Title: Effects of short-term calorie restriction on circulating DPP-4/sCD26 concentrations and body composition in patients with type 2 diabetes.

Type of manuscript: Original article for Diabetology International

Mototaka Yamauchi[1], Takafumi Tsuchiya[1], Kohzo Takebayashi[1], Toshihiko Inukai[1,2], and Koshi Hashimoto[1].

1: Department of Diabetes, Endocrinology and Hematology, Dokkyo Medical University Saitama Medical Center, 2-1-50, Minami-Koshigaya, Koshigaya, Saitama 343-8555, Japan.

2: Department of Internal Medicine, Seibu General Hospital, 884, Kamiookubo, Sakura-ku, Saitama, Saitama 333-0824, Japan.

Address all correspondence to:

Mototaka Yamauchi M.D.

Department of Diabetes, Endocrinology and Hematology

Dokkyo Medical University Saitama Medical Center

2-1-50, Minami-Koshigaya, Koshigaya, Saitama 343-8555, Japan.

Tel: +81-48-965-1111, Fax: +81-48-965-1127

Email: mototaka@dokkyomed.ac.jp

Abstract

Previous studies have shown that dipeptidyl peptidase (DPP) -4, is released from adipocytes in a differentiation-dependent manner and a marker for insulin resistance in obese individuals who have particularly high circulating DPP-4/soluble CD26 (sCD26) concentrations. In this study, we have evaluated the effects of short-term hospitalization with calorie restriction on body composition and circulating DPP-4/sCD26 concentrations in patients with type 2 diabetes. A total of 47 Japanese adults with type 2 diabetes were recruited to the study (age; 56.6 ± 13.0 years, body mass index (BMI); 27.3 ± 5.6 kg/m^2). Body composition, circulating DPP-4/sCD26 concentrations and metabolic parameters were assessed upon admission and at discharge from hospital (average of the period: 13.0 ± 2.5 days). Visceral fat area (VFA) was also assessed by dual impedance method. During hospitalization, there was a significant reduction in body weight, BMI, lean body mass, VFA and circulating DPP-4/sCD26 concentrations, but not in body fat mass. Fasting circulating DPP-4/sCD26 concentrations were significantly correlated with fasting insulin, aspartate aminotransferase, γ-glutamyltransferase (γ-GTP) levels, and HOMA-IR (r =0.477, 0.423, 0.415, 0.548, respectively), but not with VFA (r = -0.056) by liner regression analyses at base line. It was also observed a positive correlation between changes in circulating DPP-4/sCD26 concentrations and γ-GTP level, HOMA-IR, and a negative correlation between the changes

in circulating DPP-4/sCD26 concentrations and VFA significantly (r =0.300, 0.633, -0.343, respectively). In conclusion, our observations suggest that liver enzymes as well as VFA might be associated with the response of DPP-4/sCD26 concentrations.

Key words: DPP-4, soluble CD26, type 2 diabetes, body composition, visceral fat.

Introduction

Both obesity and type 2 diabetes are associated with insulin resistance [1]. Chronic nutrient excess leads to visceral adipose tissue expansion and dysfunction in an active process that involves the adipocytes, their supporting matrix, and immune cell infiltrates [2]. Visceral adipose tissue secretes a number of adipokines and cytokines leading to a proinflammatory, procoagulant and insulin resistance collectively known as the metabolic syndrome [3]. Thus, greater visceral adiposity is associated with subsequent insulin resistance [4-6]. However, there are several studies to report that subcutaneous fat accumulation is more closely related to insulin resistance in general and diabetic population [7-11]. Although normalizing overweight should lead to improve metabolic disorders, such as type 2 diabetes, dyslipidemia and hypertension, the weight loss is often accompanied by a loss in lean body mass [12]. Therefore, it should be important to consider a change in each component of the body composition as well as visceral adipose fat, when we investigate whether those components would be associated with insulin resistance and glucose metabolism.

Metabolic syndrome is associated with abdominal obesity, dyslipidemia, inflammation, insulin resistance or full-blown diabetes, and risk of developing cardiovascular diseases [3]. The visceral fat area (VFA) has been quantitatively evaluated by computed tomography (CT) or magnetic resonance imaging

(MRI) until several years ago. However, these methods have some problems such as risk of radiation, higher costs and they also require radiologists' cooperation, which comprises an obstacle in the prevention, improvement and treatment of lifestyle related diseases. Recently, a dual bioelectrical analysis (Dual BIA) that can determine VFA by measuring truncal impedance and surface impedance at the abdomen separately has been developed. As the change in estimated VFA by Dual BIA is highly correlated with that by CT, Dual BIA is useful and important for the early detection and prevention cardiovascular risks and evaluation of effectiveness of weight reduction therapy in obese patients [13].

Dipeptidyl peptidase (DPP)-4 is also known as cell surface antigen CD26 that has a costimulatory function in the immune system [14]. A soluble form of CD26 (sCD26), which lacks the cytoplasmic tail and transmembrane domain, is found in serum and it possesses DPP-4/sCD26 enzymatic activity that removes X-Pro and X-Ala dipeptides from substrates [15]. DPP-4/CD26 gene is widely expressed in many organs, including adipose and liver tissues [16]. Previous studies have shown that DPP-4/sCD26 is released from adipocytes in a differentiation-dependent manner [17] and a marker for insulin resistance in obese individuals, who have particularly high concentrations of circulating DPP-4/sCD26 [18]. On the other hand, the activity of serum DPP-4/sCD26 and the expression level of DPP-4/CD26 in liver were correlated with histopathological grade of nonalcoholic steatohepatitis (NASH) and

78 hepatosteatosis [19]. Moreover, mice lacking DPP-4 displayed a protective effect from diet-induced
79 hepatic steatosis and insulin resistance [20].
80 Interestingly, high circulating DPP-4/sCD26 concentrations may be associated with reduced
81 efficacy of a DPP-4 inhibitor, sitagliptin [21]. It is possible that reduction in circulating DPP-4/sCD26
82 concentrations may enhance the efficacy of incretin related agents. In this study, our purpose is to evaluate
83 the changes in each component of body composition, including those in VFA measured by Dual BIA, under
84 short-term hospitalization with calorie restriction and the effects on insulin secretion and sensitivity, lipid
85 metabolism and circulating DPP-4/sCD26 concentrations in type 2 diabetic patients, thereby specifying an
86 influence factor which influences the response of DPP-4/sCD26 concentrations.
87

Materials and methods

Study population

All subjects were adult Japanese with type 2 diabetes who underwent hospitalization for glycemic control. The protocol of the following studies was approved by the ethical committee on human research at Dokkyo Medical University Saitama Medical Center（approval number: 1431）according to the Declaration of Helsinki. Written informed consent was obtained from all subjects prior to enrollment.

The sample size was calculated as follows; suppose one wishes to detect a simple correlation r (r=0.4) of N observations. Using a two-sided test, 5% significance level test (α=0.05) with 80% power (β=0.2), the required sample size is approximate 47 (n=47) [22]. A total of 47 patients were recruited to this study. They were eligible to participate if they were ≥20 years old and had inadequate glycemic control (Hemoglobin A1c (HbA1c) ≥7.0%) required medical care and self-management education under hospitalization. Subjects who had already been diagnosed with type 1 diabetes, gestational diabetes and diabetes in pregnancy, and who had any acute complications such as diabetic ketoacidosis were excluded from the study.

Study protocol

All subjects were placed under calorie restriction with 25 to 30 kcal per kilogram (kg) of ideal body weight, corresponding to body mass index (BMI) of 22, per day (carbohydrate: 50-60% of total calories) during hospitalization. Body composition, circulating DPP-4/sCD26 concentrations and hemodynamic parameters were assessed upon admission and at discharge from hospital. After overnight fasting, blood samples were obtained from each subject and metabolic parameters were measured. Body composition was assessed by bioelectrical impedance analysis using multi frequency segmental body composition analyzer, MC-780 (Tanita corporation, Tokyo, Japan). Abdominal VFA was also assessed by dual impedance method using visceral fat monitor system, HDS-2000 DUALSCAN (Omron Healthcare Co., Ltd., Kyoto, Japan). Circulating DPP-4/sCD26 concentrations were measured with enzyme-linked immunosorbent assay kit (Human sCD26 platinum ELISA; Bender MedSystems GmbH, Vieena, Austria). Fasting plasma glucose (FPG) was evaluated using Glucose Auto Stat GA1160® (Arkray, Kyoto, Japan). HbA1c was evaluated by Norudia N HbA1c (Sekisui Medical Inc., Tokyo, Japan) and the normal range was 4.5% to 6.2% [NGSP: national glycohemoglobin standardization program]. Serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and serum triglyceride (TG) were measured using enzymatic assays. The Determiner L TC II® and Determiner L TG® reagents (Kyowa Medics, Tokyo, Japan) were used for measurements of TC and TG,

120 respectively. HDL-C and LDL-C were directly measured by Cholestest N HDL-C® and Cholestest
121 LDL® (Daiichi Pure Chemicals, Tokyo, Japan), respectively. Fatty acids including malondialdehyde-
122 modified LDL (MDA-LDL), remnant like particles cholesterol (RLP-C), dihomo γ-linolenic acid (DHLA),
123 and arachidonic acid (AA) were assessed using the gas-chromatograph method (SRL inc. Tokyo, Japan).
124 Plasma insulin was measured by a chemiluminescent enzyme immunoassay (CLEIA) using the lumipulse
125 Presto Insulin Kit ® (Fujirebio, Tokyo, Japan). HOMA-IR was used as an indicator of insulin resistance
126 and was calculated as follows: HOMA-IR = FPG (mg/dL) × fasting immunoreactive insulin (μU/mL)/405
127 [23].
128
129 **Statistics**
130 Data are expressed as the mean ± SD. Statistical evaluation of the differences between two
131 groups was performed by unpaired t-test. The relationships between fasting circulating DPP-4/sCD26
132 concentrations and metabolic and hemodynamic parameters were examined by linear regression and
133 Spearman's correlation coefficient analyses. For multiple regression analyses, we used the stepwise
134 regression method to find a model that is appropriate for these data. Serious multicollinearity was not
135 detected among included factors (Variance inflation factors <10). Statistical analyses were performed

using IBM SPSS Statictics 25 (IBM, Armonk, NY, USA) and R version 3.0.2 (R Foundation for Statistical Computing, http://www.r-project.org/). All P-values were two-tailed.

Results

Changes in various variables at baseline and at 2 weeks after hospitalization in patients with type 2 diabetes are presented in Table 1. During the period, a significant decrease was obtained in FPG, fasting immunoreactive insulin (IRI), fasting C-peptide and HOMA-IR by the optimization of the treatment for diabetes including the calorie restriction. A significant decrease was also obtained in body weight, BMI, VFA and lean body mass. However, no significant changes were observed in body fat mass and body fat weight during the period. We also found that the circulating DPP-4/sCD26 concentrations were significantly decreased after a 2 week-hospitalization (Table 2). As shown in Table 1, administration of sodium/glucose cotransporter 2 (SGLT2) inhibitors was initiated for 7 patients with diabetes. A recent study has demonstrated that a SGLT2 inhibitor, dapagliflozin, reduced serum levels of DPP-4/sCD26 in people with Type 2 diabetes [24]. In this study, significant reductions of DPP-4/sCD26 concentrations were observed in both groups with and without administration of SGLT2 inhibitors during the hospitalization (with SGLT2 inhibitor: at baseline 1161.3± 176.0 ng/ml, after 2 weeks 900.4±143.9 ng/ml, P=0.04, without SGLT2 inhibitor: at baseline 973.4±416.9 ng/ml, after 2 weeks 890.5±430.8 ng/ml, P=0.03). Interestingly, changes of DPP-4/sCD26 concentrations were greater in a group with SGLT-2 inhibitors than in that with SGLT-2 inhibitors, however this difference was not significant (P=0.09). Then,

155 there was a significant reduction in γ-glutamyltransferase (γ-GTP), LDL-C, HDL-C, TG, MDA-LDL,

156 RLP-C, DHLA, and AA after a 2 week-hospitalization.

157 At baseline analysis, circulating DPP-4/sCD26 concentrations were positively correlated with

158 AST, γ-GTP, fasting IRI levels and HOMA-IR (r=0.423, 0.415, 0.477, 0.548, P=0.004, 0.005, 0.004, 0.001,

159 respectively), but not with abdominal VFA (r=-0.056, P=0.72). There are no significant correlations

160 between circulating DPP-4/sCD26 concentrations and each lipid profile. We performed a stepwise

161 regression analysis that included all significant variables. In a model explaining 45.6% (R^2=0.456) of the

162 variation of circulating DPP-4/sCD26 concentrations in our 35 patients, HOMA-IR (β=0.308, P=0.05) and

163 γ-GTP (β=0.462, P=0.005) were independent determinants of circulating DPP-4/sCD26 concentrations

164 (Table 3).

165 During hospitalization, changes in circulating DPP-4/sCD26 concentrations were positively

166 correlated with changes in γ-GTP levels and HOMA-IR (r=0.300, 0.633, P=0.04, 0.005, respectively), and

167 negatively correlated with changes in VFA (r=-0.343, P=0.02). There are no significant correlations

168 between changes in circulating DPP-4/sCD26 concentrations and changes in each lipid profiles. We also

169 performed a stepwise regression analysis that excluded fasting IRI levels and HOMA-IR because

170 approximately 50% of patients were received insulin injection therapy at the end point of this study. In a

171 model explaining 19.9% (R^2=0.199) of the variation in changes in circulating DPP-4/sCD26 concentrations

172 in our 43 patients, VFA (β=-0.302, P=0.05) were independent determinants of changes in circulating DPP-

173 4/sCD26 concentrations (Table 4).

174

175 **Discussion**

176 In this study, we evaluated that the effects of a 2 week-hospitalization of type 2 diabetic patients
177 on the changes in body composition including VFA and related clinical parameters including circulating
178 DPP-4/sCD26 concentrations. We observed that body weight, BMI and lean body mass were significantly
179 decreased, but body fat mass was not changed. These results indicate that the change in lean body mass,
180 rather than that of body fat mass, may be the major factor, which contributed to body weight loss under the
181 short-term hospitalization with calorie restriction. It may be resulted from the reduction of physical
182 activity during the hospitalization with bed rest, because the physical activity has been shown to be vitally
183 important for maintaining muscle mass and function [25].

184 A previous study has reported that DPP-4/sCD26 expression levels in both subcutaneous and
185 visceral adipose tissues were positively correlated with BMI [18]. However, any significant correlations
186 between the circulating DPP-4/sCD26 concentrations and BMI were not found at baseline or after the
187 hospitalization in this study, suggesting that the circulating DPP-4/sCD26 concentrations may not
188 necessarily reflect local DPP-4/sCD26 expression levels in adipose tissues.

189 It has been also shown that circulating DPP-4/sCD26 concentrations in obese subjects with
190 insulin resistance are higher than those without insulin resistance [18]. Our data showed that the

191 circulating DPP-4/sCD26 concentrations were not correlated with HOMA-IR at baseline. However, the
192 changes in circulating DPP-4/sCD26 concentrations were positively correlated with those in HOMA-IR
193 during the hospitalization, indicating that the circulating DPP-4/sCD26 concentrations can be one of
194 markers for insulin resistance.

195 A previous report showed that the DPP-4/sCD26 expression in visceral adipose tissue was
196 significantly higher than that in subcutaneous adipose tissue and positively correlated with BMI in non-
197 diabetic population [18]. However, in the current study, we found no significant correlation between
198 circulating DPP-4/sCD26 concentrations and BMI. There is a possibility that the treatment for diabetes
199 during hospitalization may affect the circulating DPP-4/sCD26 concentrations in our study. Another
200 previous report showed that circulating DPP-4/sCD26 concentrations were positively and specifically
201 associated with the accumulation of visceral fat evaluated by CT and the presence of metabolic syndrome
202 in 135 men with type 2 diabetes [26]. On the other hand, we could not find any significant association
203 between the baseline circulating DPP-4/sCD26 concentrations and VFA or between the changes in those
204 during hospitalization, which might be attributed to the smaller participants in our study.

205 Regarding the expression and secretion of DPP-4/sCD26 in liver, a previous study reported that
206 the serum DPP-4/sCD26 activity was higher in patients with NASH than controls, and correlated with the

207 histopathological grade and hepatosteatosis but not with DPP-4/CD26 positive staining [19]. Furthermore,
208 another previous study showed that the high circulating DPP-4/sCD26 concentrations were correlated with
209 liver function but not with FPG or HbA1c in nonalcoholic fatty liver disease (NAFLD) [27]. It is
210 suggested that improving NAFLD and NASH by calorie restriction may lead to suppress the expression of
211 DPP-4 in liver, reduce circulating DPP-4/sCD26 concentrations, and ameliorate the efficacy of DPP-4
212 inhibitors. A meta regression analysis also showed that the baseline BMI is significantly correlated with
213 the HbA1c lowering efficacy of DPP-4 inhibitors [28], because obese patients should be under a situation
214 with NAFLD or NASH, thereby with possibly high circulating DPP-4/sCD26 concentrations.

215 It is not clear that how the glucose-lowering effects of DPP-4 inhibitors could be attenuated in
216 patients with elevated circulating DPP-4/sCD26 concentration. There is a possibility that the therapeutic
217 dose of DPP-4 inhibitors may be insufficient to inhibit DPP-4 activity in type 2 diabetic patients with high
218 circulating DPP-4/sCD26 concentrations. Considering another possibility, a negative correlation between
219 circulating DPP-4/sCD26 and postprandial serum C peptide was observed in a previous report [21]. A
220 high circulating DPP-4/sCD26 promotes degradation of active incretin, and might be associated with
221 impaired postprandial insulin secretion.

222 There are several limitations in this study. First, this study was conducted at a single institute.

223 Second, all subjects were diabetic patients with severe hyperglycemia, which were required hospitalization,
224 and healthy subjects were not enrolled. Therefore, the participants enrolled in this study might have
225 relatively severe diabetes and might not be representative of all Japanese diabetic patients. Third, every
226 participant received different treatments. For example, approximate 50% participants received insulin
227 injection therapy and several participants received the treatment of SGLT-2 inhibitor at discharge from the
228 hospital. It may affect varying changes in the body composition during the hospitalization. Fourth, we
229 used HOMA-IR for a marker of insulin resistance, although patients receiving insulin therapy at both
230 baseline and the end of the study were excluded from this analysis. However, there is a possibility that
231 the evaluation of insulin resistance is inaccurately at baseline, because all subjects needed to be hospitalized
232 for glycemic control and showed high fasting glucose levels. Fifth, we have tried to specify an
233 influence factor which influences the response of DPP-4/sCD26 concentrations, but the R-squared value of
234 the multivariate analysis was substantially low. There can be a problem with the regression model and
235 included independent variables did not explain much in the variation of DPP-4/sCD26 concentration.
236 In conclusion, circulating DPP-4/sCD26 concentrations are positively associated with liver
237 enzymes but not with fat mass and VFA. However, changes in circulating DPP-4/sCD26 concentrations
238 are negatively and independently associated with VFA and positively with γ-GTP. Further clinical

observations in subjects with various metabolic disorders should be necessary to clarify the role of circulating DPP-4/sCD26.

Data availability.

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments: This work was supported in part by Grants-in-Aid for Scientific Research (KAKENHI) from the Japan Society for the Promotion of Science (JSPS) [grant Numbers 19K09018 (K.H.)]; Takeda Science Foundation (K.H.).

Compliance with Ethical Standards:

Author Disclosure Statement: The authors have nothing to disclose.

Competing interests: The authors declare no competing interests.

Human rights statement: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (the Institutional Review Boards of Dokkyo Medical

University Saitama Medical Center, approval date: Nov. 19, 2014, approval number: 1431) and with Helsinki Declaration of 1964 and later versions.

Informed consent: All informed consent or substitute for it was obtained from all patients for being included in the study.

Reference

1. Reaven GM. Banting Lecture 1988. Role of insulin resistance in human disease. 1988. Nutrition. 1997; 13: 65; discussion 64, 66.

2. Revelo XS, Luck H, Winer S, Winer DA. Morphological and inflammatory changes in visceral adipose tissue during obesity. Endocr Pathol. 2014; 25:93-101.

3. Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. Nature. 2006; 444:881-7.

4. Koh H, Hayashi T, Sato KK, Harita N, Maeda I, Nishizawa Y, Endo G, Fujimoto WY, Boyko EJ, Hikita Y. Visceral adiposity, not abdominal subcutaneous fat area, is associated with high blood pressure in Japanese men: the Ohtori study. Hypertens Res. 2011; 34:565-72.

5. Hirose H, Takayama M, Iwao Y, Kawabe H. Effects of Aging on Visceral and Subcutaneous Fat Areas and on Homeostasis Model Assessment of Insulin Resistance and Insulin Secretion Capacity in a Comprehensive Health Checkup. J Atheroscler Thromb. 2016; 23:207-15.

6. DeNino WF, Tchernof A, Dionne IJ, Toth MJ, Ades PA, Sites CK, Poehlman ET. Contribution of abdominal adiposity to age-related differences in insulin sensitivity and plasma lipids in healthy nonobese women. Diabetes Care. 2001; 24:925-32.

7. Gotoh H, Gohda T, Tanimoto M, Gotoh Y, Horikoshi S, Tomino Y. Contribution of subcutaneous fat accumulation to insulin resistance and atherosclerosis in haemodialysis patients. Nephrol Dial Transplant. 2009; 24:3474-80.

8. Alba DL, Farooq JA, Lin MYC, Schafer AL, Shepherd J, Koliwad SK. Subcutaneous Fat Fibrosis Links Obesity to Insulin Resistance in Chinese Americans. J.Clin.Endocrinol.Metab. 2018; 103:3194-204.

9. Abate N, Garg A, Peshock RM, Stray-Gundersen J, Adams-Huet B, Grundy SM. Relationship of generalized and regional adiposity to insulin sensitivity in men with NIDDM. Diabetes. 1996; 45:1684-93.

10. Goodpaster BH, Thaete FL, Simoneau JA, Kelley DE. Subcutaneous abdominal fat and thigh muscle composition predict insulin sensitivity independently of visceral fat. Diabetes. 1997; 46:1579-85.

11. Kelley DE, Thaete FL, Troost F, Huwe T, Goodpaster BH. Subdivisions of subcutaneous abdominal adipose tissue and insulin resistance. Am J Physiol.Endocrinol Metab. 2000; 278:941.

12. Willoughby D, Hewlings S, Kalman D. Body Composition Changes in Weight Loss: Strategies and Supplementation for Maintaining Lean Body Mass, a Brief Review. Nutrients. 2018; 10: 10.3390/nu10121876.

13. Yamakage H, Ito R, Tochiya M, Muranaka K, Tanaka M, Matsuo Y, Odori S, Kono S, Shimatsu A, Satoh-Asahara N. The utility of dual bioelectrical impedance analysis in detecting intra-abdominal fat area in obese patients during weight reduction therapy in comparison with waist circumference and abdominal CT. Endocr J. 2014; 61:807-19.

14. Matteucci E, Giampietro O. Dipeptidyl peptidase-4 (CD26): knowing the function before inhibiting the enzyme. Curr Med Chem. 2009; 16:2943-51.

15. Durinx C, Lambeir AM, Bosmans E, Falmagne JB, Berghmans R, Haemers A, Scharpe S, De Meester I. Molecular characterization of dipeptidyl peptidase activity in serum: soluble CD26/dipeptidyl peptidase IV is responsible for the release of X-Pro dipeptides. Eur J Biochem. 2000; 267:5608-13.

16. Mentzel S, Dijkman HB, Van Son JP, Koene RA, Assmann KJ. Organ distribution of aminopeptidase A and dipeptidyl peptidase IV in normal mice. J Histochem Cytochem. 1996; 44:445-61.

17. Lamers D, Famulla S, Wronkowitz N, Hartwig S, Lehr S, Ouwens DM, Eckardt K, Kaufman JM, Ryden M, Muller S, Hanisch FG, Ruige J, Arner P, Sell H, Eckel J. Dipeptidyl peptidase 4 is a novel adipokine potentially linking obesity to the metabolic syndrome. Diabetes. 2011; 60:1917-25.

18. Sell H, Bluher M, Kloting N, Schlich R, Willems M, Ruppe F, Knoefel WT, Dietrich A, Fielding BA, Arner P, Frayn KN, Eckel J. Adipose dipeptidyl peptidase-4 and obesity: correlation with insulin resistance and depot-specific release from adipose tissue in vivo and in vitro. Diabetes Care. 2013; 36:4083-90.

19. Balaban YH, Korkusuz P, Simsek H, Gokcan H, Gedikoglu G, Pinar A, Hascelik G, Asan E, Hamaloglu E, Tatar G. Dipeptidyl peptidase IV (DDP IV) in NASH patients. Ann Hepatol. 2007; 6:242-50.

311 20. Conarello SL, Li Z, Ronan J, Roy RS, Zhu L, Jiang G, Liu F, Woods J, Zycband E, Moller DE,
312 Thornberry NA, Zhang BB. Mice lacking dipeptidyl peptidase IV are protected against obesity and
313 insulin resistance. Proc Natl Acad Sci U.S.A. 2003; 100:6825-30.

314 21. Aso Y, Ozeki N, Terasawa T, Naruse R, Hara K, Suetsugu M, Takebayashi K, Shibazaki M, Haruki
315 K, Morita K, Inukai T. Serum level of soluble CD26/dipeptidyl peptidase-4 (DPP-4) predicts the response
316 to sitagliptin, a DPP-4 inhibitor, in patients with type 2 diabetes controlled inadequately by metformin
317 and/or sulfonylurea. Transl Res. 2012; 159:25-31.

318 22. Lachin JM. Introduction to sample size determination and power analysis for clinical trials. Control
319 Clin Trials. 1981; 2:93-113.

320 23. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model
321 assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin
322 concentrations in man. Diabetologia. 1985; 28:412-19.

323 24. Aso Y, Kato K, Sakurai S, Kishi H, Shimizu M, Jojima T, Iijima T, Maejima Y, Shimomura K, Usui
324 I. Impact of dapagliflozin, an SGLT2 inhibitor, on serum levels of soluble dipeptidyl peptidase-4 in
325 patients with type 2 diabetes and non-alcoholic fatty liver disease. Int J Clin Pract. 2019; 73: e13335.

326 25. Trappe S, Creer A, Minchev K, Slivka D, Louis E, Luden N, Trappe T. Human soleus single muscle
327 fiber function with exercise or nutrition countermeasures during 60 days of bed rest. Am J Physiol Regul
328 Integr Comp Physiol. 2008; 294:939.

329 26. Tanaka S, Kanazawa I, Notsu M, Sugimoto T. Visceral fat obesity increases serum DPP-4 levels in
330 men with type 2 diabetes mellitus. Diabetes Res Clin Pract. 2016;116: 1-6.

331 27. Firneisz G, Varga T, Lengyel G, Feher J, Ghyczy D, Wichmann B, Selmeci L, Tulassay Z, Racz K,
332 Somogyi A. Serum dipeptidyl peptidase-4 activity in insulin resistant patients with non-alcoholic fatty
333 liver disease: a novel liver disease biomarker. PLoS One. 2010; 5: e12226.

334 28. Kim YG, Hahn S, Oh TJ, Kwak SH, Park KS, Cho YM. Differences in the glucose-lowering efficacy
335 of dipeptidyl peptidase-4 inhibitors between Asians and non-Asians: a systematic review and meta-
336 analysis. Diabetologia. 2013; 56:696-708.

337

Table 1. Clinical characteristics of study population			
	Baseline	After 2 weeks	P-value
N (male/female)	47 (34/13)		
Age (years)	56.6 ± 13.0		
Height (cm)	165.3 ± 9.5		
Weight (Kg)	75.3 ± 19.6	73.7 ± 18.6	2.11×10^{-7}
BMI	27.3 ± 5.6	26.8 ± 5.3	8.05×10^{-8}
Body fat mass (%)	28.8±9.5	28.8±9.2	0.995
Body fat weight (kg)	22.5±11.3	22.0±10.8	0.11
Lean body weight (kg)	52.8±12.3	51.7±11.3	0.001
Visceral fat area (cm^2)	104.0±52.5	96.3±46.2	0.009
FPG (mg/dl)	172.6 ± 53.2	111.1 ± 17.2	9.57×10^{-11}
HbA1c (%)	10.2 ± 2.4		
Fasting IRI (μU/ml)	10.4 ± 7.3***	9.7 ± 5.2*	0.006
Fasting C-peptide (ng/ml)	2.2 ± 1.3	1.8 ± 1.2**	0.002
HOMA-IR	4.3 ±3.6***	2.8 ± 1.8*	0.0002
Diabetic therapy			
DP/BG/TZ/SU/GL/AG/SG	29/20/1/9/1/7/0	32/27/11/4/7/10/7	
INS/GLP-1/NONE	8/1/6	22/3/2	

Data are mean ± SD. NA indicates that data were not available. *: n=18. **:n=44. ***:n=36

DP, DPP-4 inhibitors; BG, biguanide; TZ, thiazolidine; SU, sulfonylureas; GL, glinides; AG, α glucosidase inhibitors; SG, SGLT-2 inhibitors; INS, insulin; GLP-1, GLP-1 receptor agonist

Table 2. Changes in circulating DPP-4/sCD26 concentrations and metabolic parameters			
	Baseline	After 2 weeks	P-value
AST (IU/L)	32.3±23.1	30.4±18.9	0.48
ALT (IU/L)	43.1±38.9	39.9±30.9	0.47
γ-GTP (IU/L)	59.6±66.9	47.9±51.7	0.004
LDL-C (mg/dl)	118.0±31.9	104.2±28.8	0.00008
HDL-C (mg/dl)	41.3±9.3	39.0±8.1	0.004
TG (mg/dl)	159.2±86.1	133.8±60.5	0.02
MDA-LDL (U/L)	136.2±49.7	115.2±35.6	0.00003
RLP-C (mg/dl)	7.4±5.8	5.4±3.4	0.02
DHLA (μg/ml)	38.4±16.5	30.1±8.4	0.0002
AA (μg/ml)	207.3±58.9	192.7±45.2	0.003
EPA (μg/ml)	60.2±35.6	57.2±27.0	0.36
DHA (μg/ml)	139.8±50.9	137.3±43.7	0.54
EPA/AA	0.30±0.20	0.31±0.16	0.80
hsCRP (mg/dl)	0.25±0.34	0.22±0.36	0.90
DPP-4/sCD26 (ng//ml)	1002.6±393.9	892.0±399.4	0.003

343 MDA-LDL, malondialdehyde- modified LDL; RLP-C, remnant like particls cholesterol; DHLA, dihomo
344 γ-linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.
345

Table 3. Correlation analysis between clinical traits and circulating DPP-4 concentrations (at baseline)				
	Single correlation		Multiple regression(n=35)	
Independent parameter	*r*	P	*β*	P
BMI	-0.061	0.69	-	-
Visceral fat area	-0.056	0.72	-	-
Body fat mass	-0.017	0.91	-	-
Lean body weight	0.006	0.97	-	-
AST	0.423	0.004	-	-
ALT	0.248	0.10	-	-
γ-GTP	0.415	0.005	0.462	0.005
IRI	0.477	0.004	-	-
HOMA-IR	0.548	0.001	0.308	0.05

346

Table 4. Correlation analysis between changes in clinical traits and circulating DPP-4 concentrations during a 2 week-hospitalization				
	Single correlation		Multiple regression(n=43)	
	r	P	β	P
ΔBMI	0.223	0.13	0.245	0.12
ΔVisceral fat area	-0.343	0.02	-0.302	0.05
ΔBody fat mass	-0.056	0.71	-	-
ΔLean body weight	0.104	0.49	-	-
ΔAST	0.067	0.65	-	-
ΔALT	0.030	0.84	-	-
Δγ-GTP	0.300	0.04	0.173	0.27
ΔIRI	0.366	0.11	Not included	Not included
ΔHOMA-IR	0.633	0.005	Not included	Not included

347