High Fat and High Cholesterol Diet Induces DPP-IV Activity in Intestinal Lymph

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Abstract: Recent studies have reported that dipeptidyl-peptidase IV (DPP-IV) is correlated with diabetic conditions and also with dyslipidemia caused by overnutrition, especially a high fat diet. However, the role of DPP-IV in diabetes during dyslipidemia has been unclear. We utilized a lymph fistula rat model to determine whether intestinal lymph, which absorbs dietary fats, is affected by a chronic high-fat and high-cholesterol diet (HFHC). HFHC diet rats showed significantly higher DPP-IV activity in intestinal lymph and plasma compared to rats receiving a normal chow diet. In addition, HFHC diet rats showed significantly increased DPP-IV mRNA expression in the intestine. However, DPP–IV mRNA in the lymphocytes isolated from intestinal lymph and mesenteric lymph nodes did not show significant differences from that in the normal diet rats. In conclusion, HFHC diets increased DPP-IV expression in intestinal lymph; these results indicate the applicability of a previously unrecognized role for DPP-IV in metabolic disorders, including diabetes.

Key words: DPP-IV, lymph, high fat and high cholesterol diet

1 INTRODUCTION

Recently, dipeptidyl-peptidase IV (DPP-IV) has been investigated as a regulatory protease involved in the inactivation of a variety of proline-rich peptides, including glucagon like peptide-1 (GLP-1) and GLP-2, neuropeptides, and other chemokines. Notably, its effect on GLP-1, an insulinotropic hormone that enhances insulin secretion, has received attention as a novel therapeutic approach for type 2 diabetes. Moreover, recent studies have reported that an inhibitor of DPP-IV may have a previously unrecognized beneficial effect on diabetic conditions, including lipid metabolism. Dyslipidemia is commonly observed in patients with diabetes, and both of these conditions can develop into systemic atherosclerosis. Yang et al. demonstrated increases in DPP-IV in the intestine and kidney associated with a high fat diet, while Kirino et al. found that a high fat diet increased plasma DPP-IV activity. Lamers et al. reported that DPP-IV release was strongly correlated with adipocyte size, potentially representing an important source of DPP-IV in obesity. These results suggest that a high-fat diet may have an important role in the secretion of DPP-IV. However, in contrast to its role in diabetes, the role of DPP-IV during dyslipidemia has not been extensively investigated.

In the present study, we utilized an intestinal lymph fistula rat model to determine whether intestinal lymph was affected by a chronic high fat and high cholesterol diet. Our results are the first to demonstrate that DPP-IV activity increased in both plasma and intestinal lymph following the consumption of such a diet.

2 EXPERIMENTAL PROCEDURES

2.1 Animal Study

2.1.1 Animals and Experimental Design

All the experiments were approved by the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University. Male Sprague-Dawley (SD) rats, 6-weeks-old, were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan) and housed in an air-conditioned room with a 12-h light-dark cycle. The rats were divided randomly into two groups, viz., those that received normal chow (NC: 4.6% fat, 24.9% protein; CE-2 diet, CLEA JAPAN, Inc., Tokyo, Japan) and housed in an air-conditioned room with a 12-h light-dark cycle. The rats were divided randomly into two groups, viz., those that received normal chow (NC: 4.6% fat, 24.9% protein; CE-2 diet, CLEA JAPAN, Inc., Tokyo, Japan), and those that had a high fat and cholesterol diet (HFHC: tallow powder 20% and cholesterol 1.25% in CE-2, CLEA JAPAN, Inc., Tokyo, Japan). All the rats had free access to diet and water.
2.1.2 Collection of intestinal lymph samples

The animals were fed the NC or HF diet for 8 weeks prior to performing the analyses. Intestinal lymph samples were collected using a previously described procedure\(^5/9\). Briefly, rats were fasted overnight with free access to water prior to surgery. Following anesthesia with pentobarbital (Kyoritsu Seiyaku Corp., Tokyo, Japan; conc. 40 mg/kg), the mesenteric lymph duct was cannulated with a vinyl tube (0.5-mm internal diameter ID, 0.9-mm outer diameter OD; Natsume Seisakusho Co., Ltd., Tokyo, Japan) and a polyethylene tube (0.8-mm ID, 1.2-mm OD; Natsume Seisakusho Co., Ltd., Tokyo, Japan) was used to intubate the stomach for delivery of an isotonic solution. After cannulation surgery, the rats were placed in Bollman restraint cages and warmed with a lamp to recover from anesthesia. Then, a glucose (139 mM) and sodium chloride (85 mM) solution was infused via the feeding tube for 8 h to prevent dehydration. Lymph was collected in a conical centrifuge tube containing 0.5 M EDTA on ice for 1 h, and centrifuged at 1160 \( \times \) g for 15 min to obtain lymphocytes. The mesenteric lymph node was removed by microdissection and passed through a 70-\( \mu \)m nylon mesh to obtain a single cell suspension. The isolated lymphocytes were suspended in RPMI 1640 containing 10% fetal bovine serum (Sigma-Aldrich Co. LLC., MO, USA).

2.1.3 Collection of plasma samples

After collection of intestinal lymph, blood samples were obtained from the heart, which were then centrifuged to separate plasma.

2.2 DPP-IV enzyme activity assay

DPP-IV activity was determined as previously described\(^10/11\). Briefly, a reaction mixture (total volume: 100 \( \mu \)l) containing 71.44 \( \mu \)l of 100 mM glycine-NaOH buffer (pH 8.6), 14.28 \( \mu \)l of 10 mM glycine-proline-\( \mu \)molar tosylate (Peptide Institute, Inc., Osaka, Japan), and 14.28 \( \mu \)l of intestinal lymph or plasma was used. Instead of biological samples, the blank tubes contained 14.28 \( \mu \)l of distilled water, while the standard tubes contained 1 \( \mu \)mol of p-nitroanilide plus water to a total of 14.28 \( \mu \)l. All samples were incubated for 30 min at 37\(^\circ\)C; then, the reaction was stopped by adding 200 \( \mu \)l of 1 M acetate buffer (pH 4.07). To eliminate the color of the biological sample, we prepared sample tubes without incubation. DPP-IV activity was determined by assessing the release of p-nitroaniline by measuring absorbance at 405 nm using an ARVO\(^\text{TM}\)X3 plate reader (PerkinElmer, MA, USA). DPP-IV activity is expressed as \( \mu \)mol \( \cdot \)min\(^{-1}\) \( \cdot \)L\(^{-1}\).

2.3 Quantitative real time PCR

Total RNA was isolated using an RNeasy kit (Qiagen N.V., Germany). cDNA was synthesized using 500 ng total RNA and PrimeScript\(^\text{TM}\) RT Master Mix (Takara Bio Inc., Shiga, Japan). Real-time PCR was performed using KAPA SYBR\(^\text{®}\) FAST qPCR Kits (Kapa Biosystems, Inc., MA, USA) with an Applied Biosystems 7900 HT fast real-time PCR system (Life Technologies\(^\text{TM}\), CA, USA). The reaction conditions were as follows: 95\(^\circ\)C for 2 s and 60\(^\circ\)C for 1 min. The expression level of DPP-IV was calculated as DPP-IV/18S ribosomal RNA (18S rRNA) (\(2^\Delta Ct\)). The primer sequences for amplifying DPP-IV mRNA and 18S ribosomal RNA were as follows: rat DPP-IV forward 5’-TCTGTGACAA-CAGGGGATCA-3’ and reverse 5’-AGATCGCCATCAG-GAATA-3’, and 18S ribosomal RNA forward 5’-GTA-ACCCTTGAACCCATT-3’ and reverse 5’-CCATCCATCGGTAGTGGC-3’.

2.4 Data Analysis

All values are expressed as the mean \( \pm \) SEM. Student’s unpaired t-test was used to evaluate differences between the NC and HFHC groups. Differences were considered significant at \( p<0.05 \).

3 RESULTS

3.1 HFHC induces DPP-IV in rat intestinal lymph enzymatic activity

SD rats were fed with the HFHC diet for 8 weeks; then, intestinal lymph samples were collected to monitor DPP-IV activity. We analyzed the enzymatic activity of DPP-IV by measuring absorbance of p-nitroaniline released from glycine-proline-p-nitroanilide tosylate. After confirming the validity of this assay, DPP-IV activity in intestinal lymph samples was monitored. There was a significant induction of DPP-IV activity in intestinal lymph of rats in the HFHC group as compared with control rats (NC 9.4 \pm 1.1 \( \mu \)mol \( \cdot \)min\(^{-1}\) \( \cdot \)L\(^{-1}\); HFHC, 21.0 \pm 3.3 \( \mu \)mol \( \cdot \)min\(^{-1}\) \( \cdot \)L\(^{-1}\); \( p=0.0104 \); Fig. 1A). In addition, DPP-IV activity in plasma was higher in the HFHC group (NC, 51.1 \pm 6.5 \( \mu \)mol \( \cdot \)min\(^{-1}\) \( \cdot \)L\(^{-1}\); HFHC, 77.0 \pm 8.3 \( \mu \)mol \( \cdot \)min\(^{-1}\) \( \cdot \)L\(^{-1}\); \( p=0.0298 \); Fig. 1B).

3.2 HFHC diet induces DPP-IV mRNA expression in rat intestine

Next, we investigated the effects of the HFHC diet on the expression of DPP-IV in the intestine, as it is one of the main organs that produce DPP-IV. As shown in Fig. 2, the HFHC diet significantly increased DPP-IV mRNA expression in the intestine as compared to the NC diet. To identify cell types in the intestine related to production of DPP-IV with the HFHC diet, we analyzed the expression level of DPP-IV in lymphocytes from the mesenteric lymph node. mRNA expression of DPP-IV in lymphocytes from the mesenteric lymph node (Fig. 3A) and those in intestinal lymph (Fig. 3B) were not significantly different between the two groups.
DPP-IV has been investigated as a regulatory protease for inactivation of bioactive peptides, including GLP-1 and -2, and as a therapeutic target in type 2 diabetes. In this study, we utilized the intestinal lymph fistula rat model to examine effects of a HFHC diet on intestinal DPP-IV levels. Using this model, we demonstrated that DPP-IV activity was increased, not only in plasma, but also in the intestinal lymph, after 8 weeks of such a diet. Our findings suggested a key role for intestinal lymph in systemic up-regulation of DPP-IV after rats received an HFHC diet.

Previous studies have found that cholesterol feeding increased the development of atherosclerosis\(^{12, 13}\). However, the exact cellular mechanism responsible for the link between a high fat and high cholesterol diet and type 2 diabetes has not been extensively studied. It has been reported that increases in plasma DPP-IV were seen after feeding of animals with a high fat diet, although the cellular mechanisms are not well documented\(^5\). Our findings revealed an important role for intestinal lymph in systemic up-regulation of DPP-IV after rats received an HFHC diet.

It was previously reported that under non-diabetic conditions, DPP-IV activity in intestinal lymph is lower than that in plasma\(^{14}\), which helps to maintain the plasma concentration of the incretin family of bioactive peptides, including GLP-1 and -2\(^{15}\). Therefore, up-regulation of DPP-IV in type 2 diabetes reduces the plasma levels of GLP-1 and -2, which may contribute to development of the disease. Our results suggest that dyslipidemia may reduce incretin concentrations, which directly links dyslipidemia with type 2 diabetes. Others have noted that expression of DPP-IV is increased after consumption of a high fat diet\(^5\), and several relevant cell types, including vascular endothelial cells\(^{36}\) and adipocytes\(^7\), have been shown to express DPP-IV. Therefore, further experiments are required to elucidate the molecular mechanisms and tissues responsible for high fat and high cholesterol diet-induced up-regulation of DPP-IV expression.

As shown in Fig. 3, we failed to find significant induction of DPP-IV in lymphocytes from intestinal lymph and mesenteric lymph node, suggesting that other cell types, including enterocytes, may play an important role in high fat and high cholesterol diet-induced DPP-IV up-regulation in the intestine. Although DPP-IV expression is not up-regulated in lymphocytes, this cell type may be strongly influenced by DPP-IV. HFHC diet seemed to induce IFN-γ expression in the mesenteric lymph node as well as in intestinal lymph lymphocytes (data not shown). Molecular

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Fig. 1  DPP-IV activity in intestinal lymph and plasma from HFHC compared with NC rats. Intestinal lymph (A) and plasma (B) from HFHC and NC fed rats (lymph n = 5, plasma n = 7) were analyzed for their DPP-IV activity by release of \(\text{p-nitroaniline}\). Data are shown as the mean ± SEM. \(*p < 0.05\) vs. normal chow (Student’s t-test).

Fig. 2  The DPP-IV mRNA expression of intestine. Intestine isolated from HFHC and NC rats were analyzed for DPP-IV mRNA expression by quantitative real-time PCR. Data are shown as the mean ± SEM. \(*p < 0.05\) vs. normal chow (Student’s t-test). This experiment was performed with 4 rats that received the normal chow and 5 the HFHC diet.

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4 DISCUSSION

DPP-IV has been investigated as a regulatory protease for inactivation of bioactive peptides, including GLP-1 and -2, and as a therapeutic target in type 2 diabetes. In this study, we utilized the intestinal lymph fistula rat model to examine effects of a HFHC diet on intestinal DPP-IV levels. Using this model, we demonstrated that DPP-IV activity was increased, not only in plasma, but also in the intestinal lymph, after 8 weeks of such a diet. Our findings suggested a key role for intestinal DPP-IV in high fat diet-induced metabolic disorder and type 2 diabetes.

The present experimental diet is commonly used in studies of metabolic disorders, which develop into atherosclerosis and type 2 diabetes. Previous studies have found that cholesterol feeding increased the development of atherosclerosis\(^{12, 13}\). However, the exact cellular mechanism responsible for the link between a high fat and high cholesterol diet and type 2 diabetes has not been extensively studied. It has been reported that increases in plasma DPP-IV were seen after feeding of animals with a high fat diet, although the cellular mechanisms are not well documented\(^5\). Our findings revealed an important role for intestinal lymph in systemic up-regulation of DPP-IV after rats received an HFHC diet.

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structural analysis of DPP-IV has revealed that it is anchored in the plasma membrane by a hydrophobic membrane-spanning domain preceded by a short hydrophilic sequence at the N-terminus\(^1\); thus, it is classified as a type II transmembrane protein. Moreover, that study showed that DPP-IV is identical to CD26, a cell surface antigen for activated T-cells\(^2\). Since activated T-cells play an important role in the development of type 2 diabetes and atherosclerosis\(^3,4\), DPP-IV may function as an immuno-inflammatory modulator with consumption of a high fat and high cholesterol diet.

5 CONCLUSION

We have demonstrated that a high fat and high cholesterol diet increased DPP-IV in intestinal lymph. Our results suggest a previously unrecognized role for DPP-IV in metabolic disorders, including type 2 diabetes and atherosclerosis.

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References


![Fig. 3](image-url)

The DPP-IV mRNA expression of lymphocytes from mesenteric lymph node and intestinal lymph

The DPP-IV mRNA expression of lymphocytes from mesenteric lymph node (A) (n = 8) and lymphocytes from intestinal lymph (B) (NC; n = 4, HFHC; n = 3) were analyzed by quantitative real-time PCR. Data are shown as the mean ± SEM.

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