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Omentin-1 and NAMPT serum concentrations are higher and CK-18 levels are lower in children and adolescents with type 1 diabetes when compared to healthy age, sex and BMI matched controls

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Abstract

Background: Adipokines were shown to affect glucose homeostasis and β-cell function in patients with pancreatic dysfunction which is associated with changes in the adipose tissue secretory profile. However, information about adipokines associated with β-cell dysfunction is lacking in pediatric patients with type 1 diabetes.
Methods: (1) We compared serum concentrations of nicotinamide phosphoribosyltransferase (NAMPT), omentin-1 and caspase-cleaved cytokeratin 18 fragment M30 (CK-18) in pediatric type 1 diabetes patients (n = 245) and healthy age, sex and body mass index standard deviation score (BMI-SDS) matched controls (n = 243). (2) We investigated the influence of insulin treatment on serum concentrations of NAMPT, omentin-1 and CK-18 in groups of patients with type 1 diabetes stratified according to the duration of their disease: at onset (n = 50), ≥6 months and <5 years (n = 185), ≥5 and <10 years (n = 98), and ≥10 years (n = 52).
Results: Patients at onset compared with healthy controls demonstrated no significant differences in NAMPT levels ($p = 0.129$), whereas omentin-1 levels were elevated ($p < 0.001$) and CK-18 levels were lowered ($p = 0.034$). In contrast, NAMPT and omentin-1 were elevated and CK-18 serum levels were lower in longstanding patients compared to healthy controls ($p < 0.001$). NAMPT serum levels did not change significantly during the duration of type 1 diabetes ($p = 0.546$). At onset, omentin-1 and CK-18 levels were higher than in any group of longstanding type 1 diabetes ($p < 0.025$).
Conclusions: Altered serum levels of NAMPT, omentin-1 and CK-18 in pediatric type 1 diabetes patients indicate metabolic changes caused by adipose tissue dysregulation which do not normalize during insulin therapy.

Keywords: adipokines; glucose tolerance; inflammation; insulin; visfatin.

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Introduction

Adipokines were shown to affect glucose homeostasis and β-cell function in patients with pancreatic dysfunction [1]. They may represent a potential link between white adipose tissue and pancreatic β-cells, especially in type 1 diabetes patients. Vice versa, destruction of β-cell function in pediatric type 1 diabetes patients results in alterations of metabolic state and adipokine profile [2]. Furthermore, the disturbed β-cell function in these patients may frequently be associated with overweight; especially patients below 6 years of age and girls show increased body mass indexes (BMIs) [3] and a chronic systemic low-grade inflammatory process [4]. Due to these pathophysiological processes, the altered secretory profile of adipokines may deliver potential implications for diagnostic and therapeutical use.

We assume that type 1 diabetes in children causes changes in adipose tissue mass, structure and function, which consecutively contributes to altered secretion patterns of adipokines relevant to β-cell function. We also assume that obesity and inflamed adipose tissue cause an impaired function of β-cells. Not only the lack of endogenous insulin during onset of type 1 diabetes complicates the interaction between different metabolically active organs such as pancreas, liver and adipose tissue, but also long-term changes affect serum concentrations of adipokines. Therefore we asked if changed secretory profiles of adipokines, namely nicotinamide phosphoribosyltransferase (NAMPT), omentin-1 and caspase-cleaved cytokeratin 18 fragment M30 (CK-18), may be associated with alterations in the clinical course of type 1 diabetes.

NAMPT is a rate limiting enzyme of nicotinamide adenine dinucleotide (NAD) biosynthesis and exists in two forms with pleiotropic functions: intracellular NAMPT (iNAMPT) and extracellular NAMPT (eNAMPT) [5, 6]. iNAMPT and its product nicotinamide mononucleotide (NMN) potentiate glucose stimulated insulin secretion [7], which could play a role during progression of insulin deficiency in type 1 diabetes. eNAMPT is mainly secreted by leukocytes, but also by hepatocytes and adipocytes [8]. Release of NAMPT by adipocytes was shown to influence whole-body energy metabolism in mouse models of diet-induced obesity and aging [9, 10]. NAMPT could even play a bigger role in the pathogenesis of type 1 diabetes as a signaling mediator between metabolically active organs. eNAMPT may also contribute to high cellular NAD levels despite intracellular energy depletion. Potentially being a pro-inflammatory adipokine, changes in NAMPT serum concentrations in long-term type 1 diabetes patients may be resulting from metabolic sequels in these patients too. Previous studies showed increased [11–13] or decreased NAMPT levels [14] as well as no associations in type 1 diabetes [15].

Omentin-1 may enhance insulin-stimulated glucose transport in adipocytes and modulate protein kinase B (AKT) phosphorylation by insulin, but does not affect the basal adipocyte glucose uptake [16]. Elevated serum concentrations of omentin-1 could potentially indicate a favorable status in type 1 diabetes patients by supporting adipocytes to utilize exogenously supplemented insulin. Previous reports of serum omentin-1 levels among children were inconsistent [17–20]. Interestingly, lowered omentin-1 serum concentrations in obese children with new onset [13] or with longstanding type 1 diabetes [1] were influenced by disease duration, hemoglobin A_{1c} (HbA_{1c}) and BMI as well as postprandially secreted molecules.

Finally, we assume that pediatric patients with type 1 diabetes could be at risk for developing metabolic sequels such as non-alcoholic fatty liver disease (NAFLD) because of their susceptibility to acquire insulin resistance, abnormal lipid and adipokine profiles. CK-18 was found to be a biomarker specifically liberated from apoptotic hepatocytes [21] identifying children with obesity-related NAFLD [22].

Taken together, we hypothesize the following response of the adipokines NAMPT, omentin-1 and CK-18 in pediatric patients with type 1 diabetes: firstly, an up-regulation of eNAMPT may represent a compensatory mechanism especially by adipocytes for recovering insulin deficiency via NMN in order to counteract hyperglycemia [6]. Accordingly, NAMPT could be evaluated as a major factor conveying signals between adipose tissue and pancreatic β-cells or mediating actions of exogenously administered insulin. Secondly, elevated omentin-1 serum concentrations could indicate a metabolically favorable status. Thirdly, elevated CK-18 levels may be an early indicator of a beginning NAFLD caused by chronic systemic low-grade inflammation.

Materials and methods

Design, subjects and materials

We investigated a total of n = 245 patients with type 1 diabetes; 385 blood samplings were performed including follow-up examinations. We compared type 1 diabetes patients to healthy age, sex and BMI matched controls. We also compared type 1 diabetes patients among each other according to different disease durations. Diagnosis of type 1 diabetes was confirmed according to the criteria of the American Diabetes Association [23]. All patients presented with clinical features of type 1 diabetes, additionally showing hyperglycemia and positive testing for auto-antibodies. Diabetic ketoacidosis (defined as blood glucose >11.0 mmol/L and pH <7.30 or bicarbonate <15 mEq/L) was found only in three patients at new onset of the disease [24]. Type 1 diabetes patients were treated at the Hospital for Children and Adolescents of the University of Leipzig. All participants for the healthy control population were recruited from the Leipzig Research Center for Civilization Diseases (LIFE) child study [25, 26]. The matching criteria included: age ± 3 months, sex and BMI ± 0.1 SDS to the nearest decimal.

We investigated adipocytokine serum concentrations in 245 type 1 diabetes patients using 253 blood samples (eight participants were examined at two different examination dates and thus were matched twice independently for each examination date) and compared them to 243 healthy matched controls. The two subgroups of type 1 diabetes patients included: patients at new onset (n = 50) and with longstanding diabetes (n = 203) matched with healthy controls (n = 46 at onset and n = 197 for the longstanding group). The anthropometric and biochemical characteristics of patients and matched controls are shown in Table 1.

Table 1: Demographic characteristics and laboratory parameters of children with type 1 diabetes and their healthy age, sex and BMI-SDS matched controls (mean ± standard deviation [SD]).

Parameters	Onset T1D	Controls	p-Value	Chronic T1D	Controls	p-Value
Sex, F/M	22/28	20/26	0.959	88/115	83/114	0.806
Age, years	9.6 ± 4.6	9.6 ± 4.3	0.980	13.0 ± 3.9	13.2 ± 3.9	0.617
Height-SDS	0.11 ± 1.08	−0.05 ± 1.06	0.468	0.10 ± 1.01	−0.01 ± 1.09	0.280
BMI-SDS	−0.58 ± 1.22	−0.45 ± 1.06	0.593	0.39 ± 0.92	0.41 ± 0.95	0.831
Puberty (Tanner)						
Not reported	24	9		145	46	
1/2/3/4/5	17/5/2/0/2	21/5/4/3/4	0.275	32/4/1/0/21	48/22/16/19/46	0.159
Duration of T1D, years				4.7 ± 3.4		
Blood glucose, mmol/L	23.47 ± 11.01	4.62 ± 0.41	<0.001	9.91 ± 5.99	4.78 ± 0.39	<0.001
Cumulative HbA_{1c}, %	10.87 ± 2.36	5.12 ± 0.33	<0.001	8.16 ± 1.55	5.07 ± 0.31	<0.001
Triglycerides, mmol/L	2.73 ± 2.98	0.70 ± 0.33	<0.001	1.27 ± 1.17	0.84 ± 0.44	<0.001
Total cholesterol, mmol/L	4.68 ± 1.21	4.00 ± 0.63	0.001	4.53 ± 0.89	4.17 ± 0.75	<0.001
HDL cholesterol, mmol/L	1.23 ± 0.37	1.60 ± 0.42	<0.001	1.69 ± 0.39	1.55 ± 0.34	<0.001
LDL cholesterol, mmol/L	2.57 ± 0.91	2.27 ± 0.61	0.067	2.42 ± 0.66	2.43 ± 0.64	0.891
ALT, µkat/L	2.14 ± 8.40	0.31 ± 0.12	0.329	0.29 ± 0.12	0.33 ± 0.16	0.001
AST, µkat/L	1.14 ± 3.27	0.54 ± 0.18	0.413	0.57 ± 1.95	0.4 ± 0.13	0.467
GGT, µkat/L	0.95 ± 2.44	0.20 ± 0.05	0.308	0.25 ± 0.43	0.23 ± 0.10	0.532

T1D, type 1 diabetes mellitus; BMI-SDS, body mass index standard deviation score; HbA_{1c}, hemoglobin A_{1c}; HDL, high density lipoprotein; LDL, low density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase. Cumulative HbA_{1c} [%], mean of the existing HbA_{1c} values in the last examination year. Data were analyzed using Student's t-test.

Type 1 diabetes patients (n = 245, 385 follow-up blood samplings) were divided into four subgroups according to the duration of the disease:

- onset of type 1 diabetes prior to treatment (n = 50)
- 6 months to 5 years after onset (n = 185)
- 5–10 years (n = 98)
- ≥10 years (n = 52).

The anthropometric and biochemical characteristics of these patients are summarized in Table 2.

Exclusion criteria were other subtypes of diabetes, any endocrine disorders or syndromal diseases (trisomy 21, Turner syndrome, etc.).

Written and signed consent was obtained from at least one parent. The studies were approved by the Ethical Committee of the

Table 2: Demographic characteristics and laboratory parameters of children with type 1 diabetes according to their diabetes duration (mean ± standard deviation [SD]).

Parameters	Onset T1D	>6 months	>5 years	>10 years	p-Value
Sex, F/M	22/28	85/100	45/53	13/39	0.048
Age, years	9.6 ± 4.6	11.9 ± 4.1	13.1 ± 3.4	16.4 ± 2.0	<0.001
Height-SDS	0.11 ± 1.08	0.25 ± 1.01	0.18 ± 0.93	−0.28 ± 1.01	0.010
BMI-SDS	−0.58 ± 1.22	0.37 ± 0.91	0.34 ± 1.04	0.14 ± 0.78	<0.001
Puberty (Tanner)					
Not reported	24	127	74	40	
1/2/3/4/5	17/5/2/0/2	42/4/1/0/11	18/1/0/0/5	1/1/0/1/9	<0.001
Duration of T1D, years	0.0	2.8 ± 1.2	6.9 ± 1.4	12.4 ± 1.7	<0.001
Blood glucose, mmol/L	23.47 ± 11.01	9.5 ± 5.4	10.3 ± 6.6	9.8 ± 6.6	<0.001
Cumulative HbA_{1c}, %	10.9 ± 2.35	7.78 ± 1.15	8.42 ± 1.93	8.71 ± 1.43	<0.001
Triglycerides, mmol/L	2.73 ± 2.98	1.16 ± 1.17	1.21 ± 0.68	1.71 ± 1.86	<0.001
Total cholesterol, mmol/L	4.68 ± 1.21	4.46 ± 0.88	4.55 ± 0.77	4.59 ± 0.99	0.507
HDL cholesterol, mmol/L	1.23 ± 0.37	1.72 ± 0.38	1.71 ± 0.44	1.49 ± 0.35	<0.001
LDL cholesterol, mmol/L	2.57 ± 0.91	2.34 ± 0.65	2.44 ± 0.71	2.57 ± 0.82	0.093
ALT, µkat/L	2.14 ± 8.40	0.29 ± 0.11	0.29 ± 0.08	0.31 ± 0.17	0.001
AST, µkat/L	1.69 ± 4.32	0.44 ± 0.11	0.74 ± 2.82	0.44 ± 0.15	0.183
GGT, µkat/L	0.95 ± 2.44	0.27 ± 0.75	0.28 ± 0.62	0.28 ± 0.14	0.032

T1D, type 1 diabetes mellitus; ANOVA, analysis of variance; BMI-SDS, body mass index standard deviation score; HbA_{1c}, hemoglobin A_{1c}; HDL, high density lipoprotein; LDL, low density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase. Cumulative HbA_{1c} [%], mean of the existing HbA_{1c} values in the last examination year. Data were analyzed using the one-way ANOVA test.

University of Leipzig (reference numbers: 035-10-09112009; 264-10-19042010 and 265-10-19042010) and were performed in accordance with the Declaration of Helsinki.

Retrospective data collection

Anthropometric and laboratory data from previous examination and blood analysis were archived by official medical records and CrescNet® [27]. New onset of type 1 diabetes was defined as the day of admission, with presented typical features before the start of insulin therapy. Body mass index standard deviation score (BMI-SDS) [28], stages of puberty according to Tanner [29, 30], blood pressure and duration of diabetes were determined in all patients. Liver and lipid parameters as well as spontaneous plasma glucose levels and HbA_{1c} were measured by standard laboratory methods in the central laboratory of the University Hospital. Cumulative HbA_{1c} is defined as the mean of the existing HbA_{1c} values in the last examination year.

Laboratory methods

After initial measurements all blood samples were stored frozen at −80 °C. Adipokine concentrations were measured among the aforementioned subgroups. Serum concentrations of NAMPT (AdipoGen®, Liestal, Switzerland), omentin-1 (Biovendor®, Brno, Czech Republic) and CK-18 M30 (Peviva Apoptosense®, Sundbyberg, Sweden) were measured using commercially available sandwich enzyme linked-immunosorbent assay (ELISA) kits. For NAMPT, intra-assay coefficients of variation (CV, mean ± SD) were between 2.10 ± 0.99% and 4.54 ± 3.07%; inter-assay CVs were between 18.8% and 8.8%. For omentin-1, intra-assay CVs were between 5.91 ± 2.38% and 3.92 ± 2.57%; inter-assay CVs were between 6.05% and 5.2. For CK-18, M30 intra-assay CVs were between 6.16 ± 6.39% and 5.39 ± 5.25%; inter-assay CVs were between 10.95% and 6.26% for the same concentrations.

Statistical analyses

The statistical analysis was performed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA). Data of descriptive statistics were represented as mean ± SD. Values for NAMPT, omentin-1 and CK-18 were right skewed and therefore log transformed. In the groups of type 1 diabetes patients vs. healthy controls the differences between means were analyzed using Student's t-test on log transformed data. Correlation analyses were performed by Pearson correlation analysis. We also compared the treatment-related data of the three adipocytokines in different groups of type 1 diabetes patients by the one-way analysis of variance (ANOVA) and Tamhane post hoc test. The effects of age, sex, BMI-SDS, blood glucose levels, total cholesterol and liver parameters (alanine aminotransferase [ALT], aspartate aminotransferase [AST]) on NAMPT, omentin-1 and CK-18 were investigated by stepwise multiple regression analysis in patients with type 1 diabetes. A p-value <0.05 was considered statistically significant.

Results

NAMPT, omentin-1 and CK-18 in type 1 diabetes vs. healthy controls

Mean serum concentrations of NAMPT (mean ± SD [ng/mL]) were higher in patients with type 1 diabetes at onset (9.0 ± 17.6) than in healthy controls (2.4 ± 2.3); however, they did not reach statistical significance (p = 0.129; Table 3). In contrast, significantly higher NAMPT serum values were found for patients with long-standing type 1 diabetes (6.6 ± 11.4) compared to healthy

Table 3: Adipocytokine concentrations in children with type 1 diabetes vs. their healthy age, sex and BMI-SDS matched controls (mean ± standard deviation [SD]).

	Onset T1D	Controls	p-Value	Chronic T1D	Controls	p-Value
NAMPT, ng/mL						
Mean ± SD	9.0 ± 17.6	2.4 ± 2.3	0.129	6.6 ± 11.4	1.9 ± 2.3	<0.001
Median	2.2	1.8		3.0	1.4	
Range	68.1	12.2		76.3	20.7	
Variance	310.3	5.4		128.8	5.3	
Omentin-1, ng/mL						
Mean ± SD	468.5 ± 158.7	328.6 ± 116.6	<0.001	367.1 ± 130.6	296.5 ± 94.4	<0.001
Median	452.4	325.8		339.2	290.9	
Range	707.3	644.7		967.6	670.6	
Variance	25190.0	13590.1		17054.6	8877.6	
CK-18, U/L						
Mean ± SD	166.2 ± 280.9	177.6 ± 104.0	0.034	104.2 ± 48.3	153.6 ± 110.5	<0.001
Median	106.8	141.3		82.3	109.3	
Range	1988.6	478.4		321.79	741.9	
Variance	78881.8	10826.0		2331.7	12173.3	

T1D, type 1 diabetes mellitus; BMI-SDS, body mass index standard deviation score; NAMPT, nicotinamide phosphoribosyltransferase; CK-18, caspase-cleaved cytokeratin 18 fragment M30. Data were analyzed using Student's t-test.

controls (1.9 ± 2.3) ($p < 0.001$). The range of NAMPT values among type 1 diabetes patients was more widely distributed compared to the control groups (Figure 1A). In the group of healthy controls, NAMPT data correlated significantly with BMI ($r = 0.231$; $p < 0.001$) and BMI-SDS ($r = 0.230$; $p < 0.001$) (Supplementary Figures 1, 2).

Omentin-1 serum concentrations (mean ± SD [ng/mL]) were significantly higher in the new-onset group (468.5 ± 158.7) than in controls (328.6 ± 116.6) ($p < 0.001$) (Table 3). Longstanding patients also showed significantly elevated omentin-1 serum levels (367.1 ± 130.6) when compared to healthy controls (296.5 ± 94.4) ($p < 0.001$) (Figure 1B). In the type 1 diabetes group omentin-1 values were dependent on gender ($r = -0.165$; $p < 0.01$ with higher concentrations in girls than in boys), BMI ($r = -0.273$; $p < 0.01$), BMI-SDS ($r = -0.374$; $p < 0.01$), blood glucose levels ($r = 0.371$; $p < 0.01$), HbA_{1c} ($r = 0.323$; $p < 0.01$) and γ-glutamyl transferase (GGT) ($r = 0.154$; $p = 0.025$). Healthy controls showed significant associations with BMI ($r = -0190$; $p < 0.01$), BMI-SDS ($r = -0.257$; $p < 0.01$), stages of puberty (−0.160; $p = 0.029$), blood glucose levels ($r = 0.144$; $p = 0.031$), triglycerides ($r = -0.144$; $p = 0.030$), high density lipoprotein (HDL) cholesterol ($r = 0.230$; $p < 0.01$) and AST ($r = 0.161$; $p = 0.015$) (Supplementary Figures 1, 2).

CK-18 serum concentrations (mean ± SD [U/L]) were significantly lower in the type 1 diabetes onset group (166.2 ± 280.9) than in healthy controls (177.6 ± 104.0) ($p = 0.034$) (Table 3). There was a wide range of CK-18 values among patients at type 1 diabetes onset (75.0–2063.7). For the longstanding type 1 diabetes group (104.2 ± 48.3) we also showed significantly lower CK-18 serum levels compared to healthy controls (153.6 ± 110.5) ($p < 0.001$) (Figure 1C). Among the type 1 diabetes population, CK-18 levels were significantly correlated with BMI-SDS ($r = -0.145$; $p = 0.021$), blood glucose ($r = 0.244$; $p < 0.01$), HbA_{1c} ($r = 0.215$; $p < 0.01$), duration of diabetes ($r = -0.200$; $p < 0.01$), triglycerides ($r = 0.221$; $p < 0.01$) and HDL cholesterol ($r = -0,147$; $p = 0.019$). Even stronger correlations of CK-18 were observed with ALT ($r = 0.502$; $p < 0.01$), AST ($r = 0.302$; $p < 0.01$) and GGT ($r = 0.451$; $p < 0.01$), whereas neither ALT nor AST or GGT were significantly elevated in type 1 diabetes patients. ALT was the only parameter which was significantly lower in the type 1 diabetes population in concordance with lower CK-18 levels. However, among healthy subjects CK-18 levels significantly correlated with total cholesterol ($r = 0.154$; $p = 0.020$), low density lipoprotein (LDL) cholesterol ($r = 0.180$; $p < 0.01$) and ALT ($r = 0.148$; $p = 0.028$) (Supplementary Figures 1, 2).

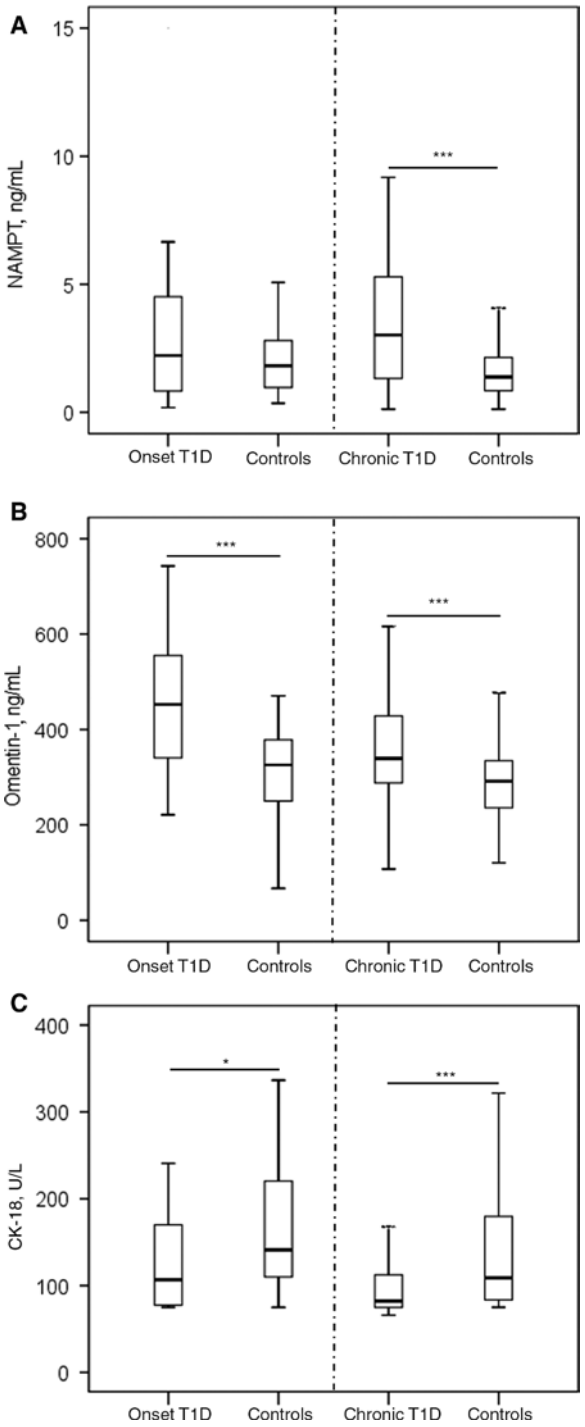

Figure 1: The boxes represent median, 1st and 3rd quartiles of NAMPT (A), omentin-1 (B) and CK-18 (C).
Whiskers represent 1.5× inter-quartile range. Outliers are not shown. NAMPT (A), omentin-1 (B) and CK-18 (C) serum levels of type 1 diabetes patients were compared to healthy age, sex and BMI-SDS matched controls. Onset T1D, patients with new onset of type 1 diabetes (n = 50); controls, healthy controls of onset type 1 diabetes patients (n = 46); chronic T1D, chronic patients with longstanding type 1 diabetes (n = 203); controls, healthy controls of longstanding type 1 diabetes patients (n = 197). Statistical significant differences by Student's t-test were shown as ***$p < 0.001$; *$p < 0.005$.

Table 4: Adipocytokine concentrations in children with type 1 diabetes according to disease duration (mean ± standard deviation [SD]).

	Onset T1D	>6 months	>5 years	>10 years	p-Value
NAMPT, ng/mL					
Mean ± SD	9.0 ± 17.6	6.7 ± 12.0	6.8 ± 13.7	5.7 ± 11.7	0.546
Median	2.2	2.9	2.7	1.9	
Range	68.1	76.3	115.4	70.7	
Variance	310.3	144.4	187.8	136.5	
Omentin-1, ng/mL					
Mean ± SD	468.5 ± 158.7	357.4 ± 128.6	379.0 ± 110.4	377.0 ± 168.5	<0.001
Median	452.4	328.8	359.1	326.0	
Range	707.3	967.6	653.2	708.8	
Variance	25190.0	16531.2	12186.4	28377.8	
CK-18, U/L					
Mean ± SD	166.2 ± 280.9	105.1 ± 46.3	105.5 ± 48.7	103.1 ± 71.0	0.001
Median	106.8	84.2	85.6	79.4	
Range	1988.6	285.0	312.7	368.0	
Variance	78881.8	2139.5	2368.3	5046.2	

T1D, type 1 diabetes mellitus; ANOVA, analysis of variance; NAMPT, nicotinamide phosphoribosyltransferase; CK-18, caspase-cleaved cytokeratin 18 fragment M30. Data were analyzed using One-way ANOVA and Tamhane post hoc tests.

Analysis of insulin replacement therapy

Results of anthropometric and descriptive data of patients with type 1 diabetes stratified according to the duration of their disease are summarized in Tables 2 and 4.

Serum NAMPT concentrations among type 1 diabetes patients were higher at onset and decreased with treatment. However, NAMPT concentrations remained statistically unimproved during insulin treatment at any time ($p = 0.546$) (Figure 2A). Multiple stepwise regression analysis demonstrated that gender, BMI-SDS, blood glucose, total cholesterol and ALT levels had no independent significant predictive value for NAMPT. In contrast, age and AST were significantly predictive (Table 5).

Omentin-1 concentrations at onset were significantly higher than values at the remaining three time-points of treatment ($p < 0.001$) (Figure 2B). The latter three groups demonstrated no differences when compared to each other. Our data suggests that treatment over 10 years does not lead to a substantial shift of the mean value into the range of controls (Figures 1B and 2B). Multiple stepwise regression analysis revealed a significant influence of blood glucose levels and BMI-SDS on omentin-1 values (Table 5). Additionally, this analysis confirms the positive correlations of omentin-1 with blood glucose, HbA_{1c} and BMI-SDS found in type 1 diabetes patients vs. healthy controls (Supplementary Figures 1, 2).

The highest levels of serum CK-18 were shown in matched healthy controls (Table 3, Figure 1C). However, among pediatric type 1 diabetes patients the highest concentrations of serum CK-18 were measured at new onset of the disease. CK-18 values decreased with increasing duration of treatment (Figure 2C). Significantly elevated CK-18 concentrations were observed only in patients at onset when compared to patients with a treatment time ≥10 years ($p = 0.025$). No further differences between the groups were detected. Our model of multiple stepwise regression analysis for CK-18 described 18.3% of the total variance ($R^2 = 0.183$). It revealed that only ALT showed a significant relationship with CK-18. Unexpectedly, the correlation of blood glucose levels with CK-18 did not reach significance (Table 5).

Discussion

The acute metabolic decompensation at new onset of type 1 diabetes did not lead to any significant changes in serum NAMPT levels in this pediatric cohort. However, patients with longstanding type 1 diabetes had significantly higher NAMPT levels when compared with controls, suggesting that NAMPT levels were not a marker for acute inflammation in type 1 diabetes but a marker for chronic changes. Additionally, we demonstrated that NAMPT serum concentrations were not substantially influenced by insulin replacement therapy in type 1 diabetes patients.

NAMPT is important for the regulation of glucose metabolism especially in adipose tissue [31]. However, we did not find any results implying a relationship between serum concentrations of NAMPT and either glucose levels and HbA_{1c} or lipid parameters. We failed to show

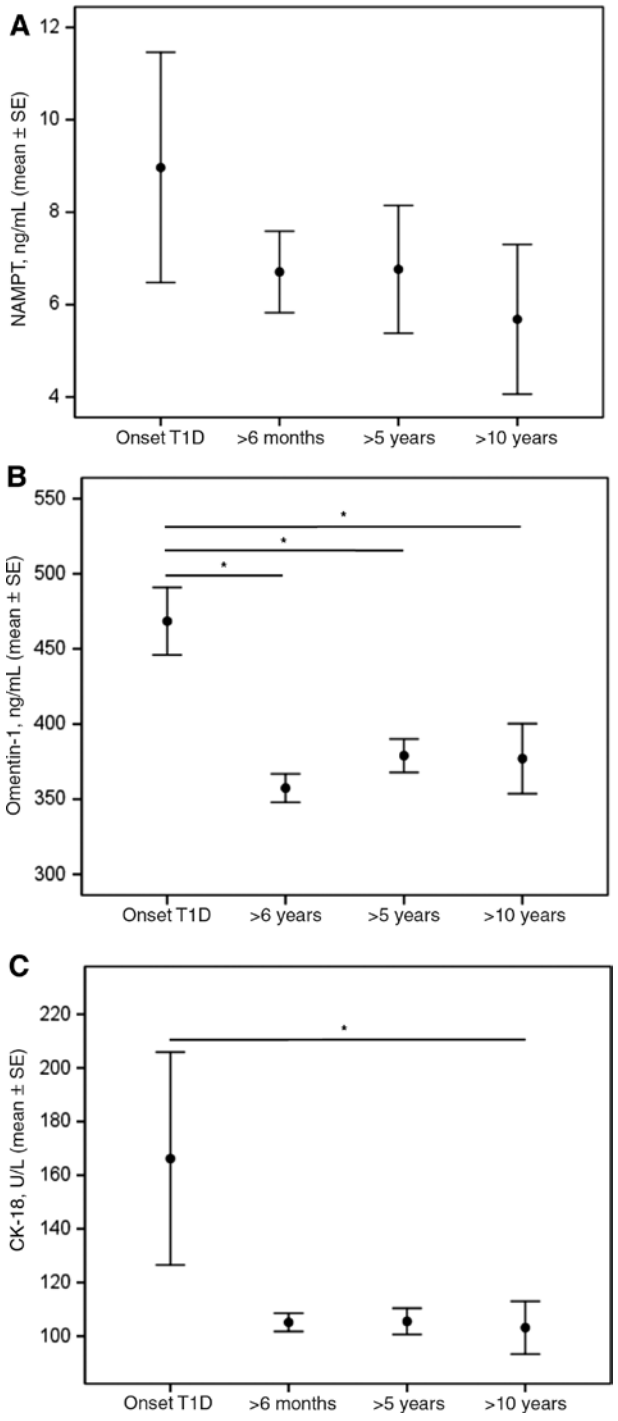

Figure 2: Mean and standard error for levels of NAMPT (A), omentin-1 (B) and CK-18 (C) from pediatric patients with new onset of type 1 diabetes; diabetes duration ≥6 months and <5 years; diabetes duration ≥5 years and <10 years; diabetes duration ≥10 years. Statistical significant differences by ANOVA and Tamhane post hoc tests were shown as *$p < 0.05$.

an up-regulation of NAMPT due to insulin depletion to restore hyperglycemia in type 1 diabetes. Instead, elevated NAMPT levels were found to be associated with adiposity as shown in the correlation with BMI-SDS in the population of healthy controls. This suggests a link to adiposity-related inflammation in islet cells, which has been proven in rodent models before [32]. Chronic fructose fed mice showed islet dysfunction accompanied by lowered secretion of eNAMPT leading to increased islet inflammation and impaired β-cell function [32]. However, a previous study reported elevated NAMPT concentrations in children with acute infectious diseases and in acute relapse of chronic inflammatory diseases, but not in stable conditions or states of low-grade inflammations such as obesity [33]. Serum concentrations of NAMPT correlated positively with inflammatory markers such as C-reactive protein (CRP) and leukocyte count, especially the neutrophil count [33]. Therefore, we conclude that elevated NAMPT concentrations in long-term type 1 diabetes patients may indicate an inflammatory state with chronic activity in our patient cohort [33]. Unfortunately, leukocyte counts or CRP levels were not examined to prove this assumption in this study.

Furthermore, the positive associations of NAMPT concentrations with AST in our longitudinal approach may suggest a higher release of enzymatically active NAMPT dimer from dysfunctional hepatocytes [34].

Previous studies investigating NAMPT serum levels in type 1 diabetes patients showed inconsistent results. Increased levels of NAMPT were shown in only 18 obese but not in 32 lean type 1 diabetes children at onset [13]. However, this study did not compare NAMPT levels of type 1 diabetes with those of healthy subjects, nor with longstanding type 1 diabetes patients. In addition, all measurements were done at a median of 7 weeks after diagnosis. Therefore, the information provided by this study is not applicable to our results because we examined serum concentrations prior to treatment and in established type 1 diabetes patients with disease durations >6 months. In adult patients with longstanding type 1 diabetes, circulating NAMPT serum levels were also found to be increased [12, 35]. NAMPT concentrations were not associated with HbA_{1c} representing glycemic control [12] which supports our available data of longstanding pediatric type 1 diabetes patients. Elevated NAMPT levels in type 1 diabetes adults were reduced after successful pancreas-kidney transplantation to levels comparable with non-diabetic healthy controls [35]. In our study, NAMPT serum concentrations did not completely normalize to levels seen in healthy participants after the start of insulin therapy, suggesting a chronic abnormal metabolic state. In another study of adult type 1 diabetes patients, circulating NAMPT levels tended to be even lower than in healthy controls

Table 5: Description of the results from stepwise multiple linear regression analysis of adipocytokine serum concentrations in children and adolescents with type 1 diabetes.

	Unstandardized coefficients		Standardized coefficients	t	Sig.
	B	Std. error	β		
Models for NAMPT					
1. (Constant)	0.400	0.033		12.292	<0.001
AST, µkat/L	0.049	0.018	0.150	2.735	0.007
					$R^2 = 0.023$
2. (Constant)	0.203	0.100		2.036	0.043
AST, µkat/L	0.048	0.018	0.148	2.707	0.007
Age, years	0.015	0.007	0.114	2.074	0.039
					$R^2 = 0.036$
Models for omentin-1					
1. (Constant)	2.479	0.014		172.697	<0.001
Blood glucose, mmol/L	0.007	0.001	0.315	5.960	<0.001
					$R^2 = 0.099$
2. (Constant)	2.497	0.015		172.148	<0.001
Blood glucose, mmol/L	0.006	0.001	0.279	5.373	<0.001
BMI-SDS	−0.035	0.008	−0.233	−4.492	<0.001
					$R^2 = 0.152$
Models for CK-18					
1. (Constant)	1.985	0.009		224.335	<0.001
ALT, µkat/L	0.035	0.004	0.427	8.492	<0.001
					$R^2 = 0.183$

NAMPT, nicotinamide phosphoribosyltransferase; AST, aspartate aminotransferase; BMI-SDS, body mass index standard deviation score; ALT, alanine aminotransferase. In the upper part of the table the parameters of the regression equation for predicting the dependent variables NAMPT, omentin-1 and CK-18 in each model are given. R^2, coefficient of determination. In the lower part of the table R^2 is given.

independent of acute hyperglycemia, exogenous insulin administration and metabolic control [15]. Moreover, significantly lower NAMPT levels in adult type 1 diabetes patients (n = 48) demonstrated an association with glycemic control reflected by HbA_{1c} [14], which could not be directly attributed to inflammatory features of NAMPT. Nevertheless, NAMPT did not appear to be an indicator of metabolic decompensation but rather a biomarker of chronic inflammation in type 1 diabetes. Besides, the use of different immunoassays needs to be considered when conflicting results of NAMPT serum concentrations have to be interpreted [36].

Omentin-1 has been previously described as having insulin-sensitizing and anti-inflammatory properties [16, 37]. Our results were in line with these assumptions as both acute and chronic hyperglycemia led to increased omentin-1 levels in type 1 diabetes children, probably to compensate for an endogenous lack in insulin production. Additionally, our data of healthy controls indicate negative associations of omentin-1 serum concentrations and the stages of puberty. Similar observations could not be stated in the type 1 diabetes patient cohort. A possible explanation for this may be the higher number of missing information for pubertal stages among type 1 diabetes patients. Interestingly, BMI-SDS was negatively correlated with omentin-1 levels in patients with type 1 diabetes and in healthy children. Furthermore, regression analysis showed a significantly positive association with blood glucose and a significantly negative association with BMI-SDS of longstanding type 1 diabetes patients. These associations were confirmed by higher omentin-1 levels in anorexic girls compared to healthy and obese participants [38] and lower serum omentin-1 levels in obese children compared to normal weight children [17]. An up-regulation of omentin-1 might be a compensatory mechanism for intracellular energy depletion caused by lowered glucose effects.

However, in pediatric type 1 diabetes patients, omentin-1 concentrations were found to be lower at onset and after treatment (n = 46) [1], whereas Redondo et al. showed only lower levels in obese (n = 18) but not in lean (n = 32) patients at onset [13]. Dayem et al. reported lower omentin-1 concentrations in patients with longstanding type 1 diabetes (n = 62) than in controls [39]. In contrast to our findings no correlation was detectable between concentrations of omentin-1 and sex, BMI, HbA_{1c}, triglycerides as well as HDL cholesterol [39]. It may be that the smaller sample size was the cause for the disagreement. Our data

support the hypothesis that omentin-1 is an adipokine with insulin-sensitizing, anti-inflammatory and probably protective features. However, as omentin-1 receptors and signaling pathways were not yet discovered, the final clarification of the role of omentin-1 in glucose and energy homeostasis remains open.

This study failed to demonstrate an elevation of serum CK-18 concentrations in pediatric type 1 diabetes patients as a sign of hepatic impairment or a beginning NAFLD caused by chronic systemic low-grade inflammation, although recent studies have proven that serum levels of CK-18 in children correlate with the severity of the liver damage caused by NAFLD [22, 40, 41]. When compared to healthy age, sex and BMI-SDS matched controls our results revealed lower CK-18 levels in the pediatric type 1 diabetes cohort both at new onset and during long-term insulin treatment. A potential explanation for these contrasting findings may be found in the design of our study. The matching of our patients for BMI-SDS eliminated differences in adipose tissue mass between cases and controls. Due to lower levels of CK-18 in pediatric type 1 diabetes patients the presence of NAFLD seems unlikely. We showed positive significant correlations of CK-18 values in our type 1 diabetes cohort with blood glucose levels, HbA_{1c} and negative correlations with disease duration as well as with BMI-SDS. Hence, a potential effect of type 1 diabetes on CK-18 levels appears to be probable. Previous studies have proven that CK-18, together with CK-8, is one of the main keratin types in pancreatic islet cells [42]. Rodent models even suggest a role for CK-8/CK-18 intermediate filament dependent interplay between insulin signaling, glucose homeostasis and liver cirrhosis [42, 43]. Our significant positive correlations of CK-18 levels with ALT are in line with previously reported results [44]. The fact that CK-18 levels at onset of type 1 diabetes were significantly higher than those of patients with a disease duration ≥10 years indicates a potential improvement of liver function in this cohort. This hypothesis is supported by previous results of decreased CK-18 levels in pediatric patients with improved liver histologies [44]. The question whether elevated CK-18 levels at onset of type 1 diabetes could be caused by apoptotic β cells still remains unanswered.

To the best of our best knowledge, our study is the first one reporting on CK-18 serum levels in pediatric type 1 diabetes patients. We have to take into account that the average diabetes duration of our patients was only 4.75 ± 3.94 years. Accordingly, metabolic sequels of type 1 diabetes such as steatohepatitis and NAFLD with excessive lipid accumulation in the liver might not be manifest yet explaining the low serum levels of CK-18. Moreover, CK-18 is primarily an apoptosis marker of hepatocytes without any specificity to underlying causes of liver cell damage.

Our study has some limitations: (1) we did not measure pro-inflammatory markers such as the number of white blood cells or the serum concentrations of CRP. CRP was shown to be associated with NAMPT serum concentrations, while white blood cells are major producers of eNAMPT. (2) With the present results we are not able to make reliable statements about causative effects of the three adipokines. (3) Additionally, there might be genetic confounders like familial obesity, familial metabolic diseases or environmental factors such as the socioeconomic circumstances of our subjects, which have been proven to influence obesity with consecutive metabolic changes. (4) Our study concept of type 1 diabetes patients lacks true longitudinal data for practical reasons. However, the high number of subjects with different disease durations supports our conclusions concerning the tendencies in adipokine serum concentrations in dependence of disease duration.

In conclusion, our data suggest that in children with type 1 diabetes, NAMPT could be a marker of chronic inflammation. Increased omentin-1 values are associated with hyperglycemia and therefore could be viewed as a marker for disturbed glucose metabolism. Finally, lower CK-18 serum concentrations may exclude relevant liver cell damage especially in terms of NAFLD in these patients.

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