Medical Research Archives

European Society of Medicine

OPEN ACCESS

Published: December 31, 2023

Citation: Secunda C, Ghimire N, et al., 2023. MKP-2 deficiency Alters Bleeding Time and Red Blood Cell Indices in Diet-Induced Obesity in Mice, Medical Research Archives, [online] 11(12). https://doi.org/10.18103/mra.v11i12.5032

DOI
https://doi.org/10.18103/mra.v11i12.5032

ISSN: 2375-1924

RESEARCH ARTICLE

MKP-2 deficiency Alters Bleeding Time and Red Blood Cell Indices in Diet-Induced Obesity in Mice

Cassandra Secunda[1], Nabin Ghimire[1], Morgan Welch[1], Urja Patel[1], Ahmed Lawan[1,*]

[1] Department of Biological Sciences, University of Alabama in Huntsville, Huntsville, Alabama 35899, USA

***Corresponding author:** al0122@uah.edu

ABSTRACT

Red blood cell dysfunction is linked to overnutrition, which is characterized by inflammation, platelet aggregation and hypercoagulability. Although the role of MAPK in platelets is well established, little is known about the connection between MKPs and red blood cell. We investigated the pathophysiological effects of MKP-2 deletion on bleeding tendencies in platelet counts, and red blood cell indices that are brought on by high-fat diet. In this study, we demonstrated that female *Mkp-2*$^{-/-}$ mice bleeding times were significantly shortened when they were fed chow diet. Male *Mkp-2*$^{-/-}$ mice on a high-fat diet show resistance to diet-obesity, which is linked to a shorter bleeding time. In high-fat diet-fed male *Mkp-2*$^{-/-}$ mice, we observed decreased levels of red blood cells, hemoglobin, and hematocrit. These data suggest that the anemia in these mice may be due to inflammation induced by obesity. When male *Mkp-2*$^{-/-}$ mice were compared to wild-type controls, their platelet counts were normal; however, the platelets derived from these mice showed increased activation of p38 MAPK and ERK and SDF-1 expression. All of these studies point to a new function for MKP-2 in red blood cell physiology and hemostasis, which may have consequences for thrombotic and hemostatic diseases.

Keywords: Mitogen-activated protein kinase, Protein tyrosine phosphatase, obesity, red blood cells, platelets, hemostasis

Introduction

Globally, the prevalence of obesity is rising with 13% of adults reported to be obese and affecting approximately 33% of adult Americans [1, 2]. Numerous metabolic disorders, such as type 2 diabetes (T2D), atherosclerosis, heart disease, fatty liver disease and multiple types of cancers are intimately related with obesity and overnutrition [2, 3]. Obesity has many complex causes, including inherited, social, environmental, physiological factors and poor dietary choices [4]. A positive correlation has been found between diet-induced obesity and pathological platelet activation [5]. Additionally, this relationship is inverse, with platelet activation declining as weight decreases [6]. High fat diets have been demonstrated to cause mice to experience pathological hypercoagulation by increasing platelet-monocyte aggregation (PMA) [7]. Metabolic stress brought on by diet-induced obesity initiates a signal cascade that activates mitogen-activated protein kinases (MAPKs) [8].

Hemostasis is the body's effective mechanism for stopping blood loss by closing off areas where there has been vascular damage. It has been demonstrated that a number of blood components are essential to this process. It is believed that platelets are essential for both hemostasis and thrombus formation [9]. The most prevalent type of blood cells are red blood cells (RBCs). There is mounting clinical and laboratory data that RBCs play an active part in development of hemostasis, thrombosis and atherosclerosis through a variety of mechanisms [9, 10]. Individuals who suffer from RBC abnormalities such as sickle cell anemia, β-thalassemia major, or hereditary stomatocytosis, or who have an elevated packed cell volume (hematocrit), are at risk of developing thrombosis [11, 12]. Studies have shown that improved hemostasis is correlated with an increase in hematocrit, particularly in cases of anemia [13-15].

Platelets and MAPKs have a complicated relationship because different MAPKs are involved in different aspects of platelet activation [16, 17]. Integrin-induced MAPK activation results in clot retraction while agonist-induced MAPK activation contributes to platelet activation [17]. Furthermore, involved in platelet granule release and the production and dissemination of thromboxane A2, MAPKs are found on platelets [18]. In addition to their roles in collagen adhesion and arachidonic acid metabolism, p38 MAPK, JNK1/2 and ERK1/2 have also been demonstrated to be involved in platelet spreading, clot retraction, and microparticle formation [19]. In a different investigation, von Willebrand factor-induced platelet aggregation was eliminated in humans by inhibiting the p38 MAPK [20]. RBCs and platelets engage in both biochemical and mechanical interactions. Under high shear, hypoxia, and acidosis, RBCs can activate platelets by exporting ATP and ADP [21, 22]. RBC export of thromboxane A2 also promotes platelet aggregation [21]. Hemolysis releases free hemoglobin (Hb) molecules that scavenge nitric oxide, causing platelet stimulation [21]. There has been inconsistent evidence linking RBC indices to obesity. Some studies found that obese subjects had higher Hb concentration than non-obese controls, while other studies found that obese subjects had lower Hb concentrations than non-obese controls [23]. The relationship between MAPKs and RBC is largely unknown. One study demonstrated enhanced JNK activity following photothrombosis [24]. By directly dephosphorylating the MAPKs, the MAPK phosphatases (MKPs) inactivate the MAPKs. It is unclear, how the MKPs regulate platelet and RBC function in the development of hemostasis and thrombosis formation in obesity.

The goal of this study is to determine the effect of MKP-2 deficiency on blood coagulation, platelet and RBC indices in response to metabolic stress of overnutrition. MKP-2 global knockout mice have been genetically characterized elsewhere [25-27].

Methods

REAGENTS AND ANTIBODIES

All reagents were purchased from standard chemical vendors. The following antibodies phospho-p38 MAPK (#4511s), phospho-ERK1/2 (#9101s), p38 MAPK (#9228s), ERK1/2 (#4696s), beta-actin (#3700s) and SDF-1 (CXCL12) (#3530s), were obtained from Cell Signaling Technology.

ANIMAL STUDIES

The University of Alabama in Huntsville Institutional Animal Care and Use Committee approved all animal studies and experiments were conducted according to the NIH's Guidelines on the Use of Laboratory Animals. MKP-2 global knockout (*Mkp-2*$^{-/-}$) and wild-type (*Mkp-2*$^{+/+}$) mice have been genetically characterized elsewhere [25]. These mice were kindly provided by Dr. Robin Plevin, University of Strathclyde, United Kingdom. The *in vivo* studies on chow, high fat diet (HFD) regiments were implemented once male and female *Mkp-2*$^{+/+}$ and *Mkp-2*$^{-/-}$ mice reached three weeks of age. Mice were either kept on a custom high fat diet (60% kcal) of purified rodent diet DN 112252 (Dyets, Inc., Bethlehem, PA, USA) or chow. Ingredients and nutritional composition of all diets are displayed in Tables 1-2. All mice used in this study were 12-16 weeks of age.

Table 1: Ingredients and nutritional content of the high fat diet

Ingredient	kcal./g	g/kg	kcal./kg
Casein	3.58	200	716
Cornstarch	3.6	0	0
Dyetrose	3.8	125	475
Sucrose	4	68.8	275
Cellulose	0	50	0
Soybean Oil	9	25	225
TBHQ	0	0.005	0
Lard	9	245	2205
Salt Mix #210088	1.6	10	16
Dicalcium Phosphate	0	13	0
Calcium Carbonate	0	5.5	0
Potassium Citrate H2O	0	16.5	0
Vitamin Mix #300050	3.92	10	39.2
L-Cystine	4	3	12
Choline Bitartrate	0	2	0

Table 2: ingredients and nutritional content of the chow diet

Ingredient	kcal./g	g/kg	kcal./kg
Casein	3.58	200	716
L-Cystine	4	3	12
Sucrose	4	350	1400
Cornstarch	3.6	315	1134
Dyetrose	3.8	35	133
Soybean Oil	9	25	225
t-Butylhydroquinone	0	0.005	0
Lard	9	20	180
Cellulose	0	50	0
Mineral Mix #210088	1.6	10	16
Dicalcium Phosphate	0	13	0
Calcium Carbonate	0	5.5	0
Potassium Citrate H2O	0	16.5	0
Vitamin Mix # 300050	3.92	10	39.2
Choline Bitartrate	0	2	0
		1055.005	3855.2

TAIL BLEED COAGULATION ASSAY

A test tube holder with room for 15 mL tubes was placed into a dry water bath. Ten minutes before the experiment, 14 mL of PBS was heated to 37°C in a dry bath and put into a 15 mL conical tube. Mice that were fed chow, high fat diet (HFD) for 12 weeks, were all put into mice restrainer on a table that allowed their tails to be hang over the water bath and into the heated tube of 1X PBS. A sharpie pen was used to mark about 3 mm of the tail, which was then cut off with scissors. The tail was then quickly taped to the open tube and inserted into the 15 mL tube containing the heated PBS, and a timer was set. As soon as the tail stopped bleeding for the first time, timing was discontinued. Once the mouse was back in its cage, the wound was cleaned and it was observed for a full day.

HEMATOLOGY ANALYSIS

Male and female *Mkp-2*$^{+/+}$ and *Mkp-2*$^{-/-}$ mice fed either HFD, diets were used for the comprehensive complete blood count (IDEXX Laboratories, Columbia, MO). Complete blood count (CBC) was obtained from EDTA-anticoagulated whole blood samples by use of the flow cytometry-based Sysmex XT-2000iV Automated Hematology Analyzer (Sysmex). The following parameters were obtained for each sample: white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (Hgb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocyte percentage, absolute reticulocyte count, leukocyte differential percentages and absolute counts, and platelet count.

IMMUNOBLOTTING

At the conclusion of the studies, isolated platelets from male and female *Mkp-2*$^{+/+}$ and *Mkp-2*$^{-/-}$ were homogenized using RIPA buffer (25 mM Tris. HCl pH 7.4, 150 mM NaCl, 5 mM EDTA, 1% NP-40, 0.1 % SDS, 1.0 % sodium deoxycholic acid), supplemented with protease and phosphatase inhibitors (5 µg/ml leupeptin, 5 µg/ml aprotinin, 1 µg/ ml pepstatin A, 1 mM PMSF, 1 mM benzamidine, 1 mM Na_3VO_3, and 10 mM NaF). Before being clarified at 20,800 g for 30 min at 4°C, homogenates were lysed for 30 min on the shaker. The Pierce BCA Protein Assay kit (Pierce, Rockford, IL) [25] was used to measure protein concentration. After protein lysates were separated using SDS-PAGE and put unto nitrocellulose membranes, phospho-specific antibodies were added, and the result was either enhanced chemiluminescence or fluorescent detection.

STATISTICAL ANALYSIS

All data represent the mean ± SEM. Differences between groups were assessed using a student's t-test or analysis of variance (ANOVA) with Bonferroni's post-test for multiple comparisons using GraphPad Prism 9.5.1 statistical software.

Results

REDUCED BLEEDING TENDENCY IN FEMALE CHOW-FED MKP-2-DEFICIENT MICE

The function of MKP-2 in hemostasis was investigated using tail bleeding assays. Figure 1 shows the average bleeding time of male *Mkp-2*$^{-/-}$ mice was 74.4±10.51 seconds which was comparable with 70.7±10.51seconds of *Mkp-2*$^{+/+}$ (**Fig. 1A**). Interestingly, the average bleeding time of female *Mkp-2*$^{-/-}$ mice was 72.2±13.68 seconds, which was significantly lower compared with 103.5±13.68 seconds in the female *Mkp-2*$^{+/+}$ (**Fig. 1B**). These results demonstrate that female *Mkp-2*$^{-/-}$ mice exhibit reduced bleeding time suggesting that MKP-2 play a role in hemostasis.

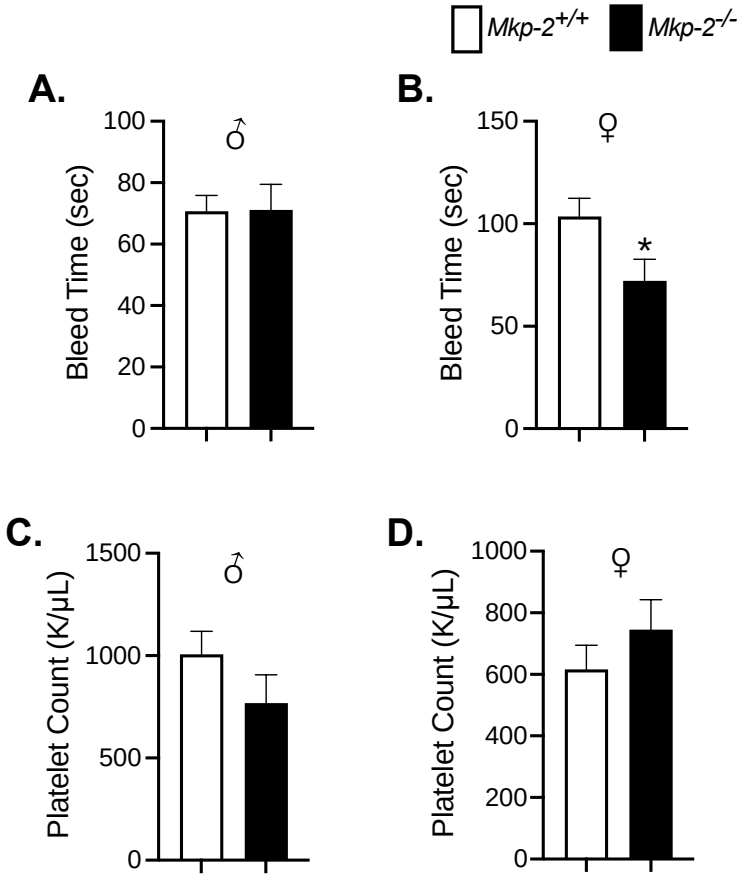

FIGURE 1. Reduced bleeding time in chow-fed female in MKP-2-deficient mice. (A) Bleeding times for chow-fed male *Mkp-2*$^{+/+}$ (n=7) and *Mkp-2*$^{-/-}$ (n=9) mice (**B**) Bleeding times for chow-fed female *Mkp-2*$^{+/+}$ (n=10) and *Mkp-2*$^{-/-}$ (n=9) mice. Platelet count in male (**C**) and female (**D**) *Mkp-2*$^{+/+}$ and *Mkp-2*$^{-/-}$ mice (n= 10 per genotype). Data are represented as mean ± SEM; *; $p < 0.05$, as determined by student t-test. Open bars, *Mkp-2*$^{+/+}$ mice; closed bars, *Mkp-2*$^{-/-}$ mice.

To follow up on the intriguing tail bleed data, platelet counts were obtained from chow-fed male and female *Mkp-2*$^{-/-}$ and *Mkp-2*$^{+/+}$ mice at age 12-16 weeks. Figure 1C show the average platelet counts for male *Mkp-2*$^{+/+}$ mice was 1046.7±144.2 K/µL compared with the *Mkp-2*$^{-/-}$ average of 866.2±144.2 K/µL (**Fig. 1D**). The average platelet count for the females is displayed in Figure 1D and was 756.6±105.7 and 752.9±105.7 K/ µL for *Mkp-2*$^{+/+}$ and *Mkp-2*$^{-/-}$ mice respectively (**Fig. 1D**). The results of the platelet count assays were not significantly different between the two genotypes and genders for the chow diet. These results suggest the reduced bleeding time in chow-fed female MKP-2-deficient mice is associated with normal platelets counts.

RESISTANCE TO DIET-INDUCED OBESITY AND REDUCED BLEEDING TIME IN MALE MKP-2-DEFICIENT MICE

Following the interesting findings on the effects of MKP-2 deficiency on chow-fed female *Mkp-2*$^{-/-}$ mice bleeding time, and the additional effects of metabolic stress has on platelets function and coagulation, we further investigated the effects of MKP-2 deficiency on bleeding time and platelet function in response to high fat diet (HFD) challenge (introduced at 3-weeks) using female and male *Mkp-2*$^{-/-}$ mice. Male and female *Mkp-2*$^{-/-}$ mice and *Mkp-2*$^{+/+}$ wild-type counterparts were placed on a HFD for 13-week and weight gain monitored weekly. Figure 2 shows that male *Mkp-2*$^{-/-}$ mice averaged a weight of 24.12 g at the 13th week and weighed significantly less than their male *Mkp-2*$^{+/+}$ controls that weighed 27 g at the same time point (**Fig. 2A**). This is consistent with our recent findings [28]. Female *Mkp-2*$^{-/-}$ mice average weight of 20.9 g was comparable to the female *Mkp-2*$^{+/+}$ average weight of 21.09 g at 13 weeks (**Fig. 2B**). These results imply that the lack of MKP-2 plays a protective role from obesity phenotypes, particularly in males.

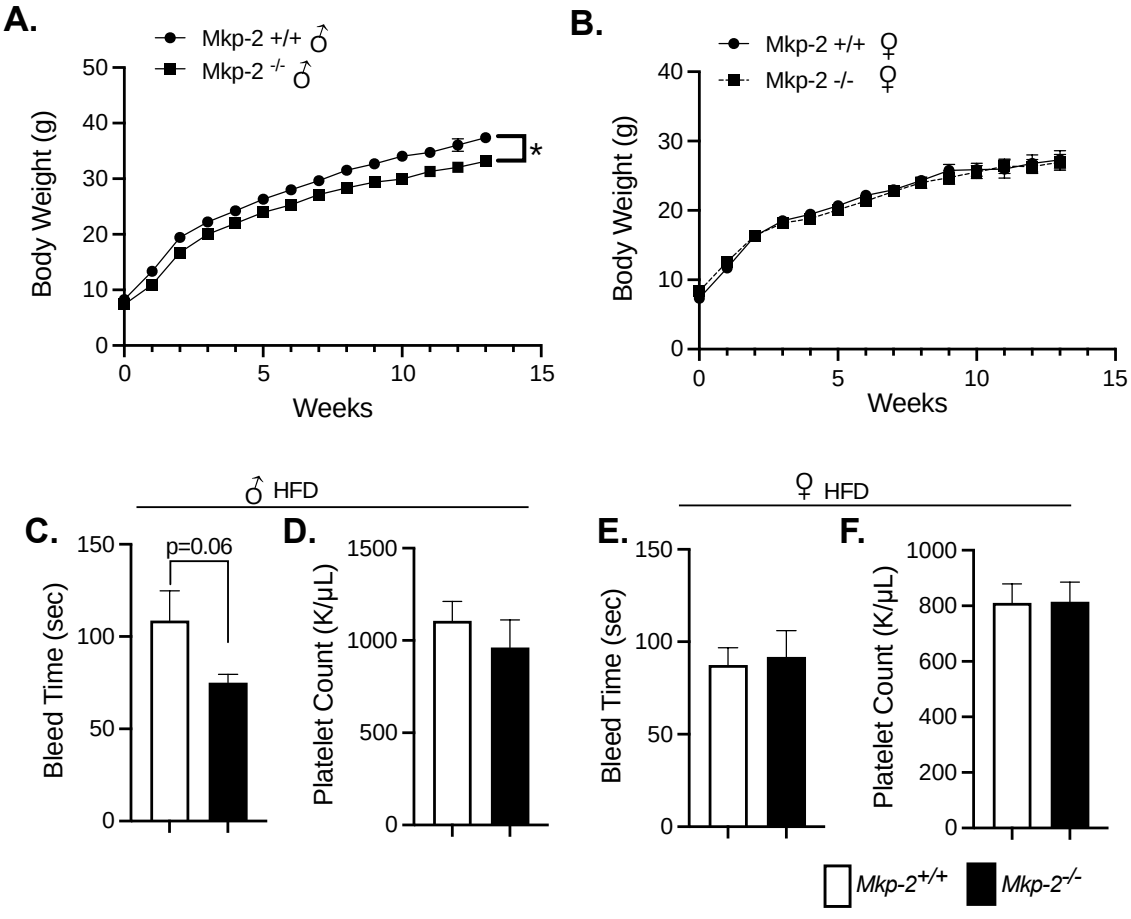

FIGURE 2. Resistance to diet-induced obesity and development of anemia in MKP-2-deficient mice. (A) Body weight of HFD-fed (**A**) male (n=12-14) and female (n=11) (**B**) *Mkp-2*$^{+/+}$ and *Mkp-2*$^{-/-}$ mice. Bleeding times and platelet counts for HFD-fed (**C** and **D**) male, (**E** and **F**) female *Mkp-2*$^{+/+}$ and *Mkp-2*$^{-/-}$ mice. Data are represented as mean ± SEM; *; $p < 0.05$, **; $p < 0.01$, ***; $p < 0.0001$, as determined by analysis of variance (ANOVA) with Bonferroni's post-test for multiple comparisons or student t-test. Open bars, *Mkp-2*$^{+/+}$ mice; closed bars, *Mkp-2*$^{-/-}$ mice.

To further investigate how obesity affects bleeding time in *Mkp-2*$^{-/-}$ mice, a tail bleed assay was conducted to observe their coagulability profiles. Interestingly, HFD male *Mkp-2*$^{-/-}$ had reduced bleed time of 75.10±17.43s compared with *Mkp-2*$^{+/+}$ mice which had an average bleed time of 108.9±17.43s (**Fig. 2C**). The female *Mkp-2*$^{-/-}$ mice had a bleed time of 91.8±17.13s that is comparable with *Mkp-2*$^{+/+}$ mice bleed time of 87.4±17.13s (**Fig. 2E**). This data shows that diet-induced obesity is associated with reduced bleeding time in male *Mkp-2*$^{-/-}$ mice. Next, we analyzed platelet counts from HFD-fed *Mkp-2*$^{-/-}$ and *Mkp-2*$^{+/+}$ mice. The results of the platelet counts were not significantly different between the two genotypes and genders (**Fig. 2D and F**). These results suggest that HFD challenge does not significantly affect the number of platelets in the blood in the absence of MKP-2. This model has limitations because definitive conclusions could not be derived from these studies since MKP-2 was deleted in a whole-body context raising the issue of counter-regulatory effects from other tissues.

MKP-2 DEFICIENCY ALTERS RED BLOOD CELL INDICES AND DEVELOPMENT OF ANEMIA IN DIET-INDUCED OBESITY

Male *Mkp-2*$^{-/-}$ mice are resistant to diet-induced obesity that is associated with reduced bleeding times. Research has demonstrated that red blood cells (RBCs) have functional characteristics that aid in hemostasis and thrombosis as well as a significant role in bleeding tendencies and complications [23, 29]. We determined the effect of MKP-2 deficiency on RBC indices in HFD-fed *Mkp-2*$^{-/-}$ mice. Male *Mkp-2*$^{-/-}$ mice exhibited significantly reduced RBC count compared with *Mkp-2*$^{+/+}$ counterparts (**Fig. 3A**). RBC counts are comparable between female HFD-fed *Mkp-2*$^{-/-}$ and *Mkp-2*$^{+/+}$ mice (**Table 3**). The analysis of hemoglobin (Hgb) levels demonstrated significantly decreased Hgb levels in male *Mkp-2*$^{-/-}$ mice in comparison with *Mkp-2*$^{+/+}$ controls (**Fig. 3B**). However, Hgb levels were similar in female HFD-fed *Mkp-2*$^{-/-}$ and *Mkp-2*$^{+/+}$ mice (**Table 3.** Furthermore, hematocrit (Hct) levels were significantly decreased in male *Mkp-2*$^{-/-}$ mice compared with *Mkp-2*$^{+/+}$ mice (**Fig. 3C**), suggesting that these mice exhibit anemia. Female HFD-fed *Mkp-2*$^{-/-}$ and *Mkp-2*$^{+/+}$ mice exhibited comparable Hct levels (**Table 3**). These observations suggest that MKP-2 plays an important role in linking diet-induced obesity and RBC function in the development of blood coagulation.

Table 3. Normal RBC indices in female MKP-2 deficient mice. Data represents mean±SEM

	Female *Mkp-2*$^{+/+}$ n=5	**Female *Mkp-2*$^{-/-}$ n=5**	***p-value***
MCH (pg)	16.72 ± 1.643	14.20 ± 1.643	0.164
HGB (g/dL)	11.76 ± 0.870	11.68 ± 0.870	0.929
HCT (%)	41.74 ± 3.398	40.10 ± 3.398	0.642
RBC (M/uL)	8.344 ± 0.667	8.226 ± 0.667	0.864

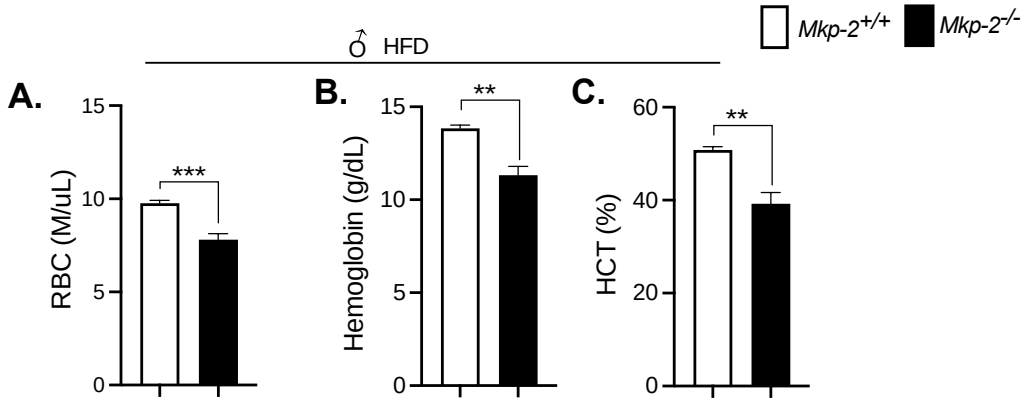

FIGURE 3. Altered red blood cell indices and development of anemia in response to a HFD in male MKP-2-deficient mice. Red blood cell count (**A**), hemoglobin levels (**B**), and hematocrit levels (**C**) from HFD-fed male *Mkp-2*$^{+/+}$ and *Mkp-2*$^{-/-}$ mice. Results represent n = 5 per genotype and data shown are the mean ± SEM; *; $p < 0.05$, **; $p < 0.01$, ***; $p < 0.0001$ as determined by student t-test . Open bars, *Mkp-2*$^{+/+}$ mice; closed bars, *Mkp-2*$^{-/-}$ mice.

ENHANCED MAPK PHOSPHORYLATION IN PLATELETS IN MALE MKP-2-DEFICIENT MICE

Understanding the role of MKP-2 in the regulation of MAPKs is crucial given the importance of MAPKs in blood coagulation. Nevertheless, despite normal platelet count, there was significant changes in MAPK signaling. Platelet lysates were analyzed through immunoblotting for the phosphorylation of p38 MAPK and ERK. Figure 4 shows that phosphorylation of p38 MAPK (**Fig. 4A, upper panel and C**) and ERK (**Fig. 4A, middle panel and C**) are significantly increased in the platelets of chow-fed male *Mkp-2*$^{-/-}$ mice compared with their wild-type counterparts (Fig. 4). The findings imply that loss of MKP-2 results in enhanced p38 MAPK and ERK phosphorylation.

ENHANCED STROMAL CELL-DERIVED FACTOR 1 (SDF-1 EXPRESSION IN PLATELETS IN MALE MKP-2-DEFICIENT MICE

We looked into the expression of SDF-1 in platelets obtained from chow-fed *Mkp-2*$^{-/-}$ mice in order to determine the signal transduction pathways crucial to platelet function. According to earlier research, *Mkp-2*$^{-/-}$ mice have higher levels of plasma circulating SDF-1 expression [28]. The immunoblot analysis of the expression of SDF-1 in platelets derived from male *Mkp-2*$^{-/-}$ and *Mkp-2*$^{+/+}$ mice is presented in Figure 4. The data indicates that male *Mkp-2*$^{-/-}$ platelets have significantly higher levels of SDF-1 protein expression compared with *Mkp-2*$^{+/+}$ controls (**Fig. 4A, lower panel and D**). This data demonstrates that the increase in SDF-1 in *Mkp-2*$^{-/-}$ mice is present in platelets.

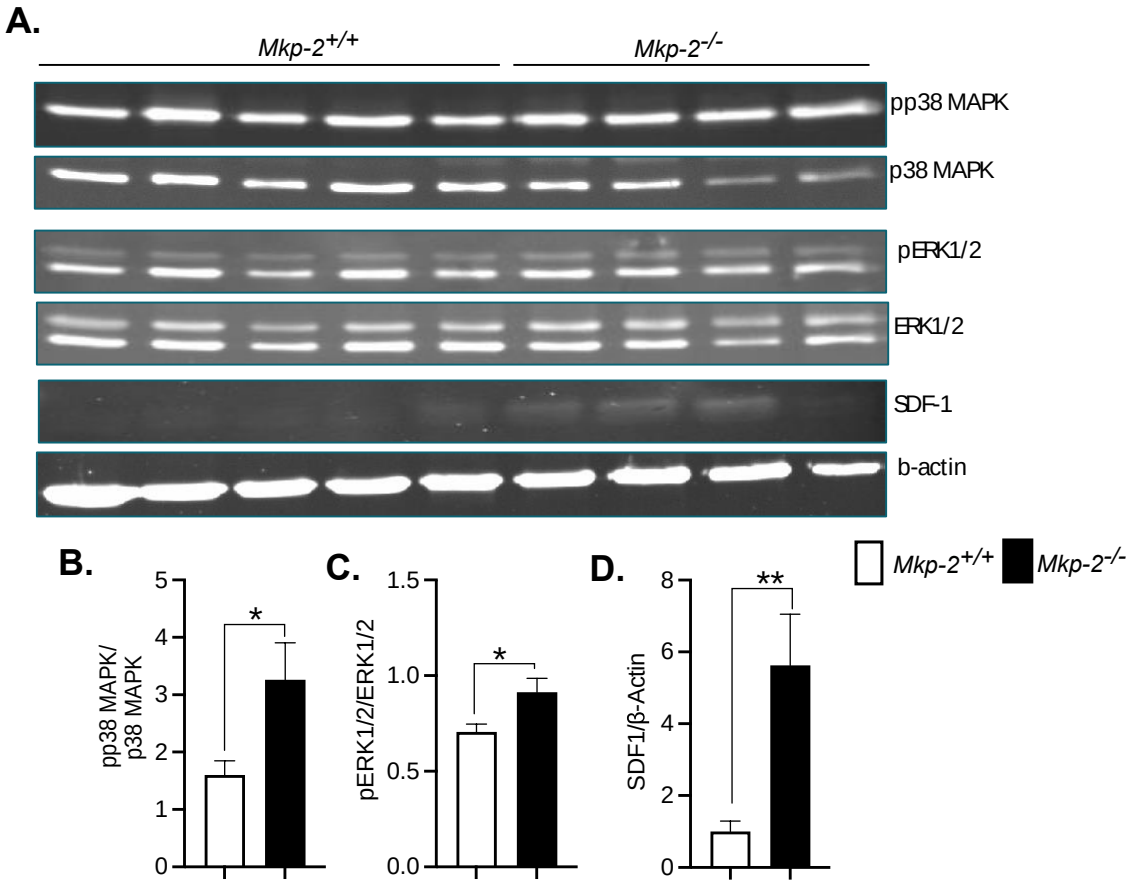

FIGURE 4. Enhanced MAPK phosphorylation and SDF-1 expr ession in the platelets of male MKP-2-deficient mice. Platelet lysates from chow-fed male *Mkp-2*$^{+/+}$ and *Mkp-2*$^{-/-}$ mice were analysed by immunoblotting (**A, B, C and D**) pp38 MAPK, pERK1/2 and SDF-1. Representative immunoblots were determined using fluorescent imaging and quantitated by densitometry for the levels of phospho-p38 MAPK/p38 MAPK, phospho-ERK1/2/ERK1/2 and SDF-1/beta-actin Results represent n = 4-5 per genotype and data shown are the mean ± SEM; *; $p < 0.05$ as determined by student t-test. Open bars, *Mkp-2*$^{+/+}$ mice; closed bars, *Mkp-2*$^{-/-}$ mice.

Discussion

Our comprehension of the mechanisms underlying the dysfunction of red blood cells, platelets, and hemorrhage is critical to our understanding of atherosclerosis, thrombosis, and bleeding, of all which are still poorly understood processes. This study details how obesity affects platelet activity, red blood cell (RBC) indices and blood coagulation in mice lacking MKP-2. We demonstrated that chow-fed female MKP-2-deficient mice exhibited reduced bleeding time. It is unexpected that the bleeding time phenotype seen in the female *Mkp-2*$^{-/-}$ mice appears to vanish when they are fed a HFD. Studies have previously demonstrated that a hypercoagulation profile may be part of the pathophysiology of obesity [30]. Perhaps overwhelming that effect and balancing the coagulation efficacy of both genotypes, the hypercoagulation brought on by HFD challenge has a far more aggressive phenotype than the hypercoagulation brought on solely by the MKP-2 deficiency. The lack of difference in bleeding time in the female HFD-fed groups could also be due to female specific sex hormones that play a role in metabolic homeostasis. Further studies are needed to determine the exact role of MKP-2 in the regulation of blood coagulation, platelet activity and RBC dysfunction in female mice.

Male *Mkp-2*$^{-/-}$ mice fed a chow diet had a comparable bleeding time with *Mkp-2*$^{+/+}$ mice. Interestingly, male *Mkp-2*$^{-/-}$ mice fed a HFD were found to have significantly decreased weight gain compared to the wild type controls. This data was previously discovered in our laboratory, and it has been verified once more [28]. Furthermore, MKP-2 deficient mice exhibited reduced bleeding time. Saturated fat-rich diets have long been linked to endothelial dysfunction, which is a prerequisite for atherosclerosis [31, 32]. However, the effects of HFD on RBCs are largely unknown as of yet. We found that RBC, Hgb and Hct levels were reduced in HFD-fed MKP-2 deficient mice suggesting that these mice exhibit anemia which could be caused by obesity-induced inflammation. According to this data, MKP-2 regulates the impact that metabolic stress has on RBC count, hematocrit, and hemoglobin levels. Some studies have demonstrated a relationship between obesity and iron deficiency [33, 34]. Whether the effects of MKP-2 deficiency also impact serum iron levels in the context of HFD remains to be established. The absence of variation in these parameters among female mice may be attributed to compensatory mechanisms triggered by female sex hormones that uphold hemoglobin homeostasis.

As the search for novel antiplatelet targets continues and as MAPK inhibitors are explored for the treatment of other diseases such as cancer therapeutics, an understanding of MAPK function in platelets is becoming more and more crucial. A thorough understanding of the upstream regulators and downstream targets is necessary and might reveal new targets for antiplatelet medications. Although *Mkp-2*$^{-/-}$ mice exhibit a gender-specific differences in blood coagulation phenotypes, platelet counts were normal. However, it will be interesting to examine platelet activation *in vitro* using fluorescent PAC-1 binding. One study demonstrated that SDF-1 does not seem to affect the overall number of megakaryocytes [35], while *Mkp-2*$^{-/-}$ mice exhibit enhanced platelet SDF-1 that correlates with reduced bleeding times. Given the role of MAPKs in hemostasis and thrombosis, there is much interest in understanding the function of MKP-2 in their regulation in platelets. MKP-2 dephosphorylates and inactivates p38 MAPK [36]. Studies demonstrated that p38 MAPK deficient mice exhibit increased tail bleeding time [37, 38], while *Mkp-2*$^{-/-}$ mice show enhanced platelet p38 MAPK activity that correlates with reduced tail bleeding time. It is noteworthy and fascinating to note that all significant findings have been found in male mice, with the exception of the chow tail bleed assay. This may be the result of physiological mechanisms unique to female mice making for the absence of MKP-2 through sex hormone pathways. These findings lend more credence to the idea that MKP-2 interacts specifically with different MAPK-directed hemostatic and thrombotic pathways in platelets.

Conclusion

In this study, we demonstrated that MKP-2 deficient mice had altered bleeding tendencies and gender specific RBC functional characteristics, indicating a potential role for MKP-2 is in the pathophysiology of thrombotic effects. Taken together, these findings imply that upregulation of MKP-2 in obesity affects hemostasis and thrombosis effects by modulating MAPK activity.

Acknowledgements

This work was supported by a grant from University of Alabama in Huntsville (#251359) to A.L.

Conflict of interests: No potential conflicts of interest relevant to this article were reported.

References

1. Arroyo-Johnson, C. and K.D. Mincey, *Obesity Epidemiology Worldwide*. Gastroenterol Clin North Am, 2016. **45**(4): p. 571-579.
2. Andolfi, C. and P.M. Fisichella, *Epidemiology of Obesity and Associated Comorbidities*. J Laparoendosc Adv Surg Tech A, 2018. **28**(8): p. 919-924.
3. Lawan, A. and A.M. Bennett, *Mitogen-Activated Protein Kinase Regulation in Hepatic Metabolism*. Trends Endocrinol Metab, 2017. **28**(12): p. 868-878.
4. Beyerlein, A., et al., *Risk factors for obesity: further evidence for stronger effects on overweight children and adolescents compared to normal-weight subjects*. PLoS One, 2011. **6**(1): p. e15739.
5. Davì, G., et al., *Platelet activation in obese women: role of inflammation and oxidant stress*. JAMA, 2002. **288**(16): p. 2008-14.
6. Simeone, P., et al., *Thromboxane-Dependent Platelet Activation in Obese Subjects with Prediabetes or Early Type 2 Diabetes: Effects of Liraglutide- or Lifestyle Changes-Induced Weight Loss*. Nutrients, 2018. **10**(12).
7. Mkandla, Z., et al., *Impaired Glucose Tolerance is Associated with Enhanced Platelet-Monocyte Aggregation in Short-Term High-Fat Diet-Fed Mice*. Nutrients, 2019. **11**(11).
8. Gehart, H., et al., *MAPK signalling in cellular metabolism: stress or wellness?* EMBO Rep, 2010. **11**(11): p. 834-40.
9. Du, V.X., et al., *New insights into the role of erythrocytes in thrombus formation*. Semin Thromb Hemost, 2014. **40**(1): p. 72-80.
10. Unruh, D., et al., *Red Blood Cell Dysfunction Induced by High-Fat Diet: Potential Implications for Obesity-Related Atherosclerosis*. Circulation, 2015. **132**(20): p. 1898-908.
11. Randi, M.L., et al., *Thrombosis and hemorrhage in thrombocytosis: evaluation of a large cohort of patients (357 cases)*. J Med, 1991. **22**(4-5): p. 213-23.
12. Stewart, G.W., et al., *Thrombo-embolic disease after splenectomy for hereditary stomatocytosis*. Br J Haematol, 1996. **93**(2): p. 303-10.
13. Tokarev, A.A., A.A. Butylin, and F.I. Ataullakhanov, *Platelet adhesion from shear blood flow is controlled by near-wall rebounding collisions with erythrocytes*. Biophys J, 2011. **100**(4): p. 799-808.
14. Schreijer, A.J., P.H. Reitsma, and S.C. Cannegieter, *High hematocrit as a risk factor for venous thrombosis. Cause or innocent bystander?* Haematologica, 2010. **95**(2): p. 182-4.
15. Klatt, C., et al., *Platelet-RBC interaction mediated by FasL/FasR induces procoagulant activity important for thrombosis*. J Clin Invest, 2018. **128**(9): p. 3906-3925.
16. Fuentes, E., S. Wehinger, and A. Trostchansky, *Regulation of Key Antiplatelet Pathways by Bioactive Compounds with Minimal Bleeding Risk*. Int J Mol Sci, 2021. **22**(22).
17. Patel, P. and U.P. Naik, *Platelet MAPKs-a 20+ year history: What do we really know?* J Thromb Haemost, 2020. **18**(9): p. 2087-2102.
18. Flaumenhaft, R., *Stressed platelets ASK1 for a MAPK*. Blood, 2017. **129**(9): p. 1066-1068.
19. Mazharian, A., et al., *Differential Involvement of ERK2 and p38 in platelet adhesion to collagen*. J Biol Chem, 2005. **280**(28): p. 26002-10.
20. Canobbio, I., et al., *A role for p38 MAP kinase in platelet activation by von Willebrand factor*. Thromb Haemost, 2004. **91**(1): p. 102-10.
21. Litvinov, R.I. and J.W. Weisel, *Role of red blood cells in haemostasis and thrombosis*. ISBT Sci Ser, 2017. **12**(1): p. 176-183.
22. Hathcock, J.J., *Flow effects on coagulation and thrombosis*. Arterioscler Thromb Vasc Biol, 2006. **26**(8): p. 1729-37.
23. Cheng, H.L., et al., *The relationship between obesity and hypoferraemia in adults: a systematic review*. Obes Rev, 2012. **13**(2): p. 150-61.
24. Armstead, W.M., et al., *RBC-coupled tPA Prevents Whereas tPA Aggravates JNK MAPK-Mediated Impairment of ATP- and Ca-Sensitive K Channel-Mediated Cerebrovasodilation After Cerebral Photothrombosis*. Transl Stroke Res, 2012. **3**(1): p. 114-21.
25. Lawan, A., et al., *Hepatic mitogen-activated protein kinase phosphatase 1 selectively regulates glucose metabolism and energy homeostasis*. Mol Cell Biol, 2015. **35**(1): p. 26-40.
26. Al-Mutairi, M.S., et al., *MAP kinase phosphatase-2 plays a critical role in response to infection by Leishmania mexicana*. PLoS Pathog, 2010. **6**(11): p. e1001192.
27. Lawan, A., et al., *Deletion of the dual specific phosphatase-4 (DUSP-4) gene reveals an essential non-redundant role for MAP kinase phosphatase-2 (MKP-2) in proliferation and cell survival*. J Biol Chem, 2011. **286**(15): p. 12933-43.
28. Fernando, S., et al., *Metabolic Impact of MKP-2 Upregulation in Obesity Promotes Insulin Resistance and Fatty Liver Disease*. Nutrients, 2022. **14**(12).
29. Purdy, J.C. and J.J. Shatzel, *The hematologic consequences of obesity*. Eur J Haematol, 2021. **106**(3): p. 306-319.
30. Chung, D.W., et al., *High-density lipoprotein modulates thrombosis by preventing von Willebrand factor self-association and*

subsequent platelet adhesion. Blood, 2016. **127**(5): p. 637-45.
31. Vita, J.A., *Endothelial function*. Circulation, 2011. **124**(25): p. e906-12.
32. Davis, N., S. Katz, and J. Wylie-Rosett, *The effect of diet on endothelial function*. Cardiol Rev, 2007. **15**(2): p. 62-6.
33. Farhangi, M.A., et al., *White blood cell count in women: relation to inflammatory biomarkers, haematological profiles, visceral adiposity, and other cardiovascular risk factors*. J Health Popul Nutr, 2013. **31**(1): p. 58-64.
34. Aigner, E., A. Feldman, and C. Datz, *Obesity as an emerging risk factor for iron deficiency*. Nutrients, 2014. **6**(9): p. 3587-600.
35. Niswander, L.M., et al., *SDF-1 dynamically mediates megakaryocyte niche occupancy and thrombopoiesis at steady state and following radiation injury*. Blood, 2014. **124**(2): p. 277-86.
36. Lawan, A., et al., *MKP-2: out of the DUSP-bin and back into the limelight*. Biochem Soc Trans, 2012. **40**(1): p. 235-9.
37. Naik, M.U., et al., *Ask1 regulates murine platelet granule secretion, thromboxane A*. Blood, 2017. **129**(9): p. 1197-1209.
38. Shi, P., et al., *Platelet-Specific p38α Deficiency Improved Cardiac Function After Myocardial Infarction in Mice*. Arterioscler Thromb Vasc Biol, 2017. **37**(12): p. e185-e196.