Brief Report

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Hypofibrinolytic phenotype in Tsumura Suzuki Obese Diabetes (TSOD) mice unrelated to hyperglycemia

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SUMMARY Obesity and diabetes mellitus are associated with increased risk of arterial thrombosis and venous thromboembolism. Tsumura Suzuki Obese Diabetes (TSOD) mice are useful models for elucidating the molecular mechanisms of these diseases. We investigated normoglycemic [Ng]-TSOD mice with a metabolic abnormality that was accompanied by a coagulative and fibrinolytic state with a phenotype that distinctly differed from that of standard TSOD mice. As in TSOD mice, plasminogen activation inhibitor-1 (PAI-1) that inhibits fibrinolysis was substantially augmented in Ng-TSOD mice, suggesting that they are hypofibrinolytic. However, blood clotting parameters were within the normal range in Ng-TSOD mice. These findings indicated that Ng-TSOD mice are novel models with a hypofibrinolytic phenotype that is not associated with hyperglycemia.

Keywords type 2 diabetes, plasma, obesity, coagulation, hypofibrinolytic state

1. Introduction

Obesity and diabetes are significant risk factors for the development of cardiac diseases, arterial thrombosis and venous thromboembolism. The status of these illnesses can be determined as altered levels of blood lipids, adipocytokines, coagulative, and fibrinolytic factors in plasma (*1*). Various mouse models of type 2 diabetes have contributed to current understanding of metabolic syndrome and its related diseases (*2*). The Tsumura Suzuki Obese Diabetes (TSOD) polygenetic mouse model of spontaneous obese Type 2 diabetes mellitus (DM) is an inbred line in which males are obese and have urinary glucose, hyperglycemia, hyperinsulinemia, increased food intake, body and fat weight (*3*). Obesity and DM are associated with increased risk of arterial thrombosis and venous thromboembolism. Effectively preventing thrombotic complications requires better understating of how prothrombotic states develop in patients with metabolic disorders. Significantly higher plasminogen activation inhibitor-1 (PAI-1) levels in prediabetic patients than in healthy persons might function as predictors of progressive diabetes (*4*). Although animal models are useful for investigating mechanisms, detailed studies of pro-thrombotic and hypofibrinolytic states are hampered by a lack of appropriate prediabetic mouse models.

Here we evaluated metabolic, coagulative and fibrinolytic parameters in normoglycemic [Ng]-TSOD mice with a metabolic abnormality as well as coagulative and fibrinolytic states to determine whether they could function as prediabetic models.

2. Materials and Methods

2.1. Animals

Sixteen-week-old Male TSOD, TSOD with undetectable urinary sugar and normoglycemia (Ng-TSOD) and non-diabetic Tsumura Suzuki Non-Obesity (TSNO) mice (Institute of Animal Reproduction, Kasumigaura, Japan) were housed at 24 ± 2°C and provided with food and water *ad libitum* for one week. Casual blood glucose levels were measured using a StatStrip Express 900

(Siemens Healthineers, Munich, Germany). Urinary sugar was evaluated using Uropaper ШG (Eiken Chemical Co., Ltd., Tokyo, Japan). Table 1 shows renal and blood glucose levels in 16-week-old TSOD and Ng-TSOD mice. Blood specimens were collected from the inferior vena cava of 17-week-old mice as described (*5*). The Animal Care and Use Committee at Teikyo University approved all experiments involving mice (Approval No: 15-046).

2.2. Measurements of biochemical parameters and blood coagulation and fibrinolytic factors

Levels of glucose, triglyceride, and total cholesterol, free fatty acid, total protein, albumin, phospholipids, aspartate aminotransferase (AST) and alanine aminotransferase (AST), were determined using Biochemical Test Kits (Fujifilm Wako Chemicals, Osaka, Japan). Insulin, adiponectin and leptin were measured using ELISA kits from their respective suppliers (Mercodia, Uppsala, Sweden, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan, and Morinaga Institute of Biological Science, Inc. Kanagawa, Japan). We analyzed tumor necrosis factor-α (TNF-α) and C-reactive protein (CRP) using the respective ELISA kits (Chondrex Inc., Woodinville, WA, USA and Assaypro, St. Charles, MO, USA).

Levels of PAI-1 antigen and active PAI-1 were measured using Total and Active Murine PAI-1 ELISA kits (Molecular Innovations Inc., Novi, MI, USA) with slight modification. Fibrinogen and antithrombin levels were measured using ELISA kits (Assaypro). Prothrombin and activated partial thrombin (APTT) times were measured using Thromborel S (Dade Behring, Liederbach, Germany) and APTT reagent (Sysmex, Kobe, Japan), respectively, as described with slight modification (*5*).

Table 1. Body weight, blood and urinary glucose levels in TSOD and Ng-TSOD mice (*n* = 6 each)

Mouse no.	Body weight (g)	Blood glucose (mg/dL)	Urinary glucose
TSOD			
1	63.2	525	+
2	62.0	470	++
3	65.6	458	+
4	64.5	501	++
5	60.3	451	+
6	64.0	477	++
Average ± SD	63.1 ± 1.9	480 ± 21	
Ng-TSOD			
1	58.1	203	-
2	56.1	183	-
3	55.8	188	-
4	58.2	175	-
5	48.7	168	-
6	57.6	172	-
Average ± SD	55.8 ± 3.6	182 ± 13	

Glucose in blood from tail veins was measured using StatStrip Express 900 blood glucose meter (Nova Biochemical Corp., Waltham, MA, USA). Urinary glucose was measured using Uropaper III (Eiken Chemical Co., Ltd., Tokyo, Japan). –, undetectable; +, 500 mg/dL; ++, 2,000 mg/dL.

2.3. Statistics

Values are expressed as means ± standard deviations (SD). Differences among the three groups were analyzed using one-way ANOVA followed by Tukey post-hoc multiple comparison tests.

3. Results and Discussion

Glucose levels were significantly lower in Ng-TSOD, than in TSOD ($p < 0.001$), but the same as those in TSNO mice (Figure 1A). Mean body weight was slightly, but significantly lower in Ng-TSOD, than in TSOD mice ($p < 0.01$; Figure 1B). Insulin levels were significantly elevated in TSOD and Ng-TSOD, compared with TSNO ($p < 0.005$; $p < 0.01$), and those of Ng-TSOD and TSNO were indistinguishable (Figure 1C).

Figure 2 shows the plasma biochemical parameters in TSOD, Ng-TSOD and TSNO mice. Triglyceride levels were significantly lower in Ng-TSOD, than in TSOD mice ($p < 0.05$). Total cholesterol levels did not significantly differ between the Ng-TSOD and TSOD

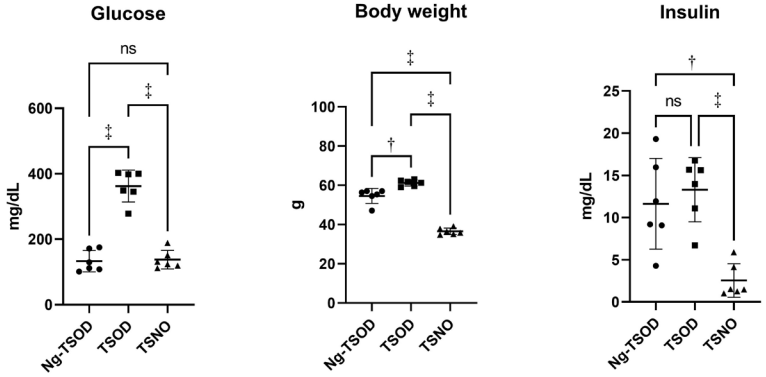

Figure 1. Body weight, glucose and insulin levels in Ng-TSOD, TSOD and TSNO mice (*n* = 6 each). Data are shown as means ± standard deviation (SD). Significantly different at *$p < 0.01$, †$p < 0.005$, ‡$p < 0.001$.

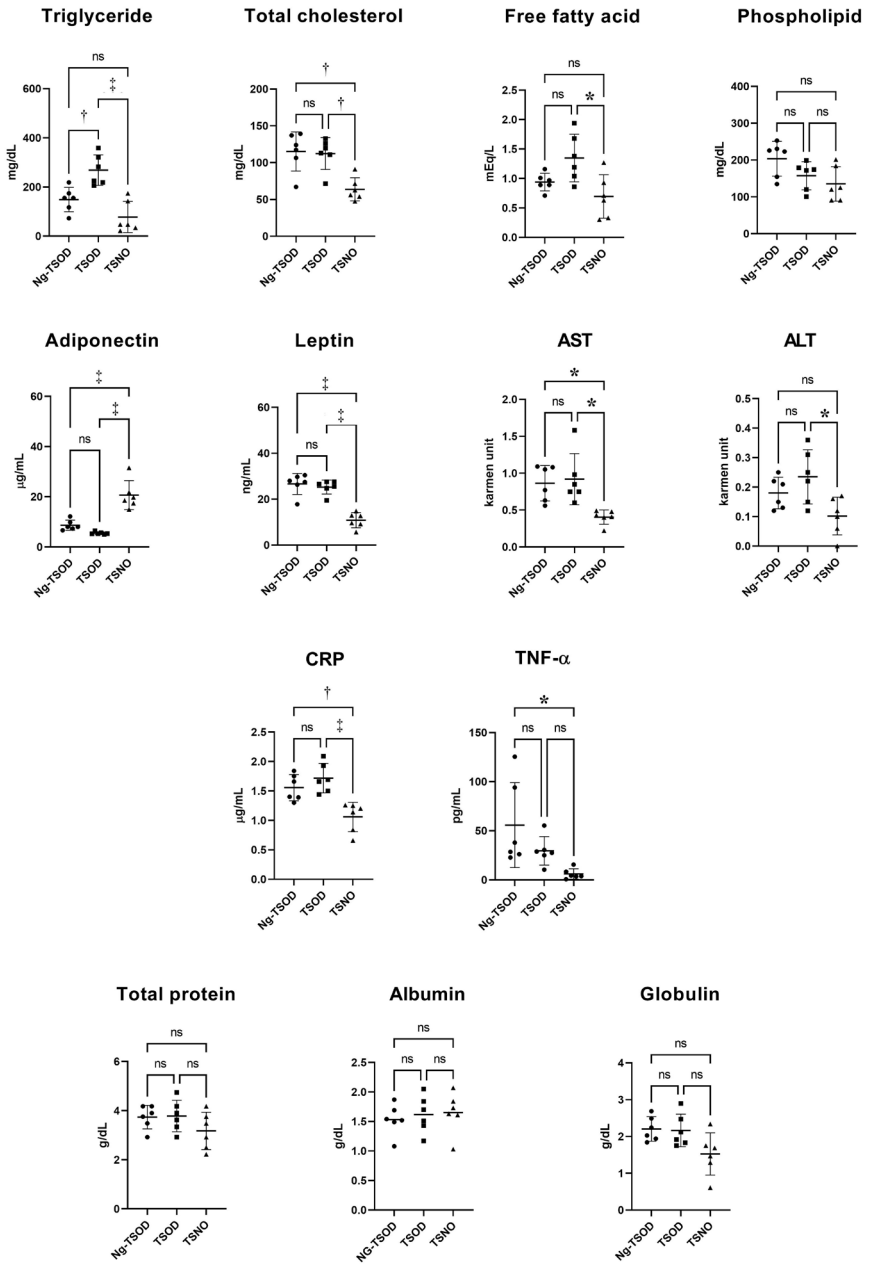

Figure 2. Plasma biochemical parameters in TSOD, Ng-TSOD and TSNO mice (n = 6 each). Data are shown as means ± standard deviation (SD). Significant differences at $^{*}p < 0.05$, $^{\dagger}p < 0.005$, $^{\ddagger}p < 0.001$.

mice, but both were significantly higher than in TSNO mice ($p < 0.01$ for both). Levels of free fatty acids did not significantly differ between Ng-TSOD and TSOD mice, and neither did adiponectin levels, both of which were significantly lower than in TSNO mice ($p < 0.001$ for both). Leptin levels did not significantly differ between Ng-TSOD and TSOD mice, but both were significantly higher than that in TSNO mice ($p < 0.001$ for both). Levels of AST did not significantly differ between Ng-TSOD and TSOD mice, but both were significantly higher than in TSNO mice ($p < 0.01$; $p < 0.01$). Levels of ALT did not significantly differ between Ng-TSOD and TSOD mice, but were significantly higher in TSOD, than in TSNO mice ($p < 0.05$) and similar between Ng-TSOD and TSNO mice. Levels of CRP did not significantly differ between Ng-TSOD and TSOD mice, but both were significantly higher than in TSNO mice ($p < 0.01$ for both). Values for TNF-α did not significantly differ between Ng-TSOD and TSOD mice, but both were significantly higher than in TSNO mice. Total protein, albumin, globulin and PL did not significantly differ among all three groups.

Figure 3 shows values for coagulation parameters that are usually measured in humans at clinical laboratories and PAI-1 in TSOD, Ng-TSOD, and TSNO mice. Levels of PAI-1 and active PAI-1 were significantly higher in Ng-TSOD and TSOD, than in TSNO mice ($p < 0.005$ and $p < 0.05$, respectively), but the difference between Ng-TSOD and TSOD mice did not reach significance. Plasminogen levels

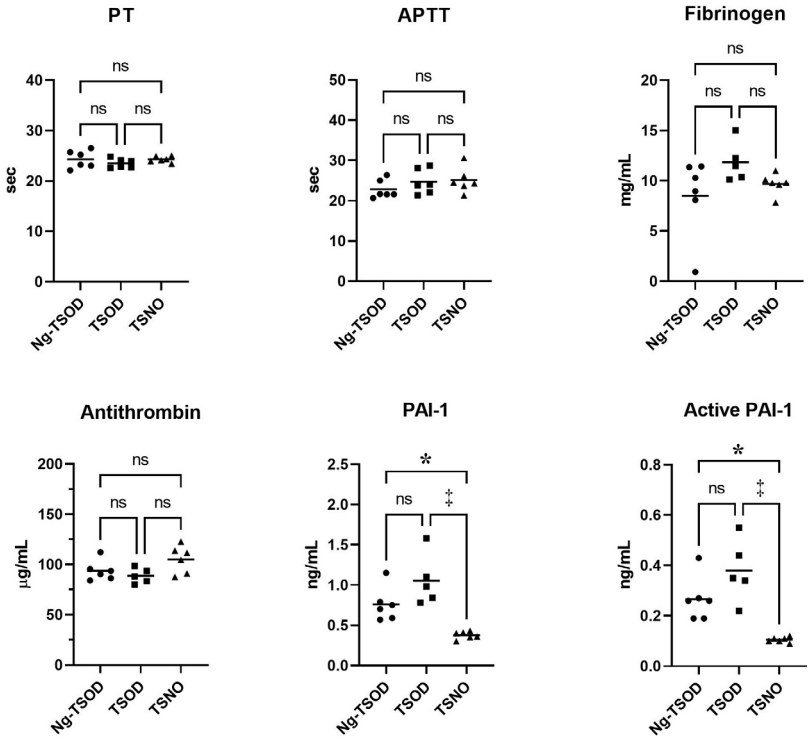

Figure 3. Plasma coagulation and fibrinolytic parameters in TSOD, Ng-TSOD and TSNO mice (n = 6 each). Data are shown as means ± standard deviation (SD). Significantly different at $^{*}p < 0.05$, $^{\dagger}p < 0.005$, $^{\ddagger}p < 0.001$.

were significantly lower and higher in Ng-TSOD, than in TSOD ($p < 0.01$) and TSNO mice ($p < 0.05$), respectively. None of prothrombin, APTT, fibrinogen and antithrombin significantly differed among the three groups.

We found that levels of insulin and body weight were significantly increased in Ng-TSOD, compared with TSNO mice, but were the same between Ng-TSOD and TSOD mice. In contrast, glucose levels were the same between Ng-TSOD and TSNO mice. We also identified levels of adiponectin, leptin and CRP in Ng-TSOD and TSOD mice, which are abnormal in nascent metabolic syndrome in humans (*6*). The genetics of Ng-TSOD and normal TSOD mice might differ because TSOD mouse models of multifactorial genetic diseases spontaneously develop various metabolic diseases (*3,7*).

Patients with diabetes are likely to develop thrombophilia due to abnormal blood coagulation and fibrinolytic factors (*8*). The primary inhibitor of tissue-type plasminogen activator (tPA) in plasma is PAI-1, which plays a key role in fibrinolysis (*9*). Increased PAI-1 is one of the main coagulation abnormalities associated with obesity and diabetes (*9*). Many factors such as insulin, glucose, lipids affect increases in blood PAI-1 activities in patients with diabetes (*10*). The present study found the same levels of total PAI-1 and active plasma PAI-1 that reflect PAI-1 activity in Ng-TSOD and TSOD mice. Nonetheless, blood clotting parameters in Ng-TSOD mice were within the normal range. Although blood glucose levels might increase with advancing age in this phenotype, these mice could serve as models of hypofibrinolysis unrelated to hyperglycemia at least until they reach the age of ~ 17 weeks.

In conclusion, we found that PAI-1 was substantially augmented in Ng-TSOD, as in TSOD mice. This finding suggested that Ng-TSOD mice are hypofibrinolytic and that Ng-TSOD mice might be novel models that could provide insight into the association of prediabetes with hypofibrinolytic states. We believe that Ng-TSOD mice have potential applications for investigating the diagnosis and treatment of prediabetes.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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