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Persons with latent autoimmune diabetes in adults express higher dipeptidyl peptidase-4 activity compared to persons with type 2 and type 1 diabetes

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Abstract

Aims: We aimed to determine serum dipeptidyl peptidase-4 (DPP-4) activity in a group of persons with latent autoimmune diabetes in adults (LADA) and to compare it with persons with type 1, type 2 diabetes and healthy controls.

Methods: DPP-4 activity measurement was performed in 67 persons (21 with type 1, 26 type 2 and 19 with LADA) and 13 healthy age and gender matched controls.

Results: Persons with LADA showed highest DPP-4 activity among the study groups (32.71±3.55 *vs* 25.37±2.84 *vs* 18.57±2.54 *vs* 18.57±2.61 U/L $p<0.001$). Mean glutamic acid autoantibody in persons with LADA was 164.32±86.28 IU/mL. It correlated with DPP-4 activity ($r=0.484$, $p=0.013$). Furthermore, DPP-4 activity correlated with waist circumference ($r=0.279$, $p=0.034$) and glycated haemoglobin A1c ($r=0.483$, $p<0.001$), as well as with LDL cholesterol ($r=0.854$, $p<0.001$) and total daily insulin dose ($r=0.397$, $p=0.001$). In the multinomial regression analysis DPP-4 activity remained associated with both LADA (prevalence ratio 1.058 (1.012-1.287), $p=0.001$) and type 1 diabetes (prevalence ratio 1.506 (1.335-1.765), $p<0.001$) while it did not show an association with type 2 diabetes (prevalence ratio 0.942 (0.713-1.988), $p=0.564$).

Conclusions: Persons with LADA express higher DPP-4 activity compared to persons with both type 1 and type 2 diabetes. The possible patophysiological role of DPP-4 in the LADA pathogenesis needs to be further evaluated.

Key words: latent autoimmune diabetes in adults, type 1 diabetes mellitus, type 2 diabetes mellitus, dipeptidy peptidase-4

1. Introduction

Diabetes mellitus reflects a group of carbohydrate, protein and fat metabolism disorders characterized by hyperglycemia which eventually leads to micro- and macrovascular complications development. Although all forms of diabetes are characterized by hyperglycemia, the pathogenic mechanisms by which hyperglycemia arises widely differ. American Diabetes Association categorized diabetes mellitus mainly as type 1, type 2 diabetes and the others [1]. Type 1 diabetes mellitus (T1DM) is an autoimmune (AI) disease in which absolute insulin deficiency and consecutive hyperglycemia results from immune-mediated destruction of insulin-secreting pancreatic islet cells [1, 2]. Although the majority of persons with T1DM experience the acute disease onset under the age of 30 years, during the last decades an accumulating body of literature led to the recognition that predominant, autoantibody mediated form of T1DM might appear at any age [2-4].

Latent autoimmune diabetes in adults (LADA) represents a form of autoimmune diabetes that resembles type 1, but has a later onset and slower progression towards absolute insulin dependency. There are controversies regarding this type of the disease and its terminology which is why several attempts have been made in order to better characterization and classification [4]. Uncertainties concern almost all aspects of this disease, including:nomenclature, diagnostic criteria, epidemiology, natural history, pathogenesis along with genetic, metabolic and immunological aspects. Clinical phenotype in persons with AI diabetes ranges from diabetic ketoacidosis to diabetes that can be controlled with diet alone [5] and the three criteria namely: age at diagnosis, autoantibody positivity and need for insulin treatment conventionally used to define adult-onset AI diabetes are non specific. Because autoantibodies to tyrosine phosphatase-like insulinoma-associated protein 2 (IA2) have been reported to be rather infrequent, the diagnosis basically relies on identifying glutamic acid decarboxylase autoantibodie (GADA), which is the best single marker for screening [6]. Time to insulin treatment is dependent on local clinical judgment and not on the disease process [7], finally, as already mentioned, the disease might appear at any age which is not possible to establish the correct diagnosis without Ab screening that is not routinely performed. Consequentially, there is no clear management strategy in terms of LADA therapy and prevention. Even though ~10% of adults with presumed type 2 diabetes (T2DM) at diagnosis in fact have LADA, so far there are only few studies evaluating

therapeutic interventions for LADA, using a hypoglycemic or an immunomodulatory agent. An ideal therapeutic approach would aim not only toobtaine a good metabolic control, but also to protect residual β-cell mass and function. Conversely, even in persons on insulin, glycaemic control is suboptimal, suggesting that insulin alone may not be sufficient [8].

Dipeptidyl peptidase-4 (DPP-4) inhibitors represent a new class of oral antidiabetic agents that have shown the potential to preserve β-cell function in mouse models of type 2 diabetes [9, 10], in persons with T2DM [11], and even in persons with impaired fasting glucose tolerance [12]. DPP-4 inhibition reduces insulitis as well, and stimulates β -cell function in a non-obese diabetic mouse model of AI diabetes, a classic model of T1DM [13, 14]. There is an increased serum DPP-4 activity [15] and expression on terminally differentiated CD4 T-cells [16] in persons with T1DM. Recent trials show that one of the DPP-4 inhibitors, sitagliptin, significantly improves glycaemic control in adult persons with T1DM [17] as well as in persons with LADA [18].

These observations provided a rationale to test the soluble serum DPP-4 activity in LADA, T1DM, T2DM and to compare it with healthy controls.

2. Materials and Methods

2.1 Study design

This was a single-centre based study undertaken at University Clinic for diabetes, endocrinology and metabolic disease Vuk Vrhovac, Zagreb, Croatia. The study population comprised clinic- and hospital-based adult persons with diabetes aged >30 years within at least one year from diabetes diagnosis coming for their comprehensive annual review screened for ICA, GAD and IA2 Abs. Persons were stratified into four groups: healthy controls (CTRL), persons with T1DM, T2DM and LADA. Histories and complete physical examination and laboratory tests were performed in all subjects in order to exclude diseases other than T1DM or medications that might affect insulin sensitivity. T1DM was defined by undetectable meal stimulated C-peptide concentrations (C-peptide <0.2 ng/mL) and positive ICA and GAD (or in combination with IA2 at least from the previous medical record if the measurement was performed in our Clinic laboratory, respectively). LADA was defined by meal stimulated C-peptide concentrations >0.2

ng/mL and positive GAD auto antibodies and further confirmed clinicallybased on the criteria a given by The Immunology of Diabetes Society [19]. Type 2 diabetes was defined as ICA, GADA and IA-2 autoantybody negative at the time of diagnosis and without any other evident cause of IR that characterises other, specific types of diabetes. All of the persons were using insulin which was administered by a basal-bolus regimen, including those with T2DM. The meal stimulated test is done with mixed meal (324 (324–528) kcal) comprising approximately 20% fat, 60% carbohydrate, and 20% protein. Patients receive subcutaneous injections of long-acting insulin analogues before going to sleep the previous day as basal, but not subcutaneous injection of rapid-acting insulin analogue as a bolus before ingestion of the test meal on the day of the study.

2.2 Study measurements

Subjects were studied after an overnight fast. Venous blood samples were collected for the determination of biochemistry panel, lipid profile status and DPP-4 activity. Basic anthropometric measurements: body mass index (BMI), waist circumference and blood pressure were performed on all study subjects.

HbA1c was measured spectrophotometrically by turbidimetric immuno-inhibition (Olympus AU600, Beckman-Coulter, USA) and were expressed in DCCT reference units.

Cholesterol and triglycerides in serum were measured by an enzymatic colorimetric method. ICA antibodies were measured by indirect immunofluorescence. Rate of positivity was calculated by determining end-point titres of samples that were converted to the units of Juvenile Diabetes Foundation (JDF-U) by comparison with a standard curve of log2 JDF units with log2 of end-point titre of standard sera. The threshold of detection was >5 JDF units. GAD and IA2 Abs were detected with an enzyme-linked immunoadsorbent assay (Euroimmun AG, Luebeck, Germany). The cut-off limit was 10 U/ml for GAD Abs and 15 U/ml for IA2 Abs. Participants were regarded as GAD and/or IA2 positive if antibody titre exceeded the reference range.

Sera samples were kept at -20°C until the analysis. DPP-4 activity was measured by a colorimetric assay procured from Sigma, St. Louis, MO, USA in a microplate reader (Cary Eclipse Varian, Agilent Technologies) at 460 nm, 37 °C in a continuous monitoring for 35 min. In this assay, DPP4 cleaves H-Gly-Pro-AMC to release a fluorescent product, 7-Amino-4-Methyl

Coumarin (AMC) which can be measured spectrophotometrically. One unit of activity was defined as the amount of enzyme which will hydrolyse the DPP-4 substrate to yield 1.0 μmol of AMC per minute at 37 °C.

The study protocol complies with the Declaration of Helsinki and was approved by the local ethics committee. Written informed consent was obtained from all participants.

2.3 Statistical analysis

According to our previous studies [20] and the expected standard deviation of sera DPP-4 activity of 2.56 U/L, the sample size was in accordance with 1-β=0.8 at a significance level of α=0.05. Baseline data were reported using descriptive statistics. Normality of distribution for continuous variables was analysed using Shapiro-Wilk test. Variables that were not normally distributed were log-transformed and described with mean and standard deviation (SD). The nominal variables were reported with absolute numbers and/or percentages. Differences between groups were examined, depending on the nature of the data, using parametric Differences between groups were examined, by One way ANOVA test, followed by Bonferroni's correction and χ 2 test for the attributive variables. Correlations between DPP-4 activity and anthropometric, biochemical and immunological activity were tested with Pearsons coefficient of correlation. The association of DPP-4 activity and type of diabetes was further tested in the multinomial regression analysis including all the variableas reaching p value <0.05 in the univariate analysis. Level of statistical significance was chosen to 0.05. Statistical analysis was performed by statistical package Statistical Package for the Social Sciences (SPSS) ver.17.0 for Windows.

3. Results

The mean age of our study population was 51.1±14.6 years with a mean duration of diabetes 20.3±11.3 years while the healthy control group was 49.7±5.5 years old. Participants were divided into four groups: 21 persons with T1DM, 26 with T2DM, 19 persons with LADA and 13

age and gender matched healthy controls (CTRL). The gender distribution was similar in-between groups (61.9% *vs* 57.7% *vs* 57.9% *vs* 53.8% males). Their baseline anthropometric and laboratory data as well as difference in-between them are given in Table 1., except for the mean DPP-4 activity of the healthy control group of 18.57±2.61 U/L (Figure 1.)

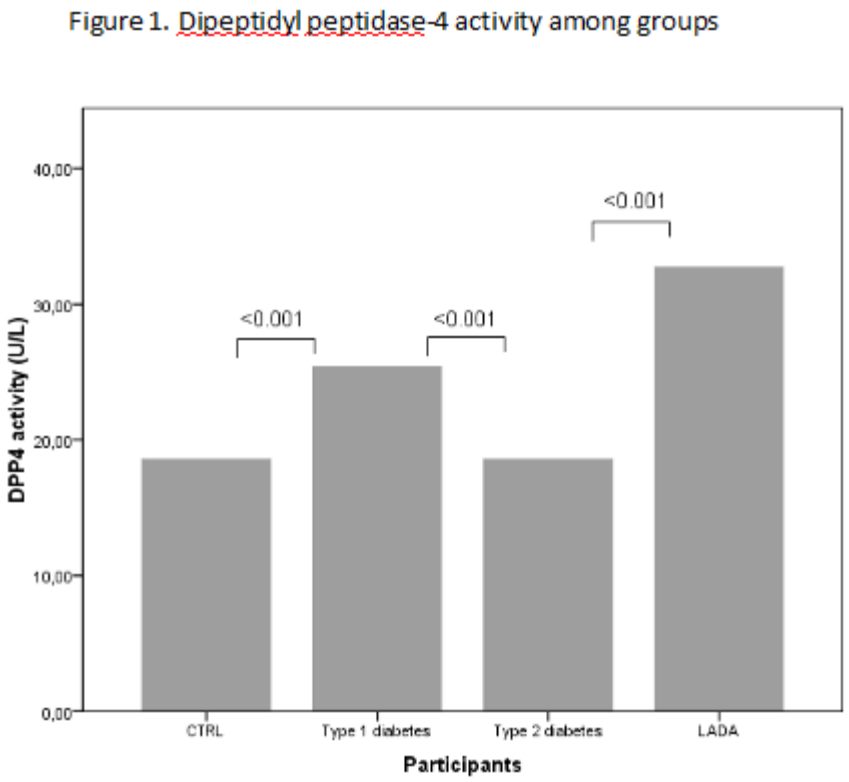
Table 1. Patients anthropometric and laboratory data

Variable	**Type 1 diabetes N=21**	**Type 2 diabetes N=26**	**LADA N=19**	**p**
Age (years)	41.6±14.9	53.7±12.5	43.4±11.4	0.09
Diabetes duration (years)	17.65±12.4	20.75±13.95	18.0±13.5	0.003
Waist circumerence (cm)	88.3±14.3	99.9±14.1	90.3±16.9	0.03
Body mass index (kg/m2)	25.9±3.5	28.6±4.5	26.3±4.6	0.07
Systolic BP (mmHg)	128.2±27.1	133.1±19.6	132.8±27.1	0.76
Dyastolic BP (mmHg)	76.6±7.8	84.3±15.1	80.8±11.7	0.15
Disease duration (years)	21.1±11.0	20.2±12.8	18.0±8.8	0.83
HbA1c (%)	7.1±1.3	7.3±1.4	7.5±1.3	0.53
HbA1c (mmol/mol)	54±9	56±8	58±9	
Total serum cholesterol (mmol/L)	4.8±0.9	5.7±1.6	5.5±2.4	0.22
HDL cholesterol (mmol/L)	1.8±0.6	1.4±0.4	1.7±0.5	0.23
LDL cholesterol (mmol/L)	2.6±0.7	3.2±2.1	3.4±1.1	0.18
Triglycerides (mmol/L)	1.03±0.52	1.97±2.86	1.27±0.71	0.21
Total daily insulin dose (IU/kg)	0.59±0.13	0.34±0.14	0.51±0.39	<0.001
DPP-4 activity (U/L)	25.37±2.84	18.57±2.54	32.71±3.55	<0.001

Legend: BP- blood pressure; HbA1c-glycated haemoglobin A1c; HDL-high density lipoprotein; LDL-low density lipoprotein; DPP-4-dipeptidy peptidase-4

Figure 1. Dipeptidyl peptidase-4 activity among groups;

Legend: CTRL-healthy control: LADA-latent autoimmune diabetes in adults



Mean ICA autoantibody titre in the group with T1DM was 117.14±83.99 JDF units while GAD titer was 461.33±102.03 IU/mL. Persons with LADA were ICA negative (<5 JDF units) while GAD autoantibody titer was 164.32±86.28 IU/mL. GAD autoantibody correlated with DPP-4 activity (r=0.484, p=0.013) while ICA titer did not (r=0.191, p=0.204). Furthermore, DPP-4 activity correlated with waist circumference (r=0.279, p=0.034) and HbA1c (r=0.483, p<0.001), as well as with LDL cholesterol (r=0.854, p<0.001) and total daily insulin dose (r=0.397, p=0.001) (Figure 2. a-e).

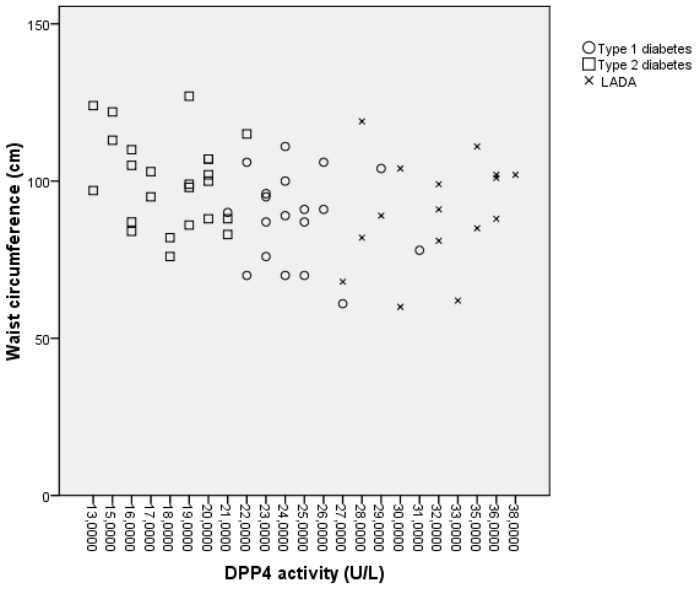
Figure 2. a) Correlations between GAD Ab titer and dipeptidyl peptidase-4 activity

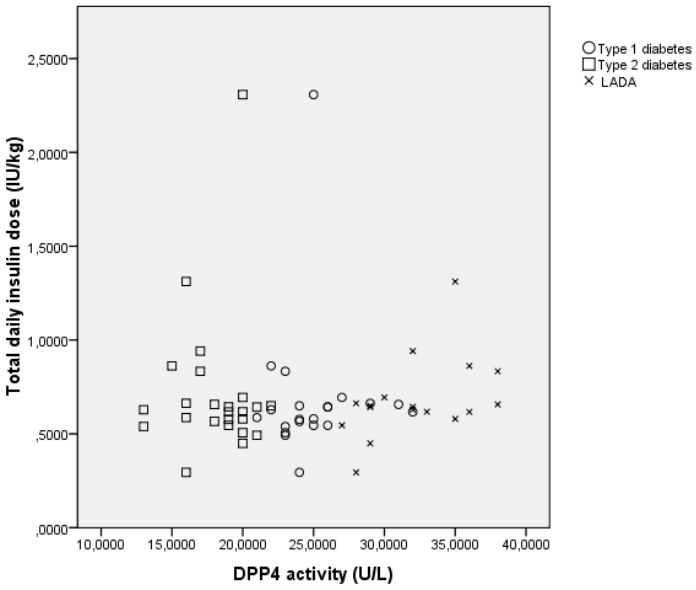
b) Correlations between waist circumference and dipeptidyl peptidase-4 activity

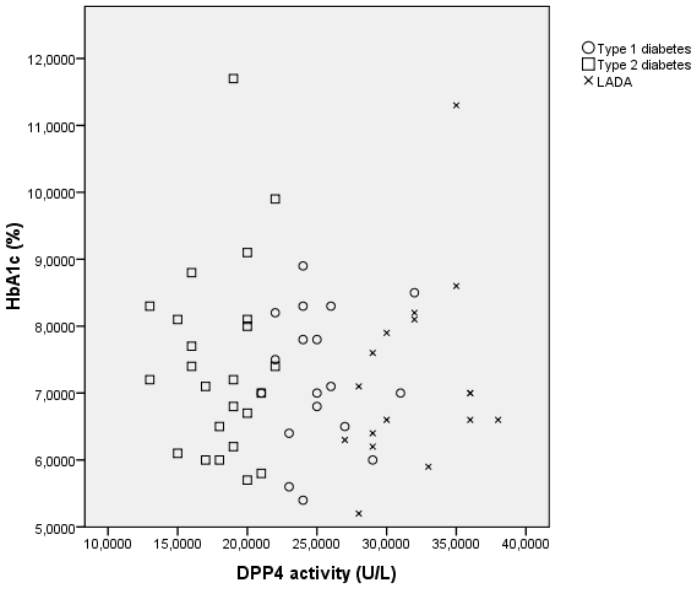
c) Correlations between total daily insulin dose and dipeptidyl peptidase-4 activity

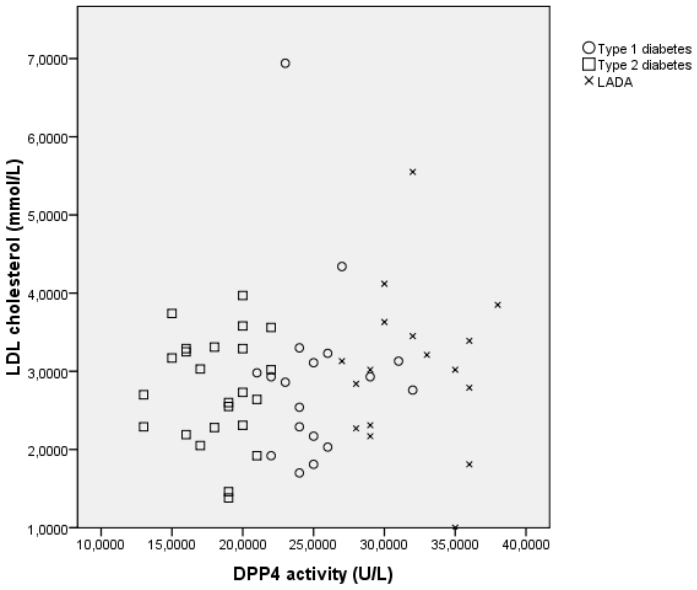
d) Correlations between glycated haemoglobin A2 and dipeptidyl peptidase-4 activity

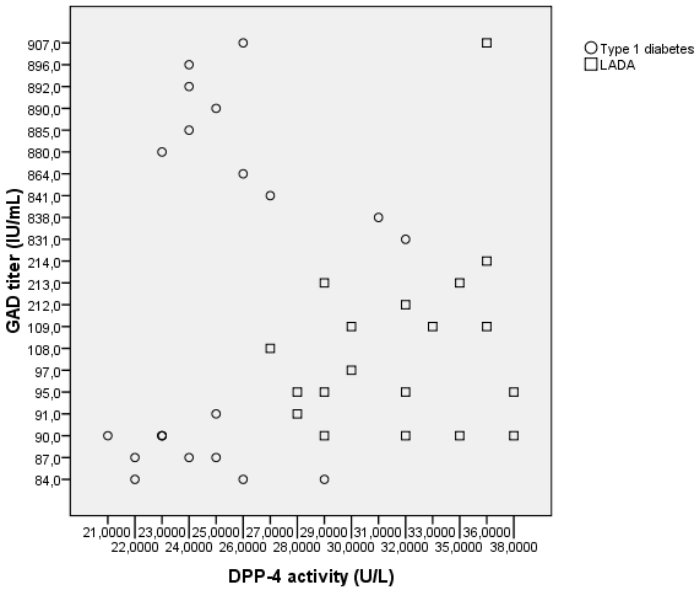
e) Correlations between low density lipoprotein cholesterol and dipeptidyl peptidase-4 activity




Type 1 diabetes
Type 2 diabetes
LADA
Total daily insulin dose (IU/kg)
2,5000
2,0000
1,5000
1,0000
,5000
,0000
10,0000
15,0000
20,0000
25,0000
30,0000
35,0000
40,0000
DPP4 activity (U/L)


Type 1 diabetes
Type 2 diabetes
LADA
HbA1c (%)
12,0000
11,0000
10,0000
9,0000
8,0000
7,0000
6,0000
5,0000
10,0000
15,0000
20,0000
25,0000
30,0000
35,0000
40,0000
DPP4 activity (U/L)





In the multinomial regression analysis DPP-4 activity remained associated with both LADA (prevalence ratio 1.058 (1.012-1.287), p=0.001) and type 1 diabetes (prevalence ratio 1.506

(1.335-1.765), p<0.001) while it did not show an association with type 2 diabetes (prevalence ratio 0.942 (0.713-1.988), p=0.564).

4. Discussion

This cross-sectional study was designed in order to examine if there is a difference in soluble serum DPP-4 activity in persons with different types of diabetes compared to healthy controls with special emphasis on persons with LADA. Our study reports several findings: 1) persons with LADA express higher DPP-4 activity compared to other types of diabetes; 2) DPP-4 activity correlates with waist circumference, LDL cholesterol, total daily insulin dose and GAD autoantibody status; 3) no significant difference was found between DPP-4 activity in persons with T2DM compared to healthy controls.

DPP-4 is ubiquitously distributed, predominantly found on endothelial and epithelial cells throughout the body [21, 22]. A fraction of soluble DPP-4 however, originates from the immune system cells which explain its altered abundance and the circulating activity in various immune mediated conditions [23] including T1DM [24]. Recent data suggest that DPP-4 activity is higher in persons with T1DM compared to healthy controls independently of islet-cell antibody status, C-peptide concentration, disease duration or HbA1c level [24] and in an inverse correlation with body mass index (BMI) and insulin sensitivity [24,25]. This is in accordance with our study results because patophysiology of LADA comprises an autoimmune component while its phenotype usually resembles T2DM. However, we also showed that DPP-4 activity correlates with GAD autoantibody titre which might confirm its meaning in the pathophysiological background of the disease. This is partially in accordance with the study of Iwbuchi et al. (2013) [25] as well as with Varga et al. (2010) who demonstrated a significantly higher DPP-4 activity in the AI form of diabetes,a strong correlation with anthropometric parameters and glycaemic control [24]. We could explain this with the fact that AI diabetes is a T-cell mediated organ-specific disease, initiated by the imbalance between pathogenic and regulatory T-lymphocytes. DPP-4 is the lymphocyte cell surface protein CD26, critical in T-cell biology as a marker of T-cell activation [24,26]. DPP-4 inhibition increased regulatory T-cells and reversed recent-onset

diabetes in a non-obese mouse model of AI diabetes [27]. Those results suggest that the DPP4 activity might be an important factor in determination of diabetes type .

Our third finding is partially in accordance with the study of Firneisz et al. [26] (2010) who determined serum DPP4 activity at fasting sate and after test meal in 41 persons with T1DM, 87 with T2DM accompanied with prominent insulin resistance (IR) and in 25 healthy volunteers. Serum DPP-4 activity was significantly higher in both fasting and postprandial state in persons with T1DM than in T2DM compared to control subjects irrespective of HbA1c or fasting serum glucose. In our study, persons with LADA had significantly higher waist circumference than persons with T1DM and required higher insulin dose than persons with T2DM which could mean that they also had higher degree of IR.

Existing studies indicate that DPP-4 inhibitors have the potential to protect β-cell function [11, 12] in persons with T2DM as well as in a mouse model of AI diabetes [15] which indicates the possible role of DPP-4 activity in the disease pathogenesis . Our present results are consistent with that hypothesis especially because LADA might represent the combination of underlying risk factors of both types of diabetes.

Because most subjects in our study had similar diabetes duration, we conclude that DPP-4 activity reflects the joint contribution of IR characteristics and β-cell function in persons with LADA. Recently Johasen et al [28] found that another DPP-4 inhibitor, linagliptin, preserves β-cell function in persons with LADA during a 2-year study. However, the beneficial metabolic effect of DPP-4 inhibitors in addition to insulin therapycould occur independently of immune effects leading secondary to reduced β-cell stress, reduced glucagon, or β-cell regeneration. Finally, because persons with LADA showed the worse glycaemic control in-between groups, it might be evident that insulin alone is inadequate in the LADA treatment

Small sample size and the cross-sectional design of the study puts the limitation of generating any general conclusions from our study. The autoantibody status was not determined at diagnosis and IR was not accessed. Furthermore, DPP-IV is a membranous enzyme expressed in endothelial cells, epithelial cells, and T cells, however, we do not know to what extent the soluble DPP-4 represents the DPP-4 activity in whole body. Thus, the small increase of DPP-4 activity in the samples from LADA patients is difficult to interpret its significance.

In conclusion, we concider increased DPP-4 activity in LADA compared to other diabetes types a reflection of combined autoimmunity and IR. A number of attractive therapeutic interventions may be envisioned for prevention of β-cell deterioration and progression towards insulin dependency. Those include hypoglycemic and immunomodulatory agents, a possibly a combination of those if provided that they are safe. Because the AI process in LADA is thought to be slower than in T1DM, there is a larger window of opportunities for intervention. DPP-4 inhibitors might represent an appropriate therapeutic approach that offers metabolic control and at the same time might improve the natural history of the disease (i.e., maintains/increases the residual β-cell mass and/or function) which needs to be evaluated in further prospective and interventional studies using DPP-4 inhibitors in persons with LADA.

5. Conflict of interest

All authors disclosed no financial or personal conflict of interest.

6. Refefences:

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2010; 33: S62–S69.
2. Bluestone JA, Herold K, Eisenbarth G. Genetics, pathogenesis and clinical interventions in type 1 diabetes. Nature 2010; 464:1293–1300
3. Bellgrau D, Eisenbarth GS. Immunobiology of autoimmunity; in Eisenbarth GS: Endocrine and Organ specific autoimmunity. Austin, TX: RG Landes, 1999:1-18.
4. Fourlanos S, Dotta F, Greenbaum CJ, Palmer JP, Rolandsson O, Colman PG, Harrison LC.: Latent autoimmune diabetes in adults (LADA) should be less latent. Diabetologia 2005; 48: 2206– 2212

5. Borg H, Gottsater A, Fernlund P, Sundkvist G. A 12-year prospective study of the relationship between islet antibodies and beta-cell function at and after the diagnosis in persons with adult-onset diabetes. Diabetes 2002; 51: 1754–1762.

6. Falorni A, Brozzetti A. Diabetes-related antibodies in adult diabetic persons. Best Pract Res Clin Endocrinol Metab 2005; 19: 119– 133

7. Brophy S, Yderstraede K, Mauricio D, Hunter S, Hawa M, Pozzilli P et al. Time to insulin initiation cannot be used in defining latent autoimmune diabetes in adults, Diabetes Care 2008; 31: 439-441.

8. Davis TME, Wright AD, Mehta ZM, Cull CA, Stratton IM, Bottazzo GF, et al. Islet autoantibodies in clinically diagnosed type 2 diabetes: prevalence and the relationship with metabolic control (UKPDS 70). Diabetologia 2005; 48:695-702.

9. Cakirca M, Karatoprak C, Zorlu M, Ksikac M, Kanat M, Cikrikcioglu MA et al. Effect of vildagliptin add-on treatment to metformin on plasma asymmetric dimethylarginine in type 2 diabetes mellitus persons. Drug Des Devel Ther. 2014; 8: 239–243.

10. Takeda Y, Fujita Y, Honjo J, Yanagimachi T, Sakagami H, Takiyama Y et al. Reduction of both β cell death and α cell proliferation by dipeptidyl peptidase-4 inhibition in a streptozotocin-induced model of diabetes in mice. Diabetologia. 2012;55:404–412.

11. D'Alessio DA, Denney AM, Hermiller LM, Prigeon RL, Martin JM, tharp WG et al . Treatment with the dipeptidyl peptidase-4 inhibitor vildagliptin improves fasting islet-cell function in subjects with type 2 diabetes. J Clin Endocrinol Metab. 2009;94:81–88.

12. Foley JE, Bunck MC, Moller-Goede DL, Poelma M, Nijpels G, Eekhoff EM et al. β Cell function following 1 year vildagliptin or placebo treatment and after 12 week washout in drug-naive persons with type 2 diabetes and mild hyperglycaemia: a randomised controlled trial. Diabetologia. 2011;54:1985–1991.

13. Utzschneider KM, Tong J, Montgomery B, Udayasankar J, Gerchman F, Marcovina SM et al. The dipeptidyl peptidase-4 inhibitor vildagliptin improves β-cell function and

insulin sensitivity in subjects with impaired fasting glucose. Diabetes Care. 2008;31:108–113.

14. Shah P, Ardestani A, Dharmadhikari G, Laue S, Schumann DM, Kerr-Conte J, et al. The DPP-4 inhibitor linagliptin restores β-cell function and survival in human isolated islets through GLP-1 stabilization. J Clin Endocrinol Metab. 2013;98:E1163–E1172.

15. Tian L, Gao J, Hao J, Zhang Y, Yi H, O'Brien TD et al. Reversal of new-onset diabetes through modulating inflammation and stimulating β-cell replication in nonobese diabetic mice by a dipeptidyl peptidase IV inhibitor. Endocrinology. 2010;151:3049–3060.

16. Jelsing J, Vrang N, van Witteloostuijn SB, Mark M, Klein T. The DPP4 inhibitor linagliptin delays the onset of diabetes and preserves β-cell mass in non-obese diabetic mice. J Endocrinol. 2012;214:381–387.

17. Varga T, Firneisz G, Nagy G, Somogyi A. Elevated serum dipeptidyl peptidase-4 activity in type 1 diabetes mellitus: a direct comparison. Orv Hetil. 2010;151:899–902.

18. Zhao Y, Yang L, Xiang Y, Huang G, Long Z, Li X,et al. Dipeptidyl peptidase 4 inhibitor sitagliptin maintains β-cell function in persons with recent-onset latent autoimmune diabetes in adults: one year prospective study.J Clin Endocrinol Metab. 2014 May;99(5):E876-80.doi: 10.1210/jc.2013-3633. Epub 2014 Jan 16.

19. Sacks DB, M. Arnold GL, Bakris DE, et al. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Diabetes Care 2011; 34(6), e61–e99

20. Duvnjak L, Blaslov K, Perković MN, Ćuća JK. Dipeptidyl peptidase-4 activity might be a link between tumour necrosis factor alpha and insulin resistance in type 1 diabetes. Endocrine. 2016 Aug;53(2):453-8

21. Sell H, Blüher M, Klöting N, Schlich R, Willems M, Ruppe F et al. 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 Dec;36(12):4083-90. doi: 10.2337/dc13-0496. [PMID: 24130353]

22. Röhrborn D, Eckel J, Sell H. Shedding of dipeptidyl peptidase 4 is mediated by metalloproteases and up-regulated by hypoxia in human adipocytes and smooth muscle cells. FEBS Lett. 2014 Nov 3;588(21):3870-7. doi: 10.1016/j.febslet.2014.08.029. Epub 2014 Sep 12. [PMID: 25217834]

23. Lambeir AM, Durinx C, Scharpé S, De Meester I. Dipeptidyl-peptidase IV from bench to bedside: an update on structural properties, functions, and clinical aspects of the enzyme DPP IV. Crit Rev Clin Lab Sci. 2003 Jun;40(3):209-94. [PMID: 12892317]

24. Varga T, Somogyi A, Barna G, Wichmann B, Nagy, G. Rácz K et al. Higher serum DPP-4 enzyme activity and decreased lymphocyte CD26 expression in type 1 diabetes. Pathol. Oncol. Res. 2011; 17, 925–930 doi: 10.1515/jpem-2013-0122. [PMID: 23817599]

25. Iwabuchi A, Kamoda T, Saito M. Nozue H, Izumi I, Hirano T et al. Serum dipeptidyl peptidase 4 activity in children with type 1 diabetes mellitus. J. Pediatr. Endocrinol. Metab. 2013: 26(11–12),1093–7

26. Firneisz G, Varga T, Lengyel G, Fehér J, Ghyczy D, Wichmann B, et al. Serum Dipeptidyl Peptidase-4 Activity in Insulin Resistant Persons with Non-Alcoholic Fatty Liver Disease: A Novel Liver Disease Biomarker. PLoS ONE 2010:5(8): e12226. doi:10.1371/journal.pone.0012226

27. Morimoto C, Schlossman SF. The structure and function of CD26 in the T-cell immune response. Immunol Rev. 1998;161:55–70.

28. Johansen OE, Boehm BO, Grill V, Torjesen PA, Bhattacharya S, Patel S et al. C-peptide levels in latent autoimmune diabetesin adults treated with linagliptin vs glimepiride: exploratory results from a 2-year double-blind randomized controlled study. Endocr Rev. 2012;33:SUN-LB1

29. Ghazi T, Rink L, Sherr JL, Herold KC. Acute metabolic effects of exenatide in persons with type 1 diabetes with and without residual insulin to oral and IV glucose challenges. Diabetes Care. 2014;37(1): 210 –216.

30. Andersen CD, Bennet L, Nystrom L, Lindblad U, Lindholm E, Groop L et al. Worse glycaemic control in LADA persons than in those with type 2 diabetes, despite a longer time on insulin therapy. Diabetologia. 2013;56:252–258.