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Mechanisms of thrombosis in obesity

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Abstract

Purpose of review—Obesity has become a worldwide epidemic that is driving increased morbidity and mortality from thrombotic disorders such as myocardial infarction, stroke, and venous thromboembolism. Effective prevention and treatment of thrombosis in obese patients is limited by an incomplete understanding of the underlying prothrombotic mechanisms and by uncertainties about risks, benefits, and dosing of anticoagulant drugs in this patient population.

Recent findings—This review summarizes our current understanding of established and emerging mechanisms contributing to the obesity-induced prothrombotic state. The mechanistic impact of chronic inflammation and impaired fibrinolysis in mediating obesity-associated thrombosis is highlighted. Recent data demonstrating the aberrant expression of adipokines and microRNAs, which appear to function as key modulators of proinflammatory and prothrombotic pathways in obesity, are also reviewed. Finally, some challenges and new approaches to the prevention and management of thrombotic disorders in obese and overweight patients are discussed.

Summary—Obesity-driven chronic inflammation and impaired fibrinolysis appear to be major effector mechanisms of thrombosis in obesity. The proinflammatory and hypofibrinolytic effects of obesity may be exacerbated by dysregulated expression and secretion of adipokines and microRNAs, which further increase the risk of thrombosis and suggest new potential targets for therapy.

Keywords

fibrinolysis; inflammation; obesity; thrombosis

INTRODUCTION

Obesity has become an epidemic in the United States. A recent American Heart Association Statistical Update estimated that the prevalence of obesity, defined as a BMI more than 30 kg/m², had reached 35% among US adults in 2010 [1[•]]. Even more alarming, the combined prevalence of obesity and overweight, defined as BMI more than 25 kg/m², was estimated to

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Conflicts of interest

There are no conflicts of interest

be 68%, and these numbers are expected to continue to rise. Over the past three decades, the prevalence of obesity in children 6–11 years of age has increased dramatically, from less than 5% to more than 20% [1^{*}]. Factors contributing to the obesity epidemic include increased calorie consumption and decreased physical activity among US citizens [1^{*}]. The worldwide prevalence of obesity is also increasing. The most recent data from the WHO estimate that the global prevalence of obesity has more than doubled since 1980 [2]. In 2008, more than 1.4 billion adults were overweight and nearly 500 million were obese [2].

A high BMI is recognized as a major risk factor for thrombotic disorders such as cardiovascular disease, stroke, and venous thromboembolism. Obesity is an established predictor of myocardial infarction independent of sex, age, and ethnicity [3,4]. Obesity also is associated with increased risk of ischemic stroke [5,6], deep vein thrombosis, and pulmonary embolism in men and women across all ethnic groups [7,8^{*}].

Although a strong association between obesity and thrombotic disease has been recognized for some time, the cellular and molecular mechanisms responsible for the prothrombotic state of obesity have only recently begun to emerge from clinical and laboratory studies. In this review, we summarize these emerging data and propose a conceptual framework for future work. We also discuss current challenges faced by clinicians in the prevention and management of thrombotic disorders in obese and overweight patients.

MAJOR PROTHROMBOTIC PATHWAYS IN OBESITY

Obesity is associated with a general dysregulation of metabolic homeostasis, resulting in insulin resistance, dyslipidemia, altered regulation of blood pressure, and increased risk of diabetes, cardiovascular disease, chronic kidney disease, and cancer [9]. Among the myriad metabolic abnormalities related to obesity, the two major pathways most responsible for obesity-induced thrombosis are chronic inflammation and impaired fibrinolysis (Fig. 1).

Chronic inflammation

There is abundant evidence that obesity is a systemic inflammatory disorder [9,10]. Chronic, low-grade inflammation is triggered by inflammatory cytokines secreted by adipocytes, leading to the recruitment of macrophages to adipose tissue [9]. Adipose-resident macrophages progressively accumulate as the fat mass grows. Macrophages are found more frequently in visceral as opposed to subcutaneous adipose tissue. Recruitment of macrophages is promoted by the expression of monocyte chemoattractant protein 1 (MCP-1; CCL2) in adipocytes and c-Jun N-terminal kinases (JNK1 and JNK2) in macrophages. Absence of JNK1 and JNK2 in myeloid cells was recently shown to prevent obesity-induced accumulation of macrophages in adipose tissue [11^{*}]. Similarly, MCP-1-deficient mice had decreased numbers of macrophages in adipose tissue, whereas adipocyte-specific MCP-1 overexpression resulted in increased macrophage accumulation [12]. Transient hypoxia developing in rapidly growing and poorly vascularized adipose tissue also contributes to the recruitment of macrophages. There is evidence that in obesity, the inflamed, remodeled adipose microenvironment promotes the polarization of macrophages to an activated, proinflammatory M1 phenotype [13]. The activated macrophages interact with adipocytes and preadipocytes to further increase the secretion and systemic circulation of inflammatory

cytokines such as tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and IL-1 β , which promote an inflammatory state in the liver and other sites, including vascular cells and blood vessels, further contributing to the maintenance of a systemic inflammatory state [14].

One of the major consequences of the chronic inflammatory state of obesity is the activation of prothrombotic signaling pathways in vascular cells. Stimulation of vascular endothelium, platelets, and other circulating vascular cells by proinflammatory cytokines leads to upregulation of procoagulant factors and adhesion molecules, downregulation of anticoagulant regulatory proteins, increased thrombin generation, and enhanced platelet activation [15]. Expression of tissue factor, a key initiator of coagulation, is stimulated in both endothelial cells and monocytes by obesity-associated cytokines such as TNF- α and IL-6 [16]. Tissue factor is a cell-surface factor that binds to coagulation factor VIIa, leading to the activation of factors IX and X and the subsequent generation of thrombin via the prothrombinase complex. Inflammatory cytokines also stimulate the expression of adhesion molecules such as P-selectin, which mediates endothelial-leukocyte and platelet-leukocyte interactions, further promoting thrombosis [17]. Chronic inflammation also is associated with dysregulation of endogenous anticoagulant mechanisms, including tissue factor pathway inhibitor, antithrombin, and the protein C anticoagulation system [15]. These alterations lead to imbalanced hemostasis and an increased risk of thrombosis.

Using gene expression profiling, Freedman *et al.* [18] have demonstrated a positive association between increased BMI and inflammatory mRNA transcript expression in human platelets. Thus, it is becoming clear that activated platelets not only are mediators of thrombosis associated with inflammatory conditions but may also function to amplify the inflammatory response in conditions such as obesity, atherosclerosis, rheumatoid arthritis, and sepsis [19].

Finally, inflammatory conditions such as obesity are associated with elevated plasma levels of certain coagulation factors, such as fibrinogen, von Willebrand factor, and factor VIII [20]. These effects are likely mediated by actions of inflammatory cytokines on hepatocytes and endothelial cells. Whether the elevated levels of coagulation factors contribute directly to thrombosis, or are simply biomarkers of inflammation, remains uncertain.

Impaired fibrinolysis

Fibrinolysis is a critical physiological process that results in the timely degradation of the fibrin clot by plasmin. The rate of fibrinolysis is highly regulated by plasminogen activator inhibitor-1 (PAI-1), a serine protease inhibitor that is secreted by vascular endothelium, the liver, and adipose tissue. PAI-1 acts as a potent, irreversible inhibitor of plasminogen activators, including tissue plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA). Both tPA and uPA convert plasminogen to plasmin, thereby promoting fibrinolysis, and PAI-1 strongly inhibits this process. Expression of PAI-1 is markedly upregulated in visceral adipose tissue in obesity [21], and human patients with central adiposity have increased circulating levels of PAI-1 [22]. Plasma levels of PAI-1 also are elevated in patients with obesity or metabolic syndrome [23]. Patients with increased BMI and waist-to-hip ratio have elevated levels of PAI-1 that can be reversed by intensive lifestyle interventions for weight loss [24,25^{*}]. Interestingly, TNF- α has been demonstrated

to upregulate PAI-1 expression [26], which suggests that elevated PAI-1 antifibrinolytic activity is linked to the chronic inflammatory state of obesity [27].

The association between obesity, elevated PAI-1 levels, and thrombosis has been investigated in mouse models. As in obese humans, plasma levels of PAI-1 are higher in obese mice compared with lean mice [28]. Nagai *et al.* [29] demonstrated that PAI-1 deficiency in mice resulted in complete abrogation of obesity-induced acceleration of middle cerebral artery occlusion (a model of ischemic stroke), suggesting that PAI-1 plays a critical mechanistic role in promoting prothrombotic effects of obesity.

MODULATORS OF THROMBOTIC PATHWAYS IN OBESITY

As discussed above and illustrated in Fig. 1, the prothrombotic state of obesity is thought to be driven in large part by chronic inflammation and impaired fibrinolysis, which can lead to endothelial dysfunction, rupture of atherosclerotic plaques, platelet hyperactivation, hypercoagulability, and delayed clot lysis. Recent work has suggested that, in addition to driving these major prothrombotic pathways, obesity also causes dysregulation of several factors that act as modulators, or ‘fine-tuners,’ of hemostatic balance. Chief among these modulators are adipokines and microRNAs (miRs).

Adipokines

Adipose tissue is not only involved in energy storage but also functions as an active paracrine and endocrine organ that secretes cytokines, hormones, and other bioactive mediators, collectively termed adipokines. Most broadly, the term adipokine refers to any bioactive substance released by adipocytes or other adipose-resident cells, such as macrophages and stromal cells [30]. Some adipokines act centrally to regulate appetite and energy expenditure, whereas other adipokines act peripherally to modulate insulin sensitivity, oxidative capacity, lipid metabolism, and vascular cell function.

The importance of adipose tissue as an endocrine organ was first recognized in 1994 with the cloning of the leptin gene [31]. Leptin is a fat-derived hormone (adipokine) that regulates both appetite and energy expenditure. Leptin receptors have been identified in many types of vascular cells, including endothelial cells, macrophages, and platelets. Clinical trials have found a strong association between plasma leptin levels and vascular thrombosis [32,33], and experimental studies in animals have established a causative role for leptin in thrombogenesis. Mice deficient in leptin or leptin receptor are protected from arterial thrombosis [34]. The pro-thrombotic effect of leptin is mediated in part via leptin receptor activation in platelets and endothelial cells. Leptin-mediated activation of human platelets stimulates the JAK2/STAT3 signaling pathway, promoting thromboxane synthesis and activation of fibrinogen receptor α IIb β 3 [35], leading to enhanced platelet aggregation [36,37]. Leptin’s effects on vascular endothelium include the upregulated expression of C-reactive protein [38] and the exacerbation of endothelial dysfunction mediated by increased activity of protein kinase C- β followed by decreased endothelial nitric oxide production [39].

Since the discovery of leptin, adipose tissue has been recognized as a metabolically active organ that can influence vascular homeostasis via the secretion of a large number of other adipokines, including some with prothrombotic or antithrombotic properties. In addition to leptin, the prothrombotic adipokines include resistin, visfatin, and the anti-fibrinolytic serpin PAI-1 (Fig. 2). Resistin was named for its association with obesity and insulin resistance [40]. Resistin can directly activate vascular endothelium, resulting in the upregulation of pro-thrombotic adhesion molecules and inflammatory mediators such as MCP-1 [41]. Visfatin was originally identified as a protein secreted by visceral fat that mimics the effects of insulin [42]. Like resistin, visfatin causes endothelial cell activation and resultant expression of prothrombotic and proinflammatory adhesion molecules [43–46]. Increased expression of visfatin in resident macrophages within atherosclerotic plaques also may promote plaque rupture and subsequent thrombosis in carotid and coronary arteries [47].

Adipose tissue also secretes some adipokines that function as counterregulatory, antithrombotic factors (Fig. 2). Adiponectin, one of the most abundant adipokines, is capable of reducing leukocyte–endothelial interactions [48,49] and inhibiting smooth muscle cell proliferation [50]. Adiponectin also stimulates nitric oxide production in endothelial cells, induces the synthesis of the anti-inflammatory cytokine IL-10 in macrophages [51], and inhibits tissue factor expression in both endothelial cells and macrophages [52,53]. Unfortunately, plasma levels of adiponectin tend to decrease as obesity progresses [30]. Apelin is another antithrombotic adipokine that, unlike adiponectin, is secreted at increased levels in obesity [54,55]. Apelin exerts protective metabolic effects in obesity-associated diseases. In mouse models, apelin has anti-inflammatory actions, increases endothelial nitric oxide bioavailability, decreases atherosclerosis, and prevents aneurysm formation [56–58]. Apelin also has been reported to decrease PAI-1 gene expression in mice [59]. The cellular actions of apelin are mediated by a G-protein-coupled receptor called APJ [56,57]. An interesting recent study suggests that, in cardiomyocytes, APJ may act as a dual receptor that responds both to apelin and to mechanical stretch [60]. When activated by apelin, APJ appears to induce a protective signaling pathway that limits stretch-induced myocardial hypertrophy [60].

microRNAs

Over the past two decades, it has become recognized that many chronic disease phenotypes involve the dysregulation of gene expression by a class of non-coding RNAs called microRNAs or miRs [61]. miRs are small (19–24 bp), evolutionarily conserved RNA molecules that normally modulate physiological processes by ‘fine-tuning’ the posttranscriptional expression of a distinct set of target genes [62]. There is growing evidence that miRs may play an important role in the pathogenesis of obesity and its thrombotic complications [63]. Obesity and diabetes are associated with aberrant miR expression patterns in plasma [64,65] and tissue [66]. Recent work by Rayner *et al.* [67] has demonstrated that miR-33 represents an important endogenous regulator of lipid metabolism and that interference with miR-33 promotes regression of atherosclerosis. Interestingly, miR-421 and miR-30c have been reported to suppress the expression of PAI-1 [68], suggesting a direct antithrombotic action. miR-126, which is markedly downregulated in obese individuals [64], also has antithrombotic effects, including the inhibition of expression

of endothelial adhesion molecules [69]. Similarly, miR-21 has been demonstrated to inhibit the expression of an endothelial antioxidant enzyme, superoxide dismutase-2, thereby promoting oxidative stress and decreasing endothelial nitric oxide bioavailability [70].

miRs also may influence gene expression in platelets. Although circulating platelets are anucleate cell fragments, they nevertheless contain residual pre-mRNA, mRNA, and miR molecules that can interact with each other to posttranscriptionally regulate gene expression in response to platelet activation [71]. In fact, because of the virtual absence of gene transcription in platelets, the regulated expression of genes in platelets is completely dependent on posttranscriptional mechanisms such as miR-mediated modulation of mRNA stability. Platelets contain several hundred miRs, as well as all of the necessary miR processing enzymes [71–74]. In a landmark study, Landry *et al.* [72] demonstrated that miR-223 downregulates the expression of the platelet ADP receptor P2Y₁₂, which is the target of clopidogrel and other thienopyridine antiplatelet drugs. Plasma levels of miR-223 are decreased in obese compared to lean individuals [64], suggesting that the miR-223/P2Y₁₂ axis may represent a causative mechanism of platelet activation in obesity. Another miR, miR-96, has been shown to regulate the expression of platelet-vesicle-associated microtubule protein-8 (VAMP8), which is an important component of platelet granule exocytosis [75].

CURRENT CHALLENGES AND FUTURE DIRECTIONS FOR THE PREVENTION AND MANAGEMENT OF THROMBOSIS IN OBESE PATIENTS

Other than weight loss, which reverses most of the prothrombotic effects of obesity, no obesity-specific therapeutic approaches to prevent or treat thrombosis have been developed. Moreover, the clinical use of standard anticoagulant and antiplatelet drugs in obese patients is limited by a paucity of information about the effects of increased BMI on their efficacy and safety. Most clinical trials investigating antithrombotic drugs have excluded patients with obesity, and there is relatively limited information available about the pharmacokinetic properties of antithrombotic drugs in obese patients.

Retrospective data suggest that obese and morbidly obese hospitalized patients require significantly higher doses of warfarin and longer times to achieve a therapeutic International Normalized Ratio compared to nonobese patients [76]. There is even more uncertainty about the dosing of low-molecular weight heparins (LMWHs), which are often administered in fixed or weight-based doses that may be capped in obese patients. Emerging evidence supports the use of higher fixed doses of LMWHs for the prevention of venous thrombosis in obese compared with lean patients [77,78], but formal dosing recommendations are not established. For the treatment of venous thrombosis, there is evidence to support the use of LMWHs in doses adjusted by total body weight, even when BMI is more than 30 [78]. The potential value of laboratory monitoring of LMWH therapy in obese patients using antifactor Xa levels is not well established or standardized [78]. Similar concerns limit the potential use of oral direct thrombin or factor Xa inhibitors, such as dabigatran, rivaroxaban, and apixaban, in obese patients. These drugs are approved for use in fixed doses, and obese patients were excluded from the efficacy and safety trials supporting their approval.

Additional studies will be necessary to determine whether these drugs can be used safely in patients with obesity.

In light of recent progress in understanding the underlying mechanisms and regulatory factors responsible for obesity-related thrombosis, there is promise that novel molecular targets for antithrombotic therapies may emerge. Given the central role of chronic inflammation in driving obesity-induced thrombotic risk, the potential to target proinflammatory pathways is attractive. Aspirin, which has anti-inflammatory as well as antiplatelet effects [79], is used commonly to reduce cardiovascular and thrombotic risk in obese patients [80]. Statins are used primarily to modulate lipoprotein profiles, but they also have anti-inflammatory antithrombotic actions that may modulate cardiovascular and thrombotic risk in obese patients, even in the absence of reductions in low-density lipoprotein cholesterol [81]. Several novel anti-inflammatory therapeutics are being developed for use in patients with type 2 diabetes and atherosclerosis [82,83], but much more work will be necessary before it is known whether any of these agents will be clinically efficacious. A major challenge is that systemic inflammatory responses are regulated by many intertwined networks and feedback mechanisms, so that targeting one specific molecule may not have a measurable effect on inflammation due to activation of bypass pathways.

PAI-1 is another attractive target for antithrombotic therapy in obesity. Fjellstrom *et al.* [84] recently reported the successful development of a small molecule inhibitor of PAI-1. Several other groups are also actively working to develop PAI-1 inhibitors [85], but these new drugs have not yet been tested in patients. Another therapeutic strategy to limit PAI-1 production is to target peroxisome proliferator-activated receptor- γ (PPAR- γ), a transcription factor that activates an antithrombotic and anti-inflammatory gene expression profile [86]. PPAR- γ is known to antagonize the activities of the proinflammatory transcription factor nuclear factor κ B (NF κ B) and downregulate PAI-1 expression. Treatment with the PPAR- γ agonist pioglitazone protects from arterial thrombosis in obese mice [87].

Pharmacological targeting of adipokines is another active area of research and development [88]. Leptin replacement therapy improves insulin sensitivity and glycemic control in patients with severe lipodystrophy and leptin deficiency [89]. In contrast, clinical trials of leptin therapy have proven to be largely ineffective in the treatment of diabetes and obesity, probably because most obese and diabetic patients have elevated leptin levels [90]. There is also concern that leptin treatment might increase thrombotic risk in obese patients due to its pro-thrombotic effects on platelets. The alternative therapeutic strategy of developing leptin receptor antagonists might be expected to be beneficial in protecting from thrombosis but have harmful metabolic effects on obesity and insulin resistance unless platelet-specific leptin receptor antagonists could be developed. Adiponectin and apelin appear to be beneficial adipokines with antithrombotic properties and, thus, are promising potential therapeutic targets in obesity [91,92]. Systemic administration of adiponectin improves endothelial function in resistant arteries of diabetic mice [93]. Apelin may decrease PAI-1 production and promote therapeutic angiogenesis [59,94,95].

Finally, the observation that miRs regulate thrombotic pathways in obesity suggests another future therapeutic strategy. Potential approaches include both enhancing miR actions by administering synthetic miR mimetics, or silencing endogenous miRs using antisense RNA oligonucleotides ('antagomiRs') [96]. Recent advances in single-stranded RNA technologies offer promise for improved potency and selectivity of miR-based therapeutics [97]. In terms of thrombosis, a particularly attractive target is miR-223, which downregulates the platelet ADP receptor [72].

CONCLUSION

The worldwide obesity epidemic is contributing to increased morbidity and mortality from thrombotic disorders. Recent data have led to an improved understanding of the underlying prothrombotic mechanisms contributing to the obesity-induced prothrombotic state, which include chronic inflammation and impaired fibrinolysis. Dysregulated expression of adipokines and miRs also appears to promote proinflammatory and prothrombotic pathways in obesity, further increasing the risk of thrombosis and suggesting new potential targets for therapy.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 480).

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KEY POINTS

- Obesity promotes a state of chronic inflammation that activates prothrombotic signaling pathways in platelets and other vascular cells.
- Impaired fibrinolysis, mediated largely by increased production of PAI-1, is a major contributing factor to thrombotic risk in obesity.
- Effective clinical use of anticoagulant and antiplatelet drugs in obese patients is limited by a relative lack of information about pharmacokinetics, efficacy, and safety.
- Emerging evidence suggests that the adverse effects of obesity on inflammation, fibrinolysis, and thrombotic risk may be modulated by adipokines and microRNAs, which represent attractive targets for antithrombotic drug development.

FIGURE 1.

Major mechanisms of obesity-associated thrombosis. Obesity promotes chronic inflammation and impaired fibrinolysis, both of which lead to an increased risk of thrombosis. The prothrombotic effects of obesity on inflammatory and antifibrinolytic pathways are modulated, or ‘fine-tuned’ by adipokines and microRNAs. Effector mechanisms include endothelial dysfunction, plaque rupture with exposure of tissue factor, activation of platelets, and delayed clot lysis.

FIGURE 2.

Aberrant adipokine expression profile in obesity. Obesity leads to disruption of the balance between prothrombotic adipokines such as leptin, plasminogen activator inhibitor-1 (PAI-1), resistin, and visfatin, and antithrombotic adipokines such as adiponectin and apelin.