RESEARCH

Depression in type 1 diabetes was associated with high levels of circulating galectin-3

Eva Olga Melin[1,2,3], **Jonatan Dereke**[4], **Maria Thunander**[1,2,5] and **Magnus Hillman**[4]

[1]Department of Clinical Sciences, Endocrinology and Diabetes, Lund University, Faculty of Medicine, Lund, Sweden
[2]Department of Research and Development, Region Kronoberg, Växjö, Sweden
[3]Region Kronoberg, Primary Care, Växjö, Sweden
[4]Department of Clinical Sciences Lund, Lund University, Faculty of Medicine, Diabetes Research Laboratory, Lund, Sweden
[5]Department of Internal Medicine, Endocrinology and Diabetes, Central Hospital, Växjö, Sweden

Correspondence should be addressed to E O Melin: eva.melin@kronoberg.se

Abstract

Objective: Neuroinflammatory responses are implicated in depression. The aim was to explore whether depression in patients with type 1 diabetes (T1D) was associated with high circulating galectin-3, controlling for metabolic variables, s-creatinine, life style factors, medication and cardiovascular complications.
Design: Cross-sectional.
Methods: Participants were T1D patients ($n = 283$, 56% men, age 18–59 years, diabetes duration ≥1 year). Depression was assessed by Hospital Anxiety and Depression Scale-depression subscale. Blood samples, anthropometrics and blood pressure were collected, and supplemented with data from medical records and the Swedish National Diabetes Registry. Galectin-3 ≥2.562 µg/l, corresponding to the 85th percentile, was defined as high galectin-3.
Results: Median (quartile$_1$, quartile$_3$) galectin-3 (µg/l) was 1.3 (0.8, 2.9) for the 30 depressed patients, and 0.9 (0.5, 1.6) for the 253 non-depressed, $P = 0.009$. Depression was associated with high galectin-3 in all the 283 patients (adjusted odds ratio (AOR) 3.5), in the 161 men (AOR 3.4), and in the 122 women (AOR 3.9). HbA1c, s-lipids, s-creatinine, blood pressure, obesity, smoking, physical inactivity, cardiovascular complications and drugs (antihypertensive, lipid lowering, oral antidiabetic drugs and antidepressants) were not associated with high galectin-3.
Conclusions: This is the first study to show an association between depression and galectin-3. Depression was the only explored parameter associated with high circulating galectin-3 levels in 283 T1D patients. High galectin-3 levels might contribute to the increased risk for Alzheimer's disease, cardiovascular and all-cause mortality observed in persons with depression. Potentially, in the future, treatment targeting galactin-3 might improve the prognosis for patients with high galectin-3 levels.

Key Words
- biomarker
- depression
- cardiovascular complications
- galectin-3
- neuroinflammation
- type 1 diabetes

Endocrine Connections (2018) **7**, 819–828

Introduction

Depression is associated with type 1 diabetes (T1D), Alzheimer's disease, increased coronary heart disease and both coronary and all-cause mortality (1, 2, 3). T1D has a serious impact on mental health, indicated by the more than doubled relative risk rate for suicide in persons with T1D (4). Several biological links between depression and somatic disorders have been demonstrated including metabolic changes, dysregulation of the hypothalamic–pituitary–adrenal axis and activation of the innate immune system causing systemic inflammation (2, 3, 5, 6, 7).

http://www.endocrineconnections.org
https://doi.org/10.1530/EC-18-0108

We have previously shown in these patients with T1D that depression was associated with inadequate glycaemic control (8), and with high midnight salivary cortisol secretion (9, 10), but not with obesity (11).

Galectin-3 is a soluble β-galactoside binding lectin, which is expressed in several cells such as activated macrophages, microglia, mast cells and in tissues such as subsets of neurons in the brain, the heart, the epithelium of the gastrointestinal and respiratory tracts, and the kidney (12, 13, 14). Galectin-3 is implicated in a variety of biological processes including immune activation, fibrosis, angiogenesis and neurodegeneration (12, 14, 15, 16, 17, 18).

Higher circulating galectin-3 concentrations have been associated with heart failure independent of aetiology, and with increased cardiovascular and all-cause mortality in the general population (14, 19, 20, 21). Galectin-3 has pleiotropic effects in CNS inflammation, combining pro-inflammatory roles with remodelling capacity in damaged CNS tissues (16). Experimental studies have shown that galectin-3 contributes to microglial activation and prolonged inflammatory responses in the brain (17). In Alzheimer's disease, increased levels of serum galectin-3 have been demonstrated (15). Significant associations have previously been found between galectin-3 and age, sex, type 2 diabetes mellitus, hypertension, hypercholesterolemia, triglycerides, LDL, BMI, renal dysfunction and smoking (19, 21, 22, 23). Metformin in type 2 diabetes mellitus has, however, been linked to lower galectin-3 levels (22). Even though the main focus of galectin-3 has been on its function as a proinflammatory mediator, there are also studies showing galectin-3 acting as a decoy to advanced glycation end-products which could indicate some kind of protective function in patients with diabetes (24).

Our hypothesis was that depression is associated with high galectin-3 levels in patients with T1D as neuroinflammatory processes are implicated in depression (2, 3, 5, 7), and since both galectin-3 and depression have been linked to Alzheimer's disease, as well as to increased cardiovascular and all-cause mortality (1, 2, 14, 17, 19, 20, 21). To our knowledge, galectin-3 has not previously been explored in depressed patients or in patients with T1D. The aim was to explore whether depression in patients with T1D was associated with galectin-3, and control for metabolic variables, s-creatinine, medication and cardiovascular complications. This knowledge could add to our current understanding of depression in T1D and prepare for better diagnostic or treatment strategies in the future.

Subjects and methods

This study has a cross-sectional design and is one of five baseline analyses (8, 9, 10, 11) for a randomized controlled trial (ClinicalTrials.gov: NCT01714986) where 'Affect School with Script Analysis' was tried against 'Basic Body Awareness Therapy' for persons with diabetes, inadequate glycaemic control and psychological symptoms (25, 26). The patients who attend the clinic every 6 months for regular follow-up visits were consecutively recruited by specialist diabetes physicians or diabetes nurses during a 9-month period, 25 March 2009 to 28 December 2009, from one hospital diabetes outpatient clinic. The catchment population was 125,000 in southern Sweden. Inclusion criteria were T1D with ≥1-year duration, in patients of 18–59 years of age. Exclusion criteria were cancer, hepatic failure, end-stage renal disease, psychotic disorder, bipolar disorder, severe personality disorder, severe substance abuse, cognitive deficiency (due to stroke, dementia or mental retardation) or inadequate knowledge of Swedish. In this study, 283 persons with T1D were included, 65% of the eligible patients (Fig. 1). A questionnaire was used to assess self-reported depression. Blood and saliva samples, anthropometrics and blood pressure were collected and supplemented with data from medical records and the Swedish National Diabetes Registry (S-NDR) (27, 28). In case of missing values, galectin-3 levels were compared between patients with and without the missing values. The study complies with the declaration of Helsinki and was approved by the Regional Ethical Review Board of Linköping University, Linköping, Sweden (Registration no. M120-07, T89-08). All participants provided written informed consent.

Self-reported depression

Self-reported depression was assessed by the Hospital Anxiety and Depression Scale-Depression subscale (HADS-D) which consists of 7 statements, with 4 response alternatives from 0 to 3. The cut-off level ≥8 points was used as recommended by the constructors of the test (29). HADS-D is a useful instrument for detecting symptoms of depression, both at an individual and a collective level, and has been demonstrated to have a good reliability and discriminant validity (30). Imputation of a missing value was performed in one case.

Blood samples

Galectin-3 was analysed using a commercial human DuoSet ELISA and supplementary ancillary kit

http://www.endocrineconnections.org
https://doi.org/10.1530/EC-18-0108

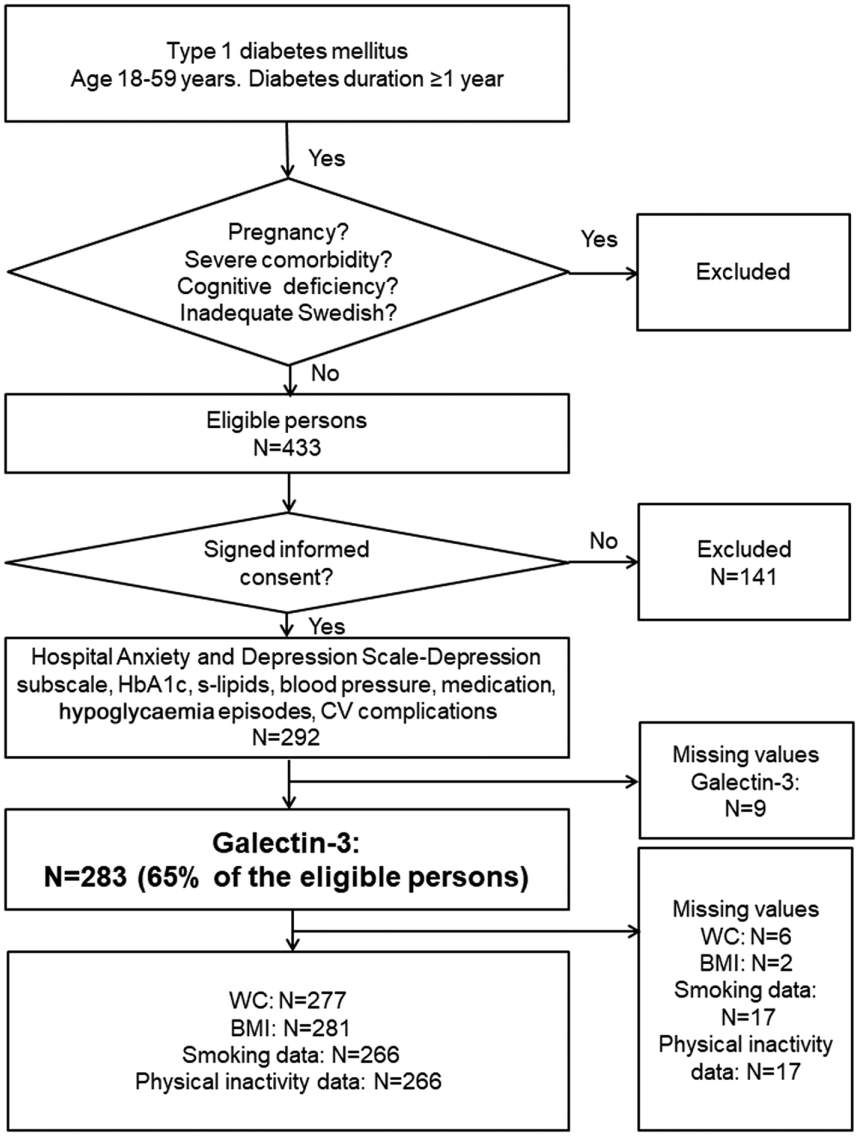

Figure 1
Flowchart describing the 283 included participants, included variables and numbers of missing values.

(R&D Systems). Patient plasma samples were diluted 1:2 in PBS supplemented with 1% BSA and run in duplicates. The ELISA analysis was performed according to the manufacturer's instructions. Absorbance was measured at 450–580 nm in a FLUOstar optima plate reader (BMG Labtech Gmbh, Ortenberg, Germany). Concentrations of unknown samples were calculated using a four-parameter logistic regression curve. The intra-assay coefficient of variation for the analysis was 4.3%.

Galectin-3 was available for 283 patients (65% of the eligible patients) (Fig. 1). Galectin-3 (µg/l) was dichotomized, and cut-off levels were determined for the 75th, the 80th, the 85th and the 90th percentiles. Galectin-3 ≥2.562 µg/l, corresponding to the 85th percentile, was defined as high galectin-3, and was used in the further statistical analyses.

The 283 patients with galectin-3 measurements were compared to the 9 patients without galectin-3 measurements. There were no differences for the prevalence of self-reported depression, abdominal obesity, general obesity, physical inactivity, smoking, cardiovascular complications, antidepressants, oral antidiabetic drugs (OADs) in addition to insulin, lipid lowering drugs (LLDs) or antihypertensive drugs (AHD); or for the medians of HbA1c, s-lipids, creatinine, systolic or diastolic blood pressure, age or diabetes duration (all *P* values ≥0.09).

Venous HbA1c (mmol/mol (%)) was analysed with HPLC – variant II, Turbo analyser (Bio-Rad). High HbA1c levels were defined as HbA1c >70 mmol/mol (>8.6%) (8).

Total cholesterol (TC), triglycerides, LDL and HDL (mmol/l) were collected after an overnight fast, and

http://www.endocrineconnections.org
https://doi.org/10.1530/EC-18-0108

measured directly (31), using the enzymatic colour test (Olympus AU®, Tokyo, Japan).

Creatinine (μmol/l) was assayed by an AU2700® instrument (Beckman Coulter, Brea, CA, USA).

Anthropometrics and blood pressure

Waist circumference (WC), weight, length and blood pressure were measured according to standard procedures by a nurse. Abdominal obesity was defined as WC ≥1.02 m for men and as WC ≥0.88 m for women. General obesity was defined as BMI ≥30 kg/m^2 for both genders (32). Galectin-3 levels did not differ between the 6 patients without and the 277 patients with WC measurements (P=0.67), and did not differ between the two patients without and the 281 patients with BMI values (P=0.11).

Episodes of hypoglycaemia

A severe episode of hypoglycaemia was defined as needing help from another person. Episodes during the last 6 months prior to recruitment were registered.

Smoking and physical inactivity

Smokers were defined as having smoked any amount of tobacco during the last year.

Physical inactivity was defined as moderate activities, such as 30 min of walking, less than once a week. There were 17 missing values both for smoking and for physical inactivity. Galectin-3 levels did not differ between the 17 without data and the 266 with data, neither for smoking (P=0.72) nor for physical inactivity (P=0.87).

Cardiovascular complications

Cardiovascular complications were defined as ischaemic heart disease, cardiac failure, stroke or transient ischemic attack.

Medication

Antidepressants were selective serotonin reuptake inhibitors (SSRIs), selective norepinephrine reuptake inhibitors (SNRIs) and/or specific serotonergic antidepressants (N06AB, N06AX16, N06AX11).

Diabetes specific treatment was divided into two groups: Insulin only (multiple daily insulin injections or continuous subcutaneous insulin infusion), or insulin combined with OAD (ATC code A10BA02). The indications for OAD prescription in addition to insulin were obesity or insulin resistance.

LLD were HMG CoA reductase inhibitors (statins) (C10AA). Indications for LLD were TC >4.5 mmol/l (>1.74 mg/dl) and/or LDL >2.5 mmol/l (>97 mg/dl) according to the Swedish national guidelines in 2009 (33).

AHD were calcium antagonists (ATC codes C08CA01-02); ACE inhibitors (ATC codes C09AA-BA); angiotensin II antagonists (ATC codes C09CA-DA); diuretics (ATC code C03A); and/or selective beta-adrenoreceptor antagonists (ATC code C07AB). Indications for AHD were systolic blood pressure >130 mmHg and/or diastolic blood pressure >80 mmHg according to the Swedish national guidelines in 2009 (33).

Statistical analysis

Analysis of data distribution using histograms revealed that age, diabetes duration, galectin-3 and triglycerides were not normally distributed. Data were presented as median values (quartile (q_1, q_3)), and analyses were performed with Mann–Whitney U test. Fisher's exact tTest (two-tailed) was used to analyse categorical data. In order to establish a cut-off level for galectin-3, the 75th, 80th, 85th and 90th percentiles of galectin-3 were entered into a backward elimination multiple logistic regression analysis with self-reported depression as the dependent variable. Crude odds ratios (CORs) for the associations with galectin-3 ≥2.562 μg/l (corresponding to the 85th percentile) were calculated. Variables with $P \leq 0.10$, and gender and age independent of P value, were entered into multiple logistic regression analyses (Backward: Wald). The Hosmer and Lemeshow test for goodness-of-fit and Nagelkerke R^2 were used to evaluate each multiple logistic regression analysis model. CIs of 95% were used. Imputation for a missing value in the HADS-D scale was performed using multinomial regression on the other variables in the scale in one case. $P<0.05$ was considered statistically significant. SPSS version 18 (IBM) was used.

Results

Median (q_1, q_3) galectin-3 (μg/l) levels were for all (n=283) 0.9 (0.6, 1.7); for the men (n=161) 1.0 (0.6, 2.0), and for the women (n=122) 0.9 (0.5, 1.4), P=0.15 for gender difference.

In Table 1, comparisons are performed between the 30 depressed and the 253 non-depressed patients. Median galectin-3 levels were significantly higher for

http://www.endocrineconnections.org
https://doi.org/10.1530/EC-18-0108

Table 1 Comparisons between 30 depressed and 253 non-depressed patients with T1D.

	Depression	No depression	*P* value[a]
n	30 (11)	253 (89)	
Gender			
Men	16 (10)	145 (90)	0.70
Women	14 (12)	108 (88)	
Galectin-3			
All	1.3 (0.8, 2.9)	0.9 (0.5, 1.6)	0.009[b]
Men	1.8 (0.9, 3.7)	0.9 (0.6, 1.9)	0.021[b]
Women	1.0 (0.8, 2.7)	0.9 (0.5, 1.4)	0.18[b]
Cardiovascular complications	4 (40)	6 (60)	0.014
Antidepressants	10 (44)	13 (56)	<0.001

Data are presented as *n* (%) or median (q_1, q_3).
[a]Fisher's exact test unless otherwise indicated; [b]Mann–Whitney *U* test.

the depressed patients compared to the non-depressed, $P=0.009$. The prevalence of cardiovascular complications was higher in the depressed patients than in the non-depressed, $P=0.014$.

In Table 2, the prevalence of depression is presented for four different galectin-3 cut-off levels, corresponding to the 75th, the 80th, the 85th and the 90th percentiles of galectin-3. The *P* values were significant for the 85th percentile ($P=0.006$), and for the 80th percentile ($P=0.026$). The association between depression and the 85th percentile of galectin-3 was significant (AOR 3.5 (CI 1.5-8.0), $P=0.004$), but not between depression and the following percentiles of galectin-3: the 75th (AOR 0.8 (CI 0.2-3.5), $P=0.73$), the 80th (AOR 1.2 (CI 0.1-20.3), $P=0.92$) or the 90th percentile (AOR 0.7 (CI 0.2-2.3), $P=0.61$).

In Table 3, baseline characteristics for all the 283 T1D patients, and comparisons between the 42 patients with galectin-3 levels ≥2.562 µg/l and the 241 patients with galectin-3 levels <2.562 µg/l are presented. Patients with galectin-3 ≥2.562 µg/l had a higher prevalence of cardiovascular complications ($P=0.045$).

In Table 4, associations with high galectin-3 (≥2.562 µg/l) are presented. Depression was associated with high galectin-3 in all the 283 patients (AOR 3.5), in the 161 men (AOR 3.4), and in the 122 women (AOR 3.9). Cardiovascular complications were only associated with high galectin-3 in simple logistic regression (COR 4.1), but not when age, depression and gender were included in the analysis.

In all the 283 patients, the association between cardiovascular complications and depression was significant: COR (CI), 6.6 (1.7-24.8), $P=0.005$; and between cardiovascular complications and age: COR (CI), 1.18 (1.06-1.31), $P=0.003$; but not between cardiovascular complications and gender, $P=0.77$.

Discussion

The main findings were that self-reported depression was associated with high galectin-3 (≥2.562 µg/l) for all, and for each gender analysed separately. There were no associations between high galectin-3 and the following explored variables: HbA1c, s-lipids, blood pressure, obesity, s-creatinine, drugs (antidepressants, OADs, LLDs or AHDs) or cardiovascular complications. There was a strong association between depression and cardiovascular complications.

The association between depression and high galectin-3 in patients with T1D is a new finding. To our knowledge, galectin-3 has neither been explored in patients with T1D previously, nor has the association between galectin-3 and depression been explored. The results supported our hypothesis that the depressed patients with T1D had higher galectin-3 levels than the non-depressed patients. Associations between high galectin-3 and potential diabetes-related confounders such as HbA1c, obesity, s-lipids, blood pressure, severe hypoglycemia, s-creatinine, smoking, physical inactivity, cardiovascular complications and drugs (antihypertensive, lipid lowering, oral antidiabetic and antidepressants) were systematically explored. Gender subanalyses were performed. Inclusion and exclusion criteria were

Table 2 The prevalence of depression calculated for four galectin-3 cut-off levels, corresponding to the 75th, 80th, 85th and 90th percentiles.

	Galectin-3 (µg/L)												
	Range	75th percentile			80th percentile			85th percentile			90th percentile		
	0.001–100.0	<1.681	≥1.681	*P* value	<2.102	≥2.102	*P* value	<2.562	≥2.562	*P* value	<3.272	≥3.272	*P* value
Depression													
All	30 (11)	18 (60)	12 (40)	0.074	19 (63)	11 (37)	0.026	20 (67)	10 (33)	0.006	24 (80)	6 (20)	0.096
Men	16 (10)	8 (50)	8 (50)	0.095	9 (56)	7 (44)	0.068	10 (62)	6 (38)	0.037	11 (69)	5 (31)	0.031
Women	14 (12)	10 (71)	4 (29)	0.28	10 (71)	4 (29)	0.11	10 (71)	4 (29)	0.056	13 (93)	1 (7)	>0.99

Data are presented as *n* (%). Fisher's exact test.

http://www.endocrineconnections.org
https://doi.org/10.1530/EC-18-0108

Table 3 Baseline characteristics for all 283 T1D patients, and comparisons between patients with galectin-3 levels below and above the 85th percentile.

	Galectin-3 (µg/L)			
	0.001–100.0	<2.562	≥2.562	*P* value[a]
n	283	241	42	
Gender				
Men	161 (57)	133 (83)	28 (17)	0.18
Women	122 (43)	108 (88)	14 (12)	
Age (years)	(18–59)	42 (31, 50)	46 (36, 54)	0.050[b]
Diabetes duration (years)	(1–55)	19 (10, 30)	24 (16, 31)	0.095[b]
HbA1c				
(mmol/mol)	(25–110)	64 (54, 72)	61 (54, 67)	0.40[b]
(%)	(4.4–12.2)	8.0 (7.1, 8.7)	7.7 (7.1, 8.3)	
TC[c] (mmol/L)	(2.1–10.9)	4.6 (4.1, 5.2)	4.6 (4.1, 5.0)	0.73[b]
Triglycerides (mmol/L)	(0.1–5.9)	0.9 (0.7, 1.3)	1.0 (0.7, 1.2)	0.42[b]
LDL[d] (mmol/L)	(0.6–8.3)	2.8 (2.4, 3.3)	2.8 (2.3, 3.3)	0.56[b]
HDL[e] (mmol/L)	(0.3–2.7)	1.5 (1.3, 1.8)	1.6 (1.3, 1.9)	0.55[b]
Creatinine (µmol/L)	(28–182)	70 (61, 78)	70 (64, 80)	0.64[b]
Abdominal obesity	46 (16)	40 (87)	6 (13)	>0.99
General obesity	34 (12)	31 (91)	3 (9)	0.44
Systolic BP (mmHg)	(90–160)	120 (110, 130)	120 (114. 131)	0.74[b]
Diastolic BP (mmHg)	(55–100)	70 (70, 75)	70 (65, 80)	0.58[b]
Hypoglycaemia (severe episodes)	12 (4)	9 (75)	3 (25)	0.40
Smoking	28 (10)	23 (82)	5 (18)	0.59
Physical inactivity	31 (12)	27 (87)	4 (13)	>0.99
Cardiovascular complications	10 (3)	6 (60)	4 (40)	0.045
Antidepressants	23 (8)	19 (83)	4 (17)	0.76
OAD[f] and insulin	15 (5)	14 (93)	1 (7)	0.71
LLD[g]	131 (46)	111 (85)	20 (15)	0.87
AHD[h]	95 (34)	79 (83)	16 (17)	0.60

[a]Fisher's exact test unless otherwise indicated. [b]Mann–Whitney *U* test. [c]Total cholesterol. [d]Low-density lipoprotein. [e]High-density lipoprotein. [f]Oral antidiabetic drugs. [g]Lipid lowering drugs. [h]Antihypertensive drugs. BP, blood pressure.

well defined. Patients with cognitive deficiencies were excluded, so no patients diagnosed with dementia were included.

One weakness was the limited number of patients within several subgroups. There were only 10 persons with cardiovascular complications, and the association between cardiovascular complications and high galectin-3 was not significant when adjusted for depression and age. The *P* value was 0.10 for the association between high galectin-3 and cardiovascular complications in the six men, indicating that this might be a type 2 error. In a larger setting, significant associations between cardiovascular complications and high galectin-3 levels could be expected (14, 19, 20, 21). Only 14 women and 16 men had self-reported depression. Despite the low number of depressed patients, the associations between depression and high galectin-3 were significant for all, and for the men and women analysed separately. The diagnosis of depression was not confirmed by a structured interview. HADS-D has, however, shown high validity for assessing depressive symptoms both at an individual and a collective level (30). We have also previously found strong associations between self-reported depression assessed by HADS-D and clinical psychiatric diagnoses, and between self-reported depression and antidepressant use in these patients (8). Another weakness is that we did not have a control group without T1D, but this was not necessary for exploring our hypothesis that galectin-3 is higher in depressed than in non-depressed patients with T1D. One difficulty we had to address in this research was that there is no established consensus regarding detrimental galectin-3 levels. Therefore, we explored the associations between depression and four different galectin-3 cut-off levels. In the further analyses, we chose the galectin-3 level that corresponded to the 85th percentile of galectin-3, as this was the level that showed the highest association with depression. Depression is associated with increased risk for cardiovascular and all-cause mortality, and with increased risk for Alzheimer's disease (1, 2). According to previous research, higher circulating galectin-3 concentrations are associated with heart failure independent of aetiology, and with increased cardiovascular and all-cause mortality in the general population (14, 19, 20, 21). Increased levels of serum galectin-3 have also been demonstrated in

http://www.endocrineconnections.org
https://doi.org/10.1530/EC-18-0108

Table 4 Associations with high galectin-3 presented for all 283 T1D patients and for each gender separately.

	High galectin-3 (≥2.562 µg/L)							
	All				Men		Women	
	COR (95% CI)	*P* value	AOR (95% CI)	*P* value[a]	AOR (95% CI)	*P* value[b]	AOR (95% CI)	*P* value[c]
Gender (men)	1.6 (0.8–3.2)	0.17	1.6 (0.8–3.3)	0.19	–	–	–	–
Age (per year)	1.03 (1.00–1.06)	0.053	1.03 (1.00–1.06)	0.095	1.02 (0.98–1.06)	0.42	1.03 (0.97–1.08)	0.32
Diabetes duration (per year)	1.02 (0.99–1.05)	0.16	–	–	–	–	–	–
Depression (HADS-D ≥8p)	3.5 (1.5–8.0)	0.004	3.5 (1.5–8.0)	0.004	3.4 (1.1–10.2)	0.032	3.9 (1.04–14.8)	0.044
HbA1c (per mmol/mol)	0.99 (0.96–1.01)	0.26	–	–	–	–	–	–
HbA1c >70 mmol/mol (>8.6%)	0.6 (0.2–1.3)	0.17	–	–				
TC[d] (per mmol/L)	0.9 (0.6–1.3)	0.51	–	–	–	–	–	–
Triglycerides (per mmol/L)	0.9 (0.6–1.5)	0.71	–	–	–	–	–	–
LDL[e] (per mmol/L)	0.7 (0.5–1.2)	0.26	–	–	–	–	–	–
HDL[f] (per mmol/L)	1.3 (0.6–3.3)	0.51	–	–	–	–	–	–
Creatinine (per µmol/L)	(0.98–1.02)	>0.99	–	–	–	–	–	–
Abdominal obesity	0.9 (0.3–2.2)	0.77	–	–	–	–	–	–
General obesity	0.5 (0.2–1.8)	0.32	–	–	–	–	–	–
Systolic BP (per mmHg)	1.00 (0.98–1.03)	0.75	–	–	–	–	–	–
Diastolic BP (per mmHg)	1.01 (0.97–1.06)	0.54	–	–	–	–	–	–
Hypoglycaemia (severe episodes)	2.0 (0.5–7.6)	0.32	–	–	–	–	–	–
Smoking	1.3 (0.4–3.5)	0.66	–	–	–	–	–	–
Physical inactivity	0.8 (0.3–2.5)	0.72	–	–	–	–	–	–
Cardiovascular complications	4.1 (1.1–15.3)	0.034	2.4 (0.6–9.8)	0.24	4.2 (0.7–23.2)	0.10	–	–
Antidepressants	1.2 (0.4–3.8)	0.72	–	–	–	–	–	–
OAD[g] and insulin	0.4 (0.05–3.1)	0.38	–	–	–	–	–	–
LLD[h]	1.1 (0.6–2.0)	0.85	–	–	–	–	–	–
AHD[i]	1.3 (0.6–2.5)	0.50	–	–	–	–	–	–

Number: [a]283; [b]161; [c]122. Hosmer and Lemeshow: [a]0.891; [b]0.218; [c]0.634. Nagelkerke R Square: [a]0.063; [b]0.042; [c]0.056. [d]Total cholesterol. [e]Low-density lipoprotein. [f]High-density lipoprotein. [g]Oral antidiabetic drugs. [h]Lipid-lowering drugs. [i]Antihypertensive drugs. BP, blood pressure.

Alzheimer's disease (15). Thus, increased galectin-3 levels might represent a biological link between depression and coronary heart and all-cause mortality, as well as between depression and Alzheimer's disease. However, as this is a cross-sectional study, it cannot be deduced whether depression is primary or secondary to increased galectin-3 levels.

We have previously shown that depression was associated with high HbA1c (8). In this study, we found no associations between high HbA1c and galectin-3, indicating that they are two independent biomarkers associated with depression. Our results differ from previous research as we did not find any associations between high galectin-3 and age, hypertension, hypercholesterolemia, triglycerides, s-LDL, BMI, s-creatinine or smoking (19, 21, 22, 23). In none of these previous studies had the results been controlled for depression, the results might have been different if they had. However, it must be observed that patients with end-stage renal disease, bipolar disorders, psychotic symptoms or stroke with secondary cognitive deficiency, were excluded which could affect the results in this study. Also, we cannot exclude that some of the metabolic variables, creatinine or cardiovascular complications could have been associated with high galectin-3 if we had chosen another galectin-3 cut-off level. There are several unanswered questions and subjects for future research. The biology of circulating galectin-3 in patients with diabetes and depression is uncertain. Matrix metalloproteinase-9 (MMP-9), which is one of the best described proteases in the CNS, targets galectin-3 for cleavage (34). Galectin-3 is, however, also a positive downstream regulator of MMP-9 expression (35). Increased levels of MMP-9 have been described during depressive episodes in patients

http://www.endocrineconnections.org
https://doi.org/10.1530/EC-18-0108

with bipolar depression (36), in patients with unipolar major depressive disorder (37), as well as in the brains of persons with Alzheimer's disease (38). MMP-9 has also been suggested to play a main role in synaptic trafficking and modification of glutamate receptors, such as the N-methyl-D-aspartate receptor (39), which is of particular interest when it comes to depression and treatment of this condition (40). Future studies should thus focus on MMP-9 in addition to galectin-3 in patients with diabetes and depression, which might give valuable information about the pathogenesis.

Whether galactin-3 could be a good diagnostic marker for depression in T1D needs to be further investigated. Additional larger studies comparing galectin-3 levels in depressed and non-depressed persons, both in persons with and without T1D will be necessary, as well as longitudinal studies. Can galectin-3 be used for the evaluation of the efficacy of the treatment for depression? It would be of interest to measure galectin-3 at the initiation of antidepressant medication or psychotherapy and follow the galectin-3 values during therapy. It would also be of interest to explore whether depressed T1D patients with high galectin-3 level have increased risk for Alzheimer's disease, renal dysfunction, cardiac failure and mortality. Which galectin-3 levels are damaging for the heart, for the brain or for the kidneys? For how long can a person have increased galectin-3 levels without risking damage to the heart or risking the development of Alzheimer's disease? Can galectin-3 be a target for treatment? In further research, we suggest that when associations between galectin-3 and somatic parameters are explored, the results should be adjusted for depression.

In conclusion, depression was the only examined variable associated with high circulating galectin-3 (≥2.562 µg/l) in the 283 patients with T1D. The association between depression and galectin-3 has not to our knowledge been explored previously, neither in persons with T1D nor in those without T1D. Higher galectin-3 levels might contribute to the increased risk for Alzheimer's disease, cardiovascular and all-cause mortality observed in patients with depression. Potentially, in the future, high galectin-3 levels could be used as indicators for depression, and treatment targeting galactin-3 might be developed to improve the prognosis for patients with high galectin-3 levels.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This research was supported by the Research and Development Fund of Health Care, Region Kronoberg, Växjö, Sweden; by the Research Council of South Eastern Sweden (FORSS), Linköping, Sweden; and by the Patient Diabetes Organisation of Southern Sweden (Sydsvenska Diabetesföreningen) Malmö, Sweden. The funding sources were not involved in the collection, analysis or interpretation of data, in the writing of the report, or in the decision to submit the article for publication.

Authors' contribution statement

E O M, J D, M T and M H participated as investigators and reviewed, edited and approved the final version of the manuscript. E O M initiated the study of depression in diabetes, performed the statistical analysis, is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. M H suggested that galectin-3 would be of interest to study. E O M and M H wrote the manuscript. J D performed and validated the galectin-3 analyses. E O M and M T organized the recruitment of patients and collection of data.

Acknowledgements

The authors are grateful to Anna Lindgren, at the Department of Mathematical Statistics, Lund University, Lund, Sweden, for her statistical skills.

References

1 Egede LE, Nietert PJ & Zheng D. Depression and all-cause and coronary heart disease mortality among adults with and without diabetes. *Diabetes Care* 2005 **28** 1339–1345. (https://doi.org/10.2337/diacare.28.6.1339)

2 Penninx BW, Milaneschi Y, Lamers F & Vogelzangs N. Understanding the somatic consequences of depression: biological mechanisms and the role of depression symptom profile. *BMC Medicine* 2013 **11** 129. (https://doi.org/10.1186/1741-7015-11-129)

3 Korczak DJ, Pereira S, Koulajian K, Matejcek A & Giacca A. Type 1 diabetes mellitus and major depressive disorder: evidence for a biological link. *Diabetologia* 2011 **54** 2483–2493. (https://doi.org/10.1007/s00125-011-2240-3)

4 Wang B, An X, Shi X & Zhang J. MANAGEMENT OF ENDOCRINE DISEASE: Suicide risk in patients with diabetes: a systematic review and meta-analysis. *European Journal of Endocrinology* 2017 **177** R169–R181. (https://doi.org/10.1530/EJE-16-0952)

5 Tynan RJ, Weidenhofer J, Hinwood M, Cairns MJ, Day TA & Walker FR. A comparative examination of the anti-inflammatory effects of SSRI and SNRI antidepressants on LPS stimulated microglia. *Brain, Behavior, and Immunity* 2012 **26** 469–479. (https://doi.org/10.1016/j.bbi.2011.12.011)

6 Ma Y, Balasubramanian R, Pagoto SL, Schneider KL, Hébert JR, Phillips LS, Goveas JS, Culver AL, Olendzki BC & Beck J. Relations of depressive symptoms and antidepressant use to body mass index and selected biomarkers for diabetes and cardiovascular disease. *American Journal of Public Health* 2013 **103** e34–e43. (https://doi.org/10.2105/AJPH.2013.301394)

7 Raison CL, Capuron L & Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends in Immunology* 2006 **27** 24–31. (https://doi.org/10.1016/j.it.2005.11.006)

8 Melin EO, Thunander M, Svensson R, Landin-Olsson M & Thulesius HO. Depression, obesity and smoking were independently associated with inadequate glycemic control in patients with type

1 diabetes. *European Journal of Endocrinology* 2013 **168** 861–869. (https://doi.org/10.1530/eje-13-0137)
9 Melin EO, Thunander M, Landin-Olsson M, Hillman M & Thulesius HO. Depression, smoking, physical inactivity and season independently associated with midnight salivary cortisol in type 1 diabetes. *BMC Endocrine Disorders* 2014 **14** 75. (https://doi.org/10.1186/1472-6823-14-75)
10 Melin EO, Thunander M, Landin-Olsson M, Hillman M & Thulesius HO. Depression differed by midnight cortisol secretion, alexithymia and anxiety between diabetes types: a cross sectional comparison. *BMC Psychiatry* 2017 **17** 335. (https://doi.org/10.1186/s12888-017-1495-8)
11 Melin EO, Svensson R, Thunander M, Hillman M, Thulesius HO & Landin-Olsson M. Gender, alexithymia and physical inactivity associated with abdominal obesity in type 1 diabetes mellitus: a cross sectional study at a secondary care hospital diabetes clinic. *BMC Obesity* 2017 **4** 21. (https://doi.org/10.1186/s40608-017-0157-1)
12 Gruson D & Ko G. Galectins testing: new promises for the diagnosis and risk stratification of chronic diseases? *Clinical Biochemistry* 2012 **45** 719–726. (https://doi.org/10.1016/j.clinbiochem.2012.04.009)
13 Nio-Kobayashi J. Tissue-and cell-specific localization of galectins, β-galactose-binding animal lectins, and their potential functions in health and disease. *Anatomical Science International* 2017 **92** 25–36. (https://doi.org/10.1007/s12565-016-0366-6)
14 Sharma UC, Pokharel S, van Brakel TJ, van Berlo JH, Cleutjens JP, Schroen B, André S, Crijns HJ, Gabius HJ & Maessen J. Galectin-3 marks activated macrophages in failure-prone hypertrophied hearts and contributes to cardiac dysfunction. *Circulation* 2004 **110** 3121–3128. (https://doi.org/10.1161/01.CIR.0000147181.65298.4D)
15 Wang X, Zhang S, Lin F, Chu W & Yue S. Elevated galectin-3 levels in the serum of patients with Alzheimer's disease. *American Journal of Alzheimer's Disease and Other Dementias* 2015 **30** 729–732. (https://doi.org/10.1177/1533317513495107)
16 Shin T. The pleiotropic effects of galectin-3 in neuroinflammation: a review. *Acta Histochemica* 2013 **115** 407–411. (https://doi.org/10.1016/j.acthis.2012.11.010)
17 Burguillos MA, Svensson M, Schulte T, Boza-Serrano A, Garcia-Quintanilla A, Kavanagh E, Santiago M, Viceconte N, Oliva-Martin MJ & Osman AM. Microglia-secreted galectin-3 acts as a toll-like receptor 4 ligand and contributes to microglial activation. *Cell Reports* 2015 **10** 1626–1638. (https://doi.org/10.1016/j.celrep.2015.02.012)
18 Yip PK, Carrillo-Jimenez A, King P, Vilalta A, Nomura K, Chau CC, Egerton AMS, Liu ZH, Shetty AJ, Tremoleda JL, *et al*. Galectin-3 released in response to traumatic brain injury acts as an alarmin orchestrating brain immune response and promoting neurodegeneration. *Scientific Reports* 2017 **7** 41689. (https://doi.org/10.1038/srep41689)
19 Ho JE, Liu C, Lyass A, Courchesne P, Pencina MJ, Vasan RS, Larson MG & Levy D. Galectin-3, a marker of cardiac fibrosis, predicts incident heart failure in the community. *Journal of the American College of Cardiology* 2012 **60** 1249–1256. (https://doi.org/10.1016/j.jacc.2012.04.053)
20 Imran TF, Shin HJ, Mathenge N, Wang F, Kim B, Joseph J, Gaziano JM & Djoussé L. Meta-analysis of the usefulness of plasma galectin-3 to predict the risk of mortality in patients with heart failure and in the general population. *American Journal of Cardiology* 2017 **119** 57–64. (https://doi.org/10.1016/j.amjcard.2016.09.019)
21 Hrynchyshyn N, Jourdain P, Desnos M, Diebold B & Funck F. Galectin-3: a new biomarker for the diagnosis, analysis and prognosis of acute and chronic heart failure. *Archives of Cardiovascular Diseases* 2013 **106** 541–546. (https://doi.org/10.1016/j.acvd.2013.06.054)
22 Weigert J, Neumeier M, Wanninger J, Bauer S, Farkas S, Scherer MN, Schnitzbauer A, Schäffler A, Aslanidis C & Schölmerich J. Serum galectin-3 is elevated in obesity and negatively correlates with glycosylated hemoglobin in type 2 diabetes. *Journal of Clinical Endocrinology and Metabolism* 2010 **95** 1404–1411. (https://doi.org/10.1210/jc.2009-1619)
23 van der Velde AR, Meijers WC, van den Heuvel ER, Bakker SJ, van Gilst WH, van der Harst P, Hillege H & de Boer RA. Determinants of temporal changes in galectin-3 level in the general population: data of PREVEND. *International Journal of Cardiology* 2016 **222** 385–390. (https://doi.org/10.1016/j.ijcard.2016.07.241)
24 Pricci F, Leto G, Amadio L, Iacobini C, Romeo G, Cordone S, Gradini R, Barsotti P, Liu FT & Di Mario U. Role of galectin-3 as a receptor for advanced glycosylation end products. *Kidney International* 2000 **58** S31–S39. (https://doi.org/10.1046/j.1523-1755.2000.07706.x)
25 Melin EO, Svensson R, Gustavsson SÅ, Winberg A, Denward-Olah E, Landin-Olsson M & Thulesius HO. Affect school and script analysis versus basic body awareness therapy in the treatment of psychological symptoms in patients with diabetes and high HbA1c concentrations: two study protocols for two randomized controlled trials. *Trials* 2016 **17** 221. (https://doi.org/10.1186/s13063-016-1347-8)
26 Melin E. Psychosomatic aspects on diabetes and chronic pain Alexithymia, depression and salivary cortisol. The Affect School and Script Analysis Therapy. PhD Thesis. Lund, Sweden: Lund University, 2014.
27 Lind M, Svensson AM, Kosiborod M, Gudbjörnsdottir S, Pivodic A, Wedel H, Dahlqvist S, Clements M & Rosengren A. Glycemic control and excess mortality in type 1 diabetes. *New England Journal of Medicine* 2014 **371** 1972–1982. (https://doi.org/10.1056/NEJMoa1408214)
28 Eeg-Olofsson K, Cederholm J, Nilsson PM, Gudbjörnsdóttir S & Eliasson B. Glycemic and risk factor control in type 1 diabetes. *Diabetes Care* 2007 **30** 496–502. (https://doi.org/10.2337/dc06-1406)
29 Zigmond AS & Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatrica Scandinavica* 1983 **67** 361–370. (https://doi.org/10.1111/j.1600-0447.1983.tb09716.x)
30 Bocéréan C & Dupret E. A validation study of the Hospital Anxiety and Depression Scale (HADS) in a large sample of French employees. *BMC Psychiatry* 2014 **14** 1–11. (https://doi.org/10.1186/s12888-014-0354-0)
31 Ridker PM. LDL cholesterol: controversies and future therapeutic directions. *Lancet* 2014 **384** 607–617. (https://doi.org/10.1016/S0140-6736(14)61009-6)
32 Tanamas SK, Permatahati V, Ng WL, Backholer K, Wolfe R, Shaw JE & Peeters A. Estimating the proportion of metabolic health outcomes attributable to obesity: a cross-sectional exploration of body mass index and waist circumference combinations. *BMC Obesity* 2016 **3** 4. (https://doi.org/10.1186/s40608-016-0085-5)
33 The National Board of Health and Welfare. Swedish National Guidelines for Diabetes, 2009. (available at: https://www.socialstyrelsen.se/english)
34 Ochieng J, Fridman R, Nangia-Makker P, Kleiner DE, Liotta LA, Stetler-Stevenson WG & Raz A. Galectin-3 is a novel substrate for human matrix metalloproteinases-2 and-9. *Biochemistry* 1994 **33** 14109–14114. (https://doi.org/10.1021/bi00251a020)
35 Dange MC, Agarwal AK & Kalraiya RD. Extracellular galectin-3 induces MMP9 expression by activating p38 MAPK pathway via lysosome-associated membrane protein-1 (LAMP1). *Molecular and Cellular Biochemistry* 2015 **404** 79–86. (https://doi.org/10.1007/s11010-015-2367-5)
36 Rybakowski JK, Remlinger-Molenda A, Czech-Kucharska A, Wojcicka M, Michalak M & Losy J. Increased serum matrix metalloproteinase-9 (MMP-9) levels in young patients during bipolar depression. *Journal of Affective Disorders* 2013 **146** 286–289. (https://doi.org/10.1016/j.jad.2012.07.019)
37 Domenici E, Willé DR, Tozzi F, Prokopenko I, Miller S, McKeown A, Brittain C, Rujescu D, Giegling I & Turck CW. Plasma protein biomarkers for depression and schizophrenia by multi analyte

profiling of case-control collections. *PLoS ONE* 2010 **5** e9166. (https://doi.org/10.1371/journal.pone.0009166)

38 Mizoguchi H, Yamada K & Nabeshima T. Matrix metalloproteinases contribute to neuronal dysfunction in animal models of drug dependence, Alzheimer's disease, and epilepsy. *Biochemistry Research International* 2011 **2011** 681385. (https://doi.org/10.1155/2011/681385)

39 Michaluk P, Mikasova L, Groc L, Frischknecht R, Choquet D & Kaczmarek L. Matrix metalloproteinase-9 controls NMDA receptor surface diffusion through integrin β1 signaling. *Journal of Neuroscience* 2009 **29** 6007–6012. (https://doi.org/10.1523/JNEUROSCI.5346-08.2009)

40 Niciu MJ, Henter ID, Luckenbaugh DA, Zarate CA Jr & Charney DS. Glutamate receptor antagonists as fast-acting therapeutic alternatives for the treatment of depression: ketamine and other compounds. *Annual Review of Pharmacology and Toxicology* 2014 **54** 119–139. (https://doi.org/10.1146/annurev-pharmtox-011613-135950)

Received in final form 2 May 2018
Accepted 14 May 2018
Accepted Preprint published online 14 May 2018