Papanas N, Pappas S, Maltezos E, Mikhailidis DP. Effects of oral hypoglycemic agents on platelet function. *J Diabetes Complications* 2015, 29:846-851
49. Shimazu T, Inami N, Satoh D, Kajiura T, Yamada K, Iwasaka T, et al. Effect of acarbose on platelet-derived microparticles, soluble selectins, and adiponectin in diabetic patients. *J Thromb Thrombolysis* 2009, 28:429-435
50. Nomura S, Omoto S, Yokoi T, Fujita S, Ozasa R, Eguchi N, et al. Effects of miglitol in platelet-derived microparticle, adiponectin, and selectin level in patients with type 2 diabetes mellitus. *Int J Gen Med* 2011, 4:539-545
51. Freedman JE. Oxidative stress and platelets. *Arterioscler Thromb Vasc Biol* 2008, 28:s11-16
52. Davì G, Ciabattoni G, Consoli A, Mezzetti A, Falco A, Santarone S, et al. *In vivo* formation of 8-iso-prostaglandin f2alpha and platelet activation in diabetes mellitus: effects of improved metabolic control and vitamin E supplementation. *Circulation* 1999, 99:224-229
53. Valentovic MA, Lubawy WC. Elevated glucose *in vivo* and in vitro adversely alters prostaglandin generation in rat aortas and platelets. *Prostaglandins Leukot Med* 1985, 19:271-277
54. Liu D, Maier A, Scholze A, Rauch U, Boltzen U, Zhao Z, et al. High glucose enhances transient receptor potential channel canonical type 6-dependent calcium influx in human platelets via phosphatidylinositol 3-kinase-dependent pathway. *Arterioscler Thromb Vasc Biol* 2008, 28:746-751
55. Gawlowski T, Stratmann B, Ruetter R, Buenting CE, Menart B, Weiss J, et al. Advanced glycation end products strongly activate platelets. *Eur J Nutr* 2009, 48:475-481
56. Assert R, Scherk G, Bumbure A, Pirags V, Schatz H, Pfeiffer AF. Regulation of protein kinase C by short term hyperglycaemia in human platelets *in vivo* and in vitro. *Diabetologia* 2001, 44:188-195
57. Corica F, Allegra A, Ientile R, Buemi M, Corsonello A, Bonanzinga S, et al. Changes in plasma, erythrocyte, and platelet magnesium levels in normotensive and hypertensive obese subjects during oral glucose tolerance test. *Am J Hypertens* 1999, 12(2 Pt 1):128-136
58. Bröijersén A, Karpe F, Hamsten A, Goodall AH, Hjemdahl P. Alimentary lipemia enhances the membrane expression of platelet P-selectin without affecting other markers of platelet activation. *Atherosclerosis* 1998, 137:107-113
59. Aznar J, Santos MT, Vallés J. Effect of postprandial lipaemia on platelet function in man evaluated in whole blood. *Thromb Res* 1987, 48:567-576

60. Freese R, Mutanen M. Postprandial changes in platelet function and coagulation factors after high-fat meals with different fatty acid compositions. *Eur J Clin Nutr* 1995, 49:658-664
61. Kälsch T, Elmas E, Nguyen XD, Kralev S, Leweling H, Klüter H, et al. Effects of alimentary lipemia and inflammation on platelet CD40-ligand. *Thromb Res* 2007, 120:703-708
62. Hyson DA, Paglieroni TG, Wun T, Rutledge JC. Postprandial lipemia is associated with platelet and monocyte activation and increased monocyte cytokine expression in normolipemic men. *Clin Appl Thromb Off J Int Acad Clin Appl Thromb* 2002, 8:147-155
63. Kälsch T, Elmas E, Nguyen XD, Leweling H, Klüter H, Borggrefe M, et al. Alimentary lipemia enhances procoagulatory effects of inflammation in patients with a history of acute myocardial infarction complicated by ventricular fibrillation. *Int J Cardiol* 2008, 123:131-137
64. Michelsen AE, Notø A-T, Brodin E, Mathiesen EB, Brosstad F, Hansen J-B. Elevated levels of platelet microparticles in carotid atherosclerosis and during the postprandial state. *Thromb Res* 2009, 123:881-886
65. Orth M, Luley C, Wieland H. Effects of VLDL, chylomicrons, and chylomicron remnants on platelet aggregability. *Thromb Res* 1995, 79:297-305
66. Knöfler R, Nakano T, Nakajima K, Takada Y, Takada A. Remnant-like lipoproteins stimulate whole blood platelet aggregation in vitro. *Thromb Res* 1995, 78:161-171
67. Esposito K, Nappo F, Giugliano F, Giugliano G, Marfella R, Giugliano D. Effect of dietary antioxidants on postprandial endothelial dysfunction induced by a high-fat meal in healthy subjects. *Am J Clin Nutr* 2003, 77:139-143
68. Xanthopoulou MN, Kalathara K, Melachroinou S, Arampatzi-Menenakou K, Antonopoulou S, Yannakoulia M, et al. Wine consumption reduced postprandial platelet sensitivity against platelet activating factor in healthy men. *Eur J Nutr* 2016, Mar 2. [Epub ahead of print]
69. Tominaga M, Eguchi H, Manaka H, Igarashi K, Kato T, Sekikawa A. Impaired glucose tolerance is a risk factor for cardiovascular disease, but not impaired fasting glucose. The Funagata Diabetes Study. *Diabetes Care* 1999, 22:920-924
70. Coutinho M, Gerstein HC, Wang Y, Yusuf S. The relationship between glucose and incident cardiovascular events. A metaregression analysis of published data from 20 studies of 95, 783 individuals followed for 12.4 years. *Diabetes Care* 1999, 22:233-240

