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The impact of a short-term high-fat diet on coagulation function in a mouse model and its role in exacerbating concanavalin A-induced liver injury

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Abstract

Background Recently, the number of patients with metabolic dysfunction-associated steatotic liver disease (MASLD) and its more advanced condition, metabolic dysfunction-associated steatohepatitis (MASH), has been increasing. These patients are at a higher risk of cardiovascular events and thromboembolism. However, the direct impact of high-fat diet (HFD), a cause of MASLD, on liver coagulation function is not well understood. Previously, we demonstrated that a short-term, 4-day intake of a HFD exacerbates concanavalin A (Con A)-induced acute liver injury in mice by promoting coagulation and inflammation. This model demonstrates that the liver exposed to a short-term HFD is vulnerable even before disease onset. In this study, using this model, we elucidated the detailed mechanisms by which short-term HFD intake promotes coagulation, considering primary and secondary hemostasis.

Methods C57BL/6 mice normally fed a normal diet (ND) were subjected to a HFD for 4 days. Liver tissue and blood samples were collected before and 4 and 24 h after Con A administration. Histological analysis, flow cytometry for platelet analysis, and blood coagulation tests related to secondary hemostasis were performed.

Results Even with short-term consumption of a HFD alone, platelet and fibrinogen levels increased in the peripheral blood and liver. Additionally, when Con A was administered to mice on a short-term HFD, an increase in P-selectin expression was observed in the liver, with no upregulation in peripheral blood platelets. Furthermore, in mice subjected to a short-term HFD and treated with Con A, prolonged prothrombin time (PT) and activated partial thromboplastin time (APTT) were observed.

Conclusions Consuming a HFD in short-term can enhance primary and secondary hemostasis, thereby increasing the risk of thrombosis. These conditions are presumed to be a risk factor that exacerbates Con A-induced liver injury. The findings provide insight into early intervention strategies for chronic liver diseases, such as MASLD and MASH.

Keywords High-fat diet, Concanavalin A, Acute liver injury, Coagulation, Platelet, Non-alcoholic fatty liver disease, Non-alcoholic steatohepatitis

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Background

In recent years, there has been a significant increase in lifestyle-related diseases associated with high-calorie diets. Concurrently, the incidence of metabolic dysfunction-associated steatotic liver disease (MASLD), a manifestation of metabolic syndrome affecting the liver, has

also increased [1, 2]. MASLD and metabolic dysfunction-associated steatohepatitis (MASH) are the recently adopted terms replacing the previously used non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) [3, 4]. MASLD progresses to MASH in about 20% of cases. This progression represents a serious health concern, with the potential to further develop into liver cirrhosis and hepatocellular carcinoma [5, 6]. MASLD patients have concurrent conditions such as diabetes, dyslipidemia, and hypertension, which highlight their increased risk of cardiovascular events and vascular thrombosis [7, 8]. However, there has been limited research on the relationship between a high-fat diet (HFD), a direct cause of MASLD, and its impact on coagulation function.

In patients with obesity-associated diabetes, prior research has shown that atherosclerosis is triggered by platelet activation [9–11]. Additionally, in mouse models subjected to a long-term HFD, which resulted in weight gain, hyperglycemia, and hyperinsulinemia, researchers have observed excessive platelet activation [12]. Moreover, it has been reported that individuals with obesity exhibit elevated plasma levels of coagulation factors (F) VII, VIII, XII, fibrinogen, and plasminogen activator inhibitor-1 (PAI-1) [13]. Based on these findings, it is reasonable to speculate that the consumption of a HFD induces obesity and systemic hypercoagulability by activating platelets and coagulation factors involved in secondary hemostasis.

We previously reported that a short-term, 4-day HFD induces vulnerability to concanavalin A (Con A)-induced liver injury in mice. A comprehensive analysis of this model revealed that short-term HFD intake increases the expression of inflammatory cytokines such as IFN-γ and TNF-α in the liver. Additionally, in the livers of mice exposed to a short-term HFD, fibrinogen deposition and the expression of tissue factor (TF) were found to be elevated, indicating the formation of a procoagulant state [14, 15]. This procoagulant state, along with the inflammatory response in the liver, is thought to exacerbate liver injury. However, the specific changes in coagulation function, particularly those affecting liver-specific or systemic primary and secondary hemostasis due to short-term HFD intake, have not yet been fully elucidated.

Con A is a lectin known to induce acute liver injury in rodents by regulating inflammatory cells and non-parenchymal liver cells [16, 17]. Additionally, by stimulating immune cells in the liver, Con A promotes inflammation that leads to coagulation abnormalities. This, in turn, predisposes the liver to widespread necrosis due to the formation of microthrombi in the hepatic sinusoids [18]. Previous research involving mice has shown that liver injury induced by Con A promotes inflammation through the macrophage-mediated accumulation of hepatic platelets. Furthermore, using anti-P-selectin antibodies, which are markers of platelet activation, has proven effective in reducing this injury [19]. The elevated expression of TF and fibrinogen, which play crucial roles in secondary hemostasis, contributes to the induction of Con A-induced liver injury. The use of heparin, known to inhibit secondary hemostasis, has been shown to significantly alleviate damage [14, 15, 18].

Given the context provided, we aimed to explore the mechanisms by which short-term HFD intake exacerbates Con A-induced liver injury. Our research specifically focuses on the role of platelets in primary hemostasis and their relationship to coagulation functions associated with secondary hemostasis. If we can identify the effects of HFD intake on coagulation function at both molecular and systemic levels, it may offer insights into early intervention strategies for treating chronic liver diseases such as MASLD and MASH. Therefore, our goal is to elucidate in greater detail the effects of HFD intake, even over short periods, on both systemic and liver-specific coagulation functions.

Methods

Animals and diets

Male C57BL/6 mice, aged 6 weeks, were obtained from Japan SLC Inc. (Shizuoka, Japan), and housed in a controlled environment with 12-h light/dark cycles at 25℃. The mice were initially fed a normal diet (ND: CE2, CLEA Japan Inc., Tokyo, Japan) as the control diet, and were subsequently switched to a HFD (High-Fat Diet 32, CLEA Japan Inc.) for 4 days, in accordance with the experimental designs (Fig. 1a). All experiments were conducted using mice that were 8 weeks old. The bleeding from the orbital veins and the sacrifice of mice were performed under anesthesia using isoflurane (Pfizer Japan Inc., Tokyo, Japan).

Model of acute liver injury induced by Con A

Two groups of mice, one fed a ND and the other a HFD for 4 days, received an intravenous administration of 10 mg/kg body weight of Con A (Sigma–Aldrich, St. Louis, MO, USA) dissolved in phosphate-buffered saline (Fujifilm Wako) (Fig. 1a). Blood samples were collected from the retro-orbital venous plexus under isoflurane anesthesia, and liver tissues were collected prior to Con A administration (0 h), as well as 4 h and 24 h post-Con A administration.

Biochemical test

The measurement of alanine aminotransferase (ALT) was conducted using serum samples at SRL Inc. (Tokyo, Japan).

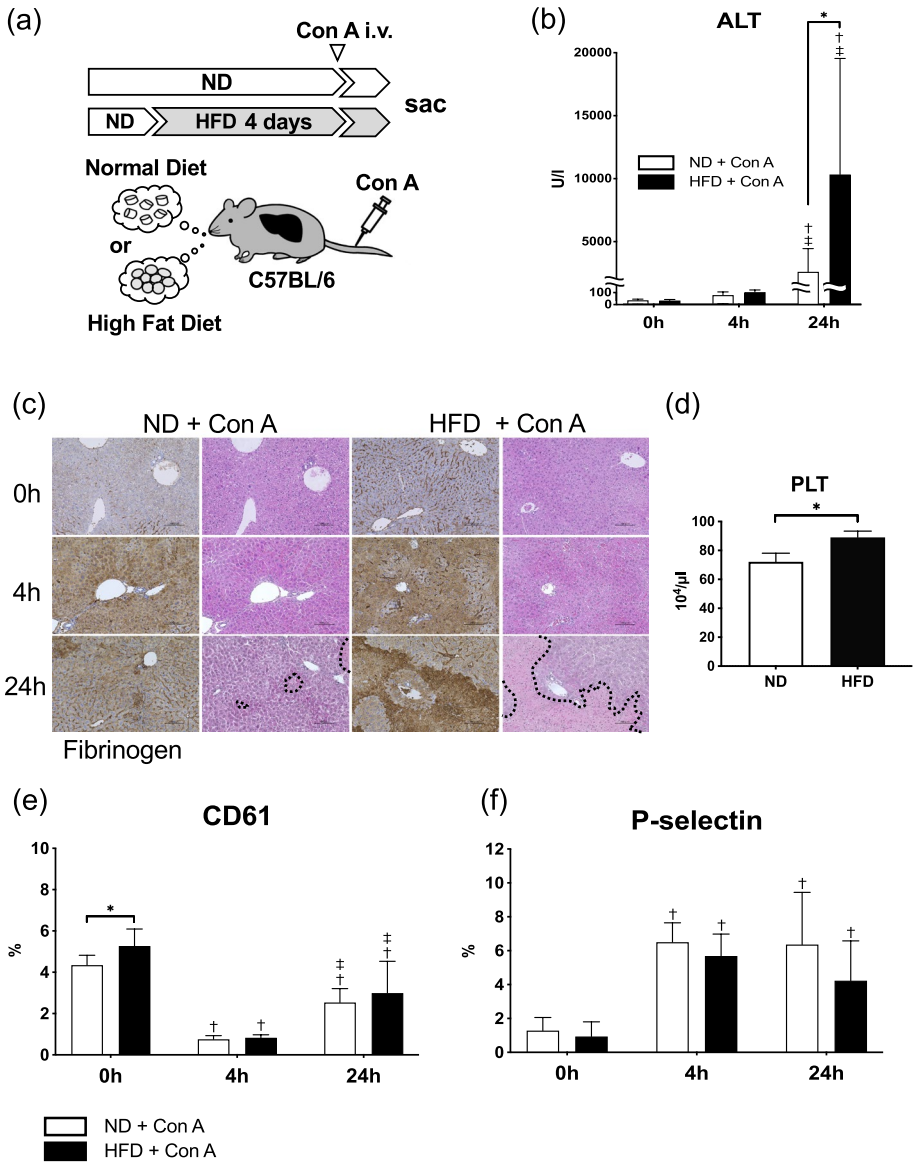

Fig. 1 A mouse model demonstrating exacerbated Con A-induced liver injury following short-term HFD intake and platelet activation changes in blood. **a** Experimental scheme for establishing a mouse model. **b** Serum ALT levels (U/L) in ND mice ($n=5$) and short-term HFD mice ($n=5$) at 0, 4, and 24 h post-Con A administration. The data are presented as means ± SD. *: $p<0.05$; †: $p<0.05$ (compared to 0 h of the same dietary model); ‡: $p<0.05$ (compared to 4 h of the same dietary model). **c** Histological images of liver tissue from mice on a ND and a short-term HFD at 0, 4, and 24 h post-Con A administration. The left panels display immunohistochemical staining for fibrinogen/fibrin, whereas the right panels demonstrate HE staining. The necrotic or degenerative area of the tissue is outlined with dashed lines. Scale bar: 100 μm. **d** Platelet counts (10^4 μl) were measured in whole blood samples from ND mice ($n=5$) and short-term HFD mice ($n=5$) using the Celltac α analyzer. The data are presented as means ± SD. * $p<0.05$. **e** Changes in CD61 expression levels in the peripheral blood of ND mice ($n=8$) and short-term HFD mice ($n=8$) were measured at 0, 4, and 24 h post-Con A administration. The data are presented as means ± SD. *: $p<0.05$; †: $p<0.05$ (compared to 0 h of the same dietary model); ‡: $p<0.05$ (compared to 4 h of the same dietary model). **f** Changes in P-selectin expression percentages in CD61-positive peripheral blood cells of ND mice ($n=6$) and short-term HFD mice ($n=6$) were measured at 0, 4, and 24 h post-Con A administration. The data are presented as means ± SD. *: $p<0.05$; †: $p<0.05$ (compared to 0 h of the same dietary model); ‡: $p<0.05$ (compared to 4 h of the same dietary model)

Histological analysis

Liver tissues from sacrificed mice were fixed in 10% formaldehyde (supplied by Japan Tanner Corporation, Osaka, Japan), embedded in paraffin, and sectioned at a thickness of 3 μm. The tissue sections were stained with hematoxylin and eosin (H&E). For immunostaining, we used a rabbit anti-fibrinogen antibody (#189,490, Abcam plc, Cambridge, UK) as the primary antibody

and SignalStain® Boost IHC Detection Reagent (HRP, Rabbit, #8114, CST, Inc.) as the secondary antibody. It is important to note that the primary antibody used for fibrinogen/fibrin immunostaining (#189,490) does not differentiate between fibrinogen and fibrin. Allophycocyanin (APC)-conjugated anti-mouse/rat CD61 antibody (catalog #104,316, BioLegend, San Diego, CA, USA) and fluorescein isothiocyanate (FITC)-conjugated anti-mouse P-selectin antibody (catalog #M130-1, Emfret Analytics, Eibelstadt, Germany) were used for fluorescence immunostaining. The proportion of the positive area in the fluorescent immunostaining image was calculated using ImageJ (Rasband, W.S., ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA).

Flow cytometric analysis of peripheral blood

Rapidly mix the peripheral blood of the mice with 3.8% sodium citrate (ERMA Inc., Saitama, Japan) in a 9:1 ratio. Gently combine 5 µL of APC anti-mouse/rat CD61 antibody (#104,316) and/or 5 µL of FITC anti-mouse P-selectin antibody (#M130-1) with 2.5 µL of whole blood per sample. Incubate the mixture in a dark environment at room temperature for 15 min. To prepare samples for flow cytometry, add 500 µL of 1% paraformaldehyde at 4 ℃. The flow cytometry analysis was conducted using the LSRFortessa X-20 system (BD Biosciences, New Jersey, USA).

Assessing platelet count in peripheral blood

Blood was collected from mice using EDTA-2 K micro blood collection tubes (BD Biosciences), and platelet counts were measured in whole blood samples using a Celltac α hematology analyzer (MEK-6558, NIHON KOHDEN, Tokyo, Japan).

Blood coagulation test

The measurements of prothrombin time (PT) in seconds, activated partial thromboplastin time (APTT) in seconds, and fibrinogen concentration in milligrams per deciliter were conducted using a COAG2NV analyzer (ERMA Inc.) on 3.8% sodium citrate plasma samples from mice.

Statistical analysis

Statistical analyses were conducted using GraphPad Prism version 9.0 (GraphPad Software, La Jolla, CA, USA). All values are presented as mean ± standard deviation (SD). The significance of differences was determined using the Student's t-test, one-way analysis of variance (ANOVA), or two-way ANOVA, depending on the study, with post-hoc Tukey's multiple comparison analysis. Statistical significance was set at $p < 0.05$.

Results

Short-term consumption of a HFD increases the count of peripheral blood platelets, yet it does not affect the degree of platelet activation induced by Con A

After acclimating the mice on an ND for more than a week, the mice were fed an HFD for 4 days, followed by an intravenous injection of Con A via the tail vein. Analyses were conducted at 0, 4, and 24 h after Con A administration (Fig. 1a). At 24 h following Con A administration, which marks the peak of Con A-induced liver damage (Fig. 1b, c), there was an increase in platelet count compared to the levels observed at 4 h post-administration in both groups. The effects of short-term HFD intake and Con A administration on peripheral blood platelet counts were investigated. First, we measured platelet counts using Celltac α to assess the impact of short-term HFD intake on these counts (Fig. 1d). The results demonstrated a significant elevation in platelet count in mice subjected to a short-term HFD intake compared to those on a ND (Fig. 1d). Subsequently, we conducted flow cytometry analysis with an APC-labeled CD61 antibody, a marker of platelet surface, to assess changes in platelet count following Con A administration (Fig. 1e). Consistent with the results obtained from Celltac α, the percentage of CD61-positive cells in the peripheral blood before Con A administration (0 h) was significantly higher in mice on a short-term HFD compared to those on a ND. Additionally, both groups exhibited a significant reduction in the percentage of CD61-positive cells at 4 h post-Con A administration relative to their pre-administration levels (Fig. 1e). However, these counts remained significantly lower than the baseline levels observed before administration (Fig. 1e). Additionally, flow cytometry analysis using FITC-labeled P-selectin, a marker of platelet activation, demonstrated a significant elevation in positivity at 4 and 24 h after Con A administration compared to baseline levels. No significant differences were observed between mice on a ND and those on a short-term HFD (Fig. 1f).

A short-term HFD and Con A administration increase platelet counts in the liver

To investigate the impact of short-term HFD intake on platelet counts in the liver following Con A administration, immunohistochemical analyses were conducted. Before the Con A injection (0 h), the livers of mice fed a short-term HFD exhibited a higher presence of platelets labeled with APC-conjugated CD61 antibody compared to those of mice on a ND (Fig. 2a). Using ImageJ to quantify the APC-positive areas in tissue images, we observed a significant difference between mice on a ND and those on a short-term HFD, prior to Con A injection (0 h) (Fig. 2b).

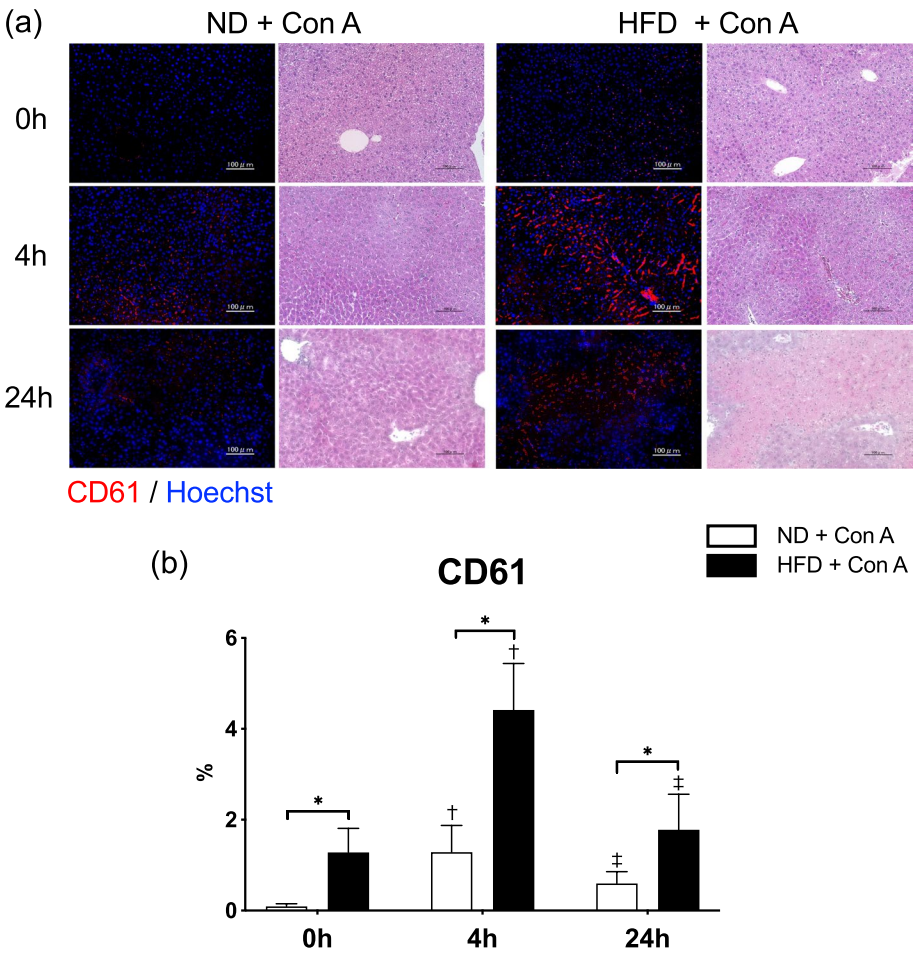

Fig. 2 Changes in liver platelet accumulation following Con A administration and short-term HFD intake. **a** Histological images of liver tissue from ND mice and short-term HFD mice at 0, 4, and 24 h after Con A administration. The left panels show immunohistochemical staining for CD61-labeled APCs, whereas the right panels show H&E staining. Scale bar: 100 μm. **b** The area positive for CD61 was measured using ImageJ software. The data ($n = 5$ for each group) are presented as means ± SD. *: $p < 0.05$; †: $p < 0.05$ (compared to 0 h of the same dietary model); ‡: $p < 0.05$ (compared to 4 h of the same dietary model)

Additionally, 4 h after Con A administration, there was a peak in platelet accumulation in the livers of mice on a short-term HFD, and at this same time point, the platelet area was significantly larger compared to that in mice on a ND (Fig. 2a, b). Additionally, 24 h after Con A injection, when the severity of Con A-induced liver injury reached its peak—as evidenced by HE staining—the area positive for APC expression decreased compared to its size 4 h post-administration (Fig. 2a, b). However, in mice subjected to a short-term HFD, the platelet area remained significantly larger compared to mice on a ND at this time point (Fig. 2a, b).

The short-term consumption of a HFD and the administration of Con A are associated with increased expression of P-selectin in the liver

To investigate the impact of short-term HFD feeding on P-selectin expression in the liver following Con A administration, immunohistochemical analyses were conducted, as P-selectin is recognized as a marker of platelet activation. Before administering Con A (0 h), there was no significant difference in the area positive for FITC-labeled P-selectin antibody between mice on a short-term HFD and those on a ND (Fig. 3a, b). However, 4 h after Con A administration, P-selectin expression in the

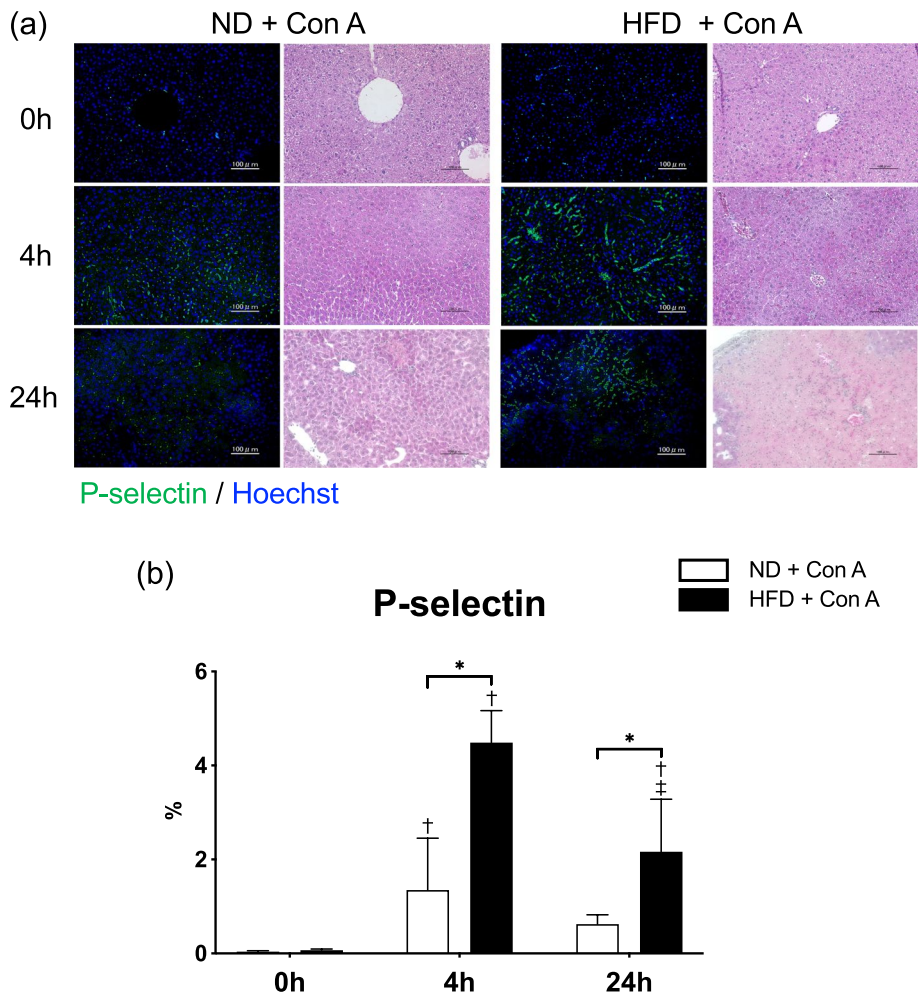

Fig. 3 Changes in liver P-selectin expression following Con A administration and short-term HFD intake. **a** Histological images of liver tissue from ND mice and short-term HFD mice at 0, 4, and 24 h after Con A administration. The left panels show immunohistochemical staining for P-selectin labeled with FITC, whereas the right panels demonstrate HE staining. Scale bar: 100 μm. **b** The area of P-selectin positivity measured using ImageJ software. The data ($n = 5$ for each group) are presented as means ± SD. *: $p < 0.05$; †: $p < 0.05$ (compared to 0 h of the same dietary model); ‡: $p < 0.05$ (compared to 4 h of the same dietary model)

livers of mice on a short-term HFD peaked, showing significantly higher levels compared to mice on a ND at the same time point (Fig. 3a, b). The expression of P-selectin decreased at 24 h post-Con A administration compared to its levels at 4 h post-administration (Fig. 3a, b). However, in mice subjected to a short-term HFD, the expression levels remained significantly elevated compared to those in ND mice 24 h after Con A administration (Fig. 3a, b).

A daily HFD intake increases fibrinogen levels in peripheral blood

The impact of short-term daily HFD consumption on peripheral blood clotting times and fibrinogen levels was examined. The PT and APTT times remained unchanged with daily HFD consumption; however, fibrinogen levels increased with each day of HFD intake, from the second day of HFD intake onward, it showed a significantly greater increase compared to ND intake (Fig. 4a).

Administration of Con A led to prolonged PT and APTT in mice on a short-term HFD compared to those on a ND

The effects of Con A administration on PT, APTT, and peripheral blood fibrinogen concentrations at 4 and 24 h post-administration were investigated in ND and short-term HFD mice. Both PT and APTT were significantly prolonged in both ND and short-term HFD mice 4 h after Con A injection, with the prolongation of APTT persisting 24 h post-Con A administration

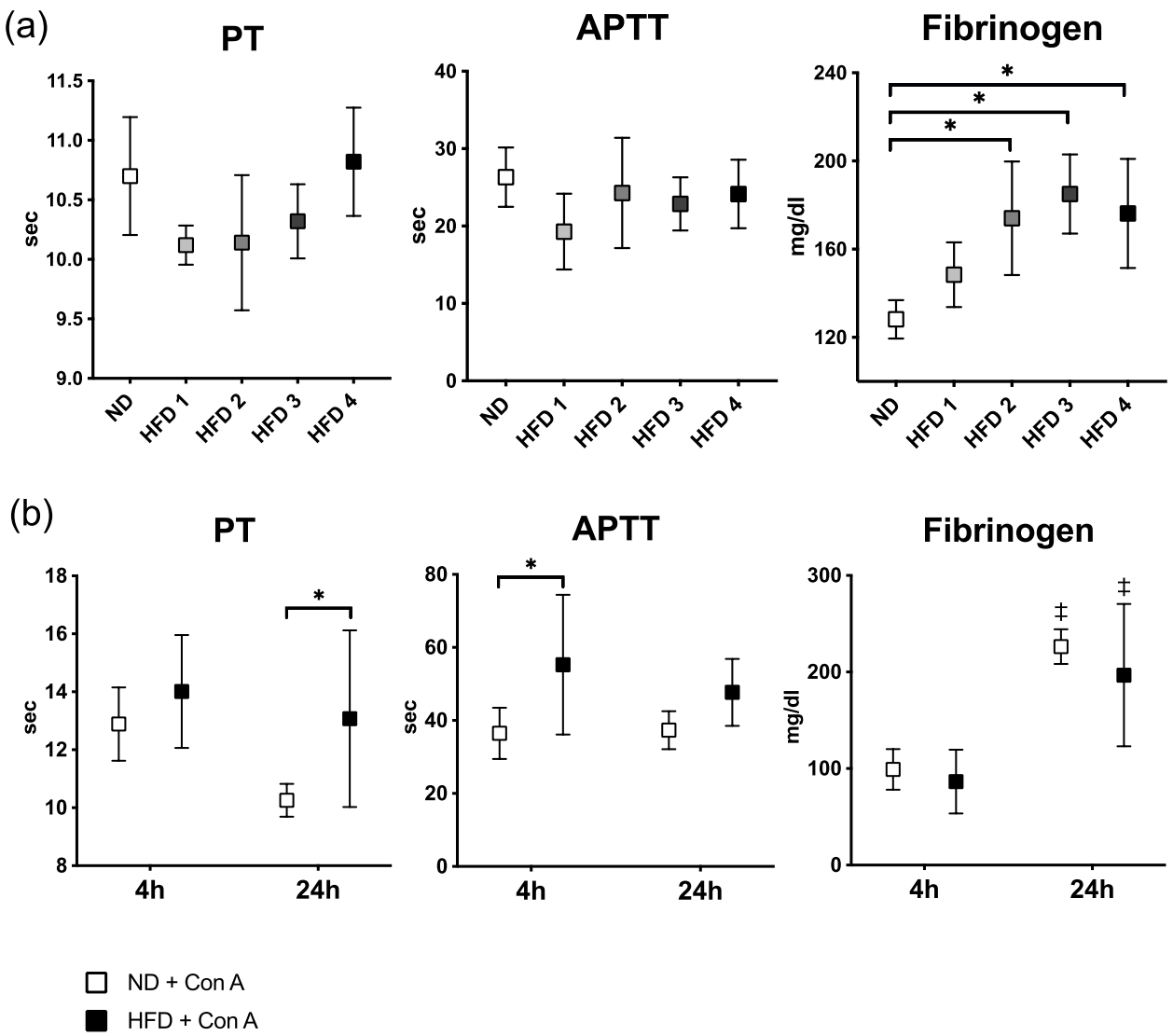

Fig. 4 Daily changes in coagulation function following HFD intake and Con A administration. **a** Daily changes in PT, (seconds), APTT (seconds), and Fibrinogen (mg/dL) associated with HFD consumption ($n=5$). The data are presented as means ± SD. *: $p<0.05$. **b** Changes in PT, APTT, and fibrinogen levels at 4 h and 24 h after Con A administration in mice fed with a ND or a HFD for 4 days ($n=8$ per group). The data are presented as means ± SD. *: $p<0.05$; ‡: $p<0.05$ (compared to 4 h of the same dietary model)

(Table 1). Fibrinogen levels sharply decreased in mice on a short-term HFD 4 h after Con A administration. However, 24 h post-administration, fibrinogen significantly increased in both ND and HFD mice, as shown in Table 1. PT was significantly prolonged in mice on a short-term HFD compared to those on a ND 24 h after administering Con A (Fig. 4b). In mice subjected to a short-term HFD, APTT was significantly prolonged compared to mice on a ND 4 h after Con A injection, as shown in Fig. 4b. There was no significant difference in fibrinogen levels between the ND mice and those on a short-term HFD after Con A administration.

Discussion

This study suggests that consuming a HFD for just 4 days induces a procoagulant state in the liver, characterized by an increase in platelets and activation of P-selectin. Concurrently, short-term consumption of a HFD has been demonstrated to enhance coagulation by elevating fibrinogen levels in the blood. Additionally, inducing Con A liver injury in the short-term HFD model significantly

Table 1 PT/APTT and Fibrinogen levels in mice on a ND and short-term HFD post-Con A administration

	+Con A		
PT (sec)	0 h	4 h	24 h
ND	10.7 ± 0.49	12.9 ± 1.26†	10.3 ± 0.57
HFD	10.8 ± 0.45	14.0 ± 1.95†	13.1 ± 3.05
	+Con A		
APTT (sec)	0 h	4 h	24 h
ND	26.3 ± 3.84	36.5 ± 7.00†	37.3 ± 5.20†
HFD	24.1 ± 4.43	55.3 ± 19.1†	47.7 ± 9.17†
	+Con A		
Fibrinogen (mg/dl)	0 h	4 h	24 h
ND	128 ± 8.70	99.1 ± 21.1†	226 ± 18.0†‡
HFD	176 ± 24.7*	86.5 ± 33.0†	197 ± 73.7†‡

Changes in PT/APTT and fibrinogen levels in mice on a ND and short-term HFD following Con A administration at 0, 4, and 24 h

The data are presented as means ± SD

* $p < 0.05$ (compared to ND at the same time point)

† $p < 0.05$ (compared to 0 h of the same dietary model)

‡ $p < 0.05$ (compared to 4 h of the same dietary model)

prolonged PT and APTT, indicating an enhancement of secondary hemostasis. Therefore, short-term consumption of a HFD appears to promote both primary and secondary hemostasis, thereby exacerbating Con A-induced liver injury.

Previous research has reported an elevation in platelet count among overweight and obese individuals, indicating a positive relationship between long-term HFD intake and platelet counts in humans [20, 21]. Conversely, it is well-documented that liver dysfunction, resulting from conditions such as chronic fatty liver or cirrhosis, leads to a decrease in platelet count [22–24]. In this study, the significant increase in platelet count observed after 4 days of HFD consumption suggests that short-term HFD intake does not induce a reduction in platelet count through liver dysfunction but rather increases platelet count, potentially enhancing transient primary hemostasis mediated by platelets. It is well-documented that administering Con A to mice results in the accumulation of platelets in the liver prior to an increase in ALT levels [25]. Our previous study demonstrated that liver inflammation induced by Con A peaks 24 h after administration, and that mice on a short-term HFD develop more severe liver injury than those on a ND [14, 15]. In this study, using flow cytometry, we observed changes in the percentage of CD61-positive platelets in peripheral blood at 4 and 24 h post-Con A administration; however, no significant differences were found between the ND and HFD mice corresponding to the severity of liver injury. This suggests that platelet dynamics in peripheral blood and the liver following Con A administration do not necessarily align. Considering that both the platelet counts and the percentage of platelets in peripheral blood were significantly higher in the HFD mice compared to the ND mice prior to Con A administration, it is highly probable that platelet consumption was accelerated in the HFD mice following Con A administration.

P-selectin, also referred to as GMP-140 or CD62P, is a transmembrane glycoprotein located in the alpha granule membranes of platelets and in the Weibel-Palade body membranes of endothelial cells [26]. When activated, P-selectin of these cells translocate to the cell surface concurrent with granule release [26]. Previous research has indicated the therapeutic potential of P-selectin antibodies in treating Con A-induced liver injury [25]. However, in this study, flow cytometry analysis of platelet P-selectin expression in peripheral blood revealed no significant difference in P-selectin expression levels between ND and short-term HFD mice following Con A administration. This finding suggests that Con A-induced upregulation of P-selectin expression may be specific to the liver.

Platelet accumulation and P-selectin expression in the livers of mice fed a ND and those on a short-term HFD were subsequently investigated using fluorescence immunostaining. Before the administration of Con A, significant platelet accumulation was observed in the livers of mice on a short-term HFD. Indeed, a study has reported a significant increase in platelet accumulation in the livers of mice fed a choline-deficient HFD for 6 months [27]. However, there was no significant difference in P-selectin expression prior to Con A administration between the ND and short-term HFD mice. This suggests that short-term HFD intake leads to an increase in platelets within the liver; however, there was no concurrent increase in platelet activity or endothelial cell activation. 4 h after administering Con A, platelet counts in the liver peaked, showing significantly higher levels in mice on a short-term HFD compared to those on a ND. Additionally, at this time point, the expression of P-selectin was significantly higher in mice on a short-term HFD compared to those on a ND. Indeed, prior research has shown that P-selectin expression in the livers of mice increases following administration of Con A [19]. Our results suggest that platelet activation in the liver of mice on a short-term HFD was significantly enhanced by Con A stimulation.

Previous studies have shown that IFN-γ and TNF-α, produced by Con A-stimulated cells, enhance TF and PAI-1 expressions in hepatic macrophages and sinusoidal endothelial cells. This upregulation induces a procoagulant state, leading to thrombosis and extensive hepatic necrosis within the sinusoids [18]. Our previous study also demonstrated that short-term HFD consumption

increased hepatic TF and PAI-1 expressions following Con A administration, indicating a procoagulant state in the short-term HFD model [14, 15]. Additionally, PT and APTT are used as indicators of the activity of the extrinsic and intrinsic pathways of the coagulation cascade, respectively. In this study, we primarily measured PT, APTT, and fibrinogen levels in peripheral blood from mice on a ND and those on a short-term HFD. These measurements represent components of secondary hemostasis. The PT and APTT times remained unchanged following HFD loading, while fibrinogen levels increased daily. Additionally, these findings align with our earlier research, which demonstrated that short-term consumption of a HFD leads to increased fibrinogen deposition in hepatic sinusoids [14, 15]. This strongly supports the hypothesis that short-term consumption of a HFD contributes to a procoagulant state by elevating fibrinogen levels, thereby serving as a risk factor for Con A-induced liver injury. In mice fed a short-term HFD, PT was significantly prolonged 24 h after Con A administration, whereas APTT was significantly prolonged 4 h post-Con A administration, compared to mice on a ND. Thus, a short-term HFD is anticipated to influence both intrinsic and extrinsic coagulation pathways in the development of Con A-induced liver injury. The fibrinogen levels post-Con A administration exhibited a temporary decrease at 4 h, suggesting transient fibrinogen consumption due to thrombus formation. There was no significant difference between the short-term HFD mice and the ND mice 4 h after Con A administration. Nevertheless, given the notable difference in fibrinogen levels prior to Con A administration, it can be inferred that fibrinogen consumption post-Con A administration differs significantly between short-term HFD mice and ND mice. Previous research has shown that long-term HFD lead to the activation of F VII after postprandially [28, 29]. Thus, several coagulation factors in addition to fibrinogen may play a role in the initiation and progression of Con A-induced liver injury. Given that the procoagulant state induced by short-term HFD consumption increases vulnerability to subsequent inflammatory stimuli, further comprehensive studies on the effects of short-term HFD on coagulation factors could elucidate the mechanisms contributing to the development of chronic liver diseases, including MASLD and MASH.

Conclusions

This study has demonstrated that even short-term consumption of a HFD induces a procoagulant state in the liver by increasing platelet counts and activating P-selectin, thereby enhancing primary hemostasis. Simultaneously, the increase in fibrinogen levels enhances secondary hemostasis, ultimately leading to a higher propensity for thrombus formation. This is presumed to exacerbate Con A-induced liver injury. These findings suggest that anticoagulant therapy may be crucial in early intervention strategies for preventing and treating chronic liver diseases such as MASLD and MASH, particularly during the pre-clinical stage.

Abbreviations

MASLD	Metabolic dysfunction-associated steatotic liver disease
MASH	Metabolic dysfunction-associated steatohepatitis
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
HFD	High-fat diet
F	Coagulation factors
PAI-1	Plasminogen activator inhibitor-1
Con A	Concanavalin A
TF	Tissue factor
ND	Normal diet
ALT	Alanine aminotransferase
H&E	Hematoxylin and eosin
APC	Allophycocyanin
FITC	Fluorescein isothiocyanate
ANOVA	Analysis of variance
PT	Prothrombin time
APTT	Activated partial thromboplastin time
SD	Standard deviation

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Authors' contributions

Study concept and design: E.N., T.Y. and M.N.; Acquisition of data: E.N., Y.T. and S.O.; Analysis and interpretation of data: E.N., N.H. and M.N.; Drafting of the manuscript: E.N.; Critical revision of the manuscript: Y.T., T.Y., S.O., N.H., M.N.; Statistical analysis: E.N.; Obtained funding: E.N.; Administrative, technical, or material support: T.Y. and M.N.; Study supervision: M.N.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

All procedures involving animals in this study were conducted in accordance with protocols approved by the Aichi Medical University Animal Care and Use Committee (approval number 2023–53).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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