Journal of Diabetes and Its Complications 31 (2017) 1408–1414

Contents lists available at ScienceDirect

Journal of Diabetes and Its Complications

journal homepage: WWW.JDCJOURNAL.COM

# Hand, shoulder and back stiffness in long-term type 1 diabetes; cross-sectional association with skin collagen advanced glycation end-products. The Dialong study

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ARTICLE INFO

*Article history:*
Received 13 March 2017
Received in revised form 30 May 2017
Accepted 15 June 2017
Available online 22 June 2017

*Keywords:*
Long-term complications
Type 1 diabetes mellitus
Advanced glycation end-products
Musculoskeletal disorders
Hemoglobin A
Glycosylated

ABSTRACT

*Aims:* We aimed to: (i) estimate the prevalence of Dupuytren's disease, trigger finger, carpal tunnel syndrome and frozen shoulder; (ii) assess stiffness of the hand, shoulder and back; and (iii) explore the association of joint stiffness with both long-term $HbA_{1c}$ and collagen advanced glycation end-products (AGEs) in long-term type 1 diabetes mellitus (T1DM).
*Methods:* Patients with T1DM from 1970 or earlier attending a specialized diabetes center were included in this cross-sectional controlled study. We collected $HbA_1/HbA_{1c}$ measurements from 1980 to 2015 and data on hand and shoulder diagnoses and joint stiffness through interviews, charts, and standardized examination. Skin biopsies were analyzed for collagen AGEs by liquid chromatography-mass spectrometry.
*Results:* Lifetime prevalence of hand and shoulder diagnoses in the diabetes group ($n = 102$) ranged from 37%–76% (frozen shoulder) versus 11%–15% in controls ($n = 73$) ($p < 0.001$). There was an association between joint stiffness and long-term $HbA_{1c}$ (odds ratio 2.01 [95% CI 1.10–3.7]) and the AGEs methyl-glyoxal-lysine-dimer (odds ratio 1.68 [95% CI 1.03–2.73]) and pentosidine (odds ratio 1.81 [95% CI 1.04–3.16]).
*Conclusions:* Patients with T1DM >45 years had a very high prevalence of hand and shoulder diagnoses versus controls. Joint stiffness was associated with collagen AGEs. However, joint biopsies and prospective studies must explore this association further.



## 1. Introduction

Several musculoskeletal diagnoses affecting the shoulder and hand have been associated with both type 1 and type 2 diabetes.[1–3] These diagnoses include adhesive capsulitis (frozen shoulder), carpal tunnel syndrome (CTS), Dupuytren's disease (DD), and stenosing tenosynovitis (trigger finger), and can have a major impact on quality of life in long-term type 1 diabetes.[3,4] They have been associated with the duration of diabetes, age, gender, microvascular complications and to a varying degree glucose control.[2,3,5] Due to improved treatment over the last 30–40 years, we now have a large cohort of persons with long-term type 1 diabetes, and these complications are becoming clinically more important with advancing age. Currently there are no recommendations for routine clinical examination of these conditions,[6] thus they may be under-estimated by health care practitioners. Limited joint mobility of the hand was first described as a complication of type 1 diabetes in 1957 by Lundbaek[7] and Rosenbloom et al. in 1974.[8] Stiffness has also been reported to affect several other joints in diabetes including the small joints of the feet, shoulder, hip, ankle and spine resulting in general stiffness.[9] Previous research in the field is limited for reasons that include lack of a control group, vague diagnostic criteria, primary focus on type 2 diabetes and/or lack of long-term survivors of type 1 diabetes.[2,3]

The formation of Advanced Glycation Endproducts (AGEs) and their cross-linking of collagen have been postulated as underlying mechanisms for these musculoskeletal disorders.[1,3] Few studies have examined this association, and there have been inconsistent findings.[10–12] Studies have shown an association between both higher concentration of collagen and circulating AGEs with microvascular and macrovascular complications in diabetes, suggesting a role for

Funding: This project was funded by the Oslo Diabetes Research Center, the Norwegian Diabetics' Center, Sophies Minde Ortopedi AS (19/2014) and the Norwegian Diabetes Association.
Conflicts of interest: None.

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http://dx.doi.org/10.1016/j.jdiacomp.2017.06.007
1056-8727/

AGEs in these complications.[13,14] However, it is unclear whether the pathogenic factors for stiffness formation in diabetes are similar to those causing microvascular complications. Several new AGEs have been discovered over the years, some of which are known to be cross-linkers of collagen; mainly glucosepane and to a smaller degree pentosidine and methyl-glyoxal-lysine dimer (MOLD).

We conducted a cross-sectional controlled study of patients with type 1 diabetes >45 years duration with a control group. We have previously reported the prevalence of frozen shoulder in our study population.[15] In the current study, our aims were to: (i) estimate both the lifetime and point prevalence of CTS, DD and trigger finger; (ii) assess joint stiffness of the hand, shoulder and back; and (iii) explore the association between joint stiffness and both collagen AGEs and mean $HbA_{1c}$ over 30 years.

## 2. Research design and methods

### 2.1. Study design and subjects

This cross-sectional controlled study was conducted in 2015. A retrospective chart review was performed. We recruited patients from the Norwegian Diabetics' Center (NDC) Oslo, Norway. Patients are referred from general practitioners or hospitals in the region and receive all diabetes-related follow up here. Type 1 diabetes was clinically defined as a medical history characteristic of type 1 diabetes, $HbA_{1c} > 6.5\%$ (48 mmol/mol) and lack of insulin production as evidenced by a fasting c-peptide concentration < 0.2 pmol/ml. All patients who were attending the NDC in 2015 with type 1 diabetes diagnosed in 1970 or earlier (n = 136) were invited to the study. Most participants had attended NDC for >30 years. Our cohort of patients with diabetes was asked to find participants for the control group by either asking their spouse or a close friend. Exclusion criteria were 1st degree relatives and a known diagnosis of diabetes or $HbA_{1c} > 6.5\%$ (48 mmol/mol). The regional ethics committee approved the study (project no. 2014/851), and written informed consent was obtained from all participants.

### 2.2. Procedure

Background data with regard to diabetes complications, comorbidities and medication lists were collected from patient charts at NDC, interviews and examination during the first visit. Participants were also asked to bring an updated medication list or any relevant discharge letters. A medical doctor (K.B.H. or T.J.B.) performed the interviews and a skin punch biopsy, and one research nurse measured blood pressure, height, weight and tested for peripheral neuropathy by standard monofilament and vibration tests of the feet. Within three weeks, the participants attended for fasting blood tests, urine sample, retina photos, joint examination, questionnaires and shoulder x-rays.

### 2.3. Demographic factors and comorbidities

Education level was reported in four categories and dichotomized into college/university education or not. Height and weight were measured with participants in light clothing and without shoes and body mass index (BMI) calculated by standard formula. Blood pressure was recorded while sitting after resting for a minimum of 10 min as the average of the last two out of three readings. Smoking habits were reported as daily, former or never. We identified participant use of HMG-CoA reductase inhibitors (statins) and ACE-inhibitors or angiotensin II receptor blockers (ARB) as well as a past history of cardiovascular disease or arthritic comorbidities through chart review, discharge letters and interviews.

### 2.4. Musculoskeletal evaluation

All participants filled out questionnaires asking for previous hand and shoulder diagnoses and the short form of Disabilities of the Arms, Shoulders and Hands (QuickDASH) questionnaire. QuickDASH is a region-specific, patient-reported outcome measure that consists of 11 questions regarding symptoms and upper limb disability. The total score ranges from 0 to 100 (best to worst). QuickDASH is translated and validated for use for the Norwegian population.[16]

#### 2.4.1. Hand and shoulder diagnoses

The lifetime prevalence of frozen shoulder, DD, trigger finger and CTS was based on information collected from chart review, interviews, questionnaires and clinical examination. We also documented any surgical intervention of the conditions. To find the point prevalence of these diagnoses a standardized clinical examination was performed by one board qualified doctor in physical medicine and rehabilitation (N.G.J.) blinded for group affiliation. The diagnostic criteria used for frozen shoulder were reduced passive glenohumeral range of motion of at least 20° in two planes and normal shoulder x-rays. The point prevalence in this cohort was recently reported.[15] To diagnose CTS, typical symptoms and at least one positive neurological test were necessary.[17] DD was diagnosed as present when one or more fingers were both contracted in flexion and had a palpable string of palmar fibromatosis in the same array.[18,19] Scars from earlier operations were not counted for as having DD. A trigger finger diagnosis required a locking sensation of one or more fingers to be present in flexion and the ability to extend it with a palpable click.[20] All diagnoses were rated as present or absent.

#### 2.4.2. Joint stiffness

We had four categories of joint stiffness: hand, shoulder, back and full stiffness. The prayer sign test was performed to assess overall hand stiffness.[8] Usually the palmar side of the hands and fingers will have full contact when opposed. A positive prayer sign was present when a subject was unable to achieve this because of flexion contracture of several finger joints.[9] For stiffness of the back we measured the fingertip-to-floor distance[21] which has shown satisfactory reliability.[22] The test was performed with the subjects standing, asked to touch the floor while bending forward with straight legs. The distance from fingertips to floor was measured in centimeters. Participants testing ≥75th percentile of the control group, were classified as having a stiff back. This corresponded to ≥2 cm for women and ≥16 cm for men. Subjects with frozen shoulder on examination were classified as having a stiff shoulder. The full stiffness category included participants who experienced all three forms of joint stiffness.

### 2.5. Analysis of skin collagen AGEs and skin autofluorescence

A punch biopsy with a diameter of 3 mm was obtained from each participant in sterile technique from the upper lateral part of the nates after administration of a local anesthetic. The sample was immediately put in a sterile container, flushed with nitrogen gas and transferred to a temperature of −80 °C. All samples were analyzed for the following AGEs (at Case Western Reserve University, Cleveland, Ohio, United States): Fructose-lysine, glucosepane, carboxymethyl-hydroxy-lysine (CML), carboxyethyl-hydroxy-lysine (CEL), pentosidine, MOLD, fluorophore LW-1, and methylglyoxal hydroimidazolone (MG-H1). For these analyses, the skin biopsy collagen was extracted and enzymatically digested into free amino acids as previously described.[23] However, for the sequential digestion procedure, several enzymes were substituted and the order differed from what was previously used as follows: collagenase (same), pronase (same), 1% enterokinase/peptidase (Sigma E0885), 1% prolidase (Sigma P6675). Additionally, liquid chromatography mass

spectrometric quantitation was done with a TSQ Quantum Access ESI MS/MS instrument loaded with Xcalibur v2 software (Thermo Fisher Scientific).[23] The analyst was blinded with regard to group affiliation. All participants also had skin autofluorescence measured non-invasively by the autofluorescence reader (AGE reader).[24]

### 2.6. $HbA_{1c}$ variables

Longitudinal $HbA_1$ and $HbA_{1c}$ values were available from the early 1980s up until 2015. An average of 73.4 measurements (SD = 28.3) per subject were taken at different time intervals. "Estimated Full Duration $HbA_{1c}$" was calculated from both all available $HbA_1$ (converted to $HbA_{1c}$) and $HbA_{1c}$ measurements and an estimate of the mean $HbA_{1c}$ from diagnosis up until the first measurement (see online supplemental material). Blood samples taken during the study were analyzed for $HbA_{1c}$ ("Current $HbA_{1c}$") at OUHU (HPLC) Tosoh G8, (Tosoh Corporation), ref. range 4.0–6.0% (20–42 mmol/mol).

### 2.7. Statistical analysis

Based on the power analysis, (type 1 error 5%, power of 90%, expected prevalence in the diabetes group of 36% and the control group 9%[2]) we needed 56 participants in each group to detect a significant difference in the prevalence of hand and shoulder diagnoses. Clinical characteristics and the presence of hand and shoulder diagnoses were compared between the groups using two-tailed Student's *t*-test or Mann Whitney *U* test for continuous and $\chi^2$ for categorical data. For stiffness explanatory variables, logistic regression was performed using univariate, adjusted, and multiple regressions with stepwise backward elimination. Standardized values for AGEs and $HbA_{1c}$ variables were calculated. The adjusted model was AGEs adjusted for Estimated Full Duration $HbA_{1c}$. Apart from the 8 AGEs, the following variables were examined: Age, duration, gender, BMI, microvascular complications (peripheral neuropathy, retinopathy and albuminuria), cardiovascular disease, Estimated Full Duration $HbA_{1c}$, and AGE reader. Variables with a $p < 0.20$ in the univariate or adjusted (AGEs) analyses were included in the full model. We made a separate composite variable of AGEs that were significantly or borderline significantly associated with stiffness in the adjusted model. To adjust for multiple tests, we also used the Benjamini-Hochberg procedure with a false discovery rate of 0.1.[25] All analyses were performed using IBM SPSS Statistics version 23 (IBM SPSS Inc., Armonk, NY: IBM Corp.).

## 3. Results

Out of 136 eligible patients with type 1 diabetes, 105 agreed to participate. However, 102 completed the musculoskeletal evaluation (75%) and two refused the skin biopsies leaving a total of 100 (74%) patients for the full analyses. In the control group, 73 completed the study. Clinical characteristics are presented in Table 1. The median diabetes duration was 49 years and mean ± SD Estimated Full Duration $HbA_{1c}$ 8.0 ± 0.8% (63.5 ± 8.6 mmol/mol). The mean current $HbA_{1c}$ of 7.4 ± 0.8% (57.8 ± 8.6 mmol/mol) in the diabetes group was similar to the average $HbA_{1c}$ for all Norwegian patients with type 1 diabetes with >45 years duration registered in the Norwegian Diabetes register of 7.6 ± 0.9% (59.3 ± 10.4 mmol/mol) ($p = 0.08$). Our study groups differed significantly with regard to history of cardiovascular disease, presence of peripheral neuropathy, blood pressure and use of statins and ACE-inhibitors/ARBs. The diabetes group had a significantly higher median score (17.1 (min–max 0–73) vs. 4.5 (0–64), $p < 0.001$) on the QuickDASH questionnaire as recently reported.[15] Table 1 also shows results of skin collagen AGE measurements. The diabetes group had higher values for all AGEs ($p < 0.001$) apart from MOLD.

Fig. 1a shows the distribution of the lifetime prevalence of hand and shoulder diagnoses. In the diabetes group, the lifetime prevalence of frozen shoulder was 76%, DD 63%, trigger finger 42% and CTS 37% compared to 14%, 15%, 11% and 12% in the control group respectively (all $p < 0.001$). 94% of the diabetes group had one or more of the diagnoses. The point prevalence was 63% for DD versus 15% ($p < 0.001$), 8% for trigger finger versus 1% ($p = 0.056$), and 3% for CTS versus 1% ($p = 0.391$). As recently reported, 59% in the diabetes group had a stiff shoulder (frozen shoulder) compared to 0% in the control group ($p < 0.001$).[15] Fifty percent in the diabetes group had a stiff hand (positive prayer sign) and 43% a stiff back compared to 4% and 27% in the control group (Fig. 1b). Eighteen percent in the diabetes group had stiff hands, shoulders and back (full stiffness) and none in the control group. Forty-four percent in the diabetes group had previous surgical intervention of these hand and shoulder diagnoses versus 6% in the control group ($p < 0.001$).

Logistic regression analyses were performed on the diabetes group with hand, back, shoulder or full stiffness as outcome variables (Table 2).

**Table 1**
Participant characteristics.

| | Diabetes (n = 102) | Controls (n = 73) | p-Value |
|---|---|---|---|
| *Demographics* | | | |
| Age, years | 61.9 ± 7.1 | 62.6 ± 7.0 | 0.545 |
| Gender, male | 51 (50) | 33 (45) | 0.531 |
| College education | 63 (62) | 54 (74) | 0.091 |
| BMI | 26.2 ± 4.01 | 25.8 ± 4.30 | 0.559 |
| Blood pressure | | | |
| Systolic | 146 ± 19.6 | 137 ± 19.3 | 0.003 |
| Diastolic | 75 ± 8.3 | 81 ± 9.3 | <0.001 |
| Smoking | | | |
| Daily | 4 (4) | 6 (8) | 0.479 |
| Former | 40 (39) | 28 (38) | |
| *Diabetes related factors* | | | |
| Current $HbA_{1c}$, %, mmol/mol | 7.4 ± 0.8, 57.8 ± 8.6 | 5.5 ± 0.28, 36.4 ± 3.1 | <0.001 |
| Estimated full duration $HbA_{1c}$, % mmol/mol | 8.0 ± 0.8, 63.5 ± 8.6 | – | |
| Diabetes duration in years, median (min-max) | 49 (45–67) | – | |
| Persistent albuminuria | 17 (17) | 4 (6)[a] | 0.010 |
| eGFR <60 | 9 (9) | 2 (3) | 0.102 |
| Retinopathy | | | |
| None | 5 (5) | | |
| Background | 53 (52) | | |
| Proliferative | 64 (63) | | |
| Neuropathy | 64 (63) | 15 (21) | <0.001 |
| Cardiovascular disease | 21 (21) | 6 (8) | 0.026 |
| *Medication use* | | | |
| Insulin pump | 45 (44) | – | |
| Statins | 55 (54) | 11 (15) | <0.001 |
| ACE-inhibitors/ARB | 51 (50) | 15 (21) | <0.001 |
| Daily NSAIDs | 2 (2) | 3 (4) | 0.494 |
| *Arthritic comorbidities* | | | |
| Psoriasis arthritis % | 1 (1) | 2 (3) | 0.377 |
| Rheumatoid arthritis % | 2 (2) | 0 (0) | 0.229 |
| *Investigations* | | | |
| QuickDASH, median (min–max) | 17,1 (0–73) | 4,5 (0–64) | <0.001 |
| Skin intrinsic fluorescence | 2,77 (0.52) | 2.23 (0.47) | <0.001 |

| Collagen AGEs (pmol/mg) | (n = 100) | (n = 73) | p-Value |
|---|---|---|---|
| Glucosepane | 6480 ± 1247 | 3417 ± 598 | <0.001 |
| Pentosidine | 30.3 ± 10.3 | 18.5 ± 5.9 | <0.001 |
| LW-1 | 1058 ± 654 | 474 ± 318 | <0.001 |
| CML | 287.6 ± 59.9 | 197.4 ± 42.3 | <0.001 |
| CEL | 30.0 ± 7.3 | 23.5 ± 5.9 | <0.001 |
| Fructose-lysine | 5717 ± 944 | 3408 ± 344 | <0.001 |
| MG-H1 | 445 ± 192 | 301 ± 130 | <0.001 |
| MOLD | 1.27 ± 1.00 | 1.22 ± 0.94 | 0.745 |

Data are mean ± SD or n (%) unless otherwise stated.
[a] Based on one urine sample measuring albumin:creatinine ratio.

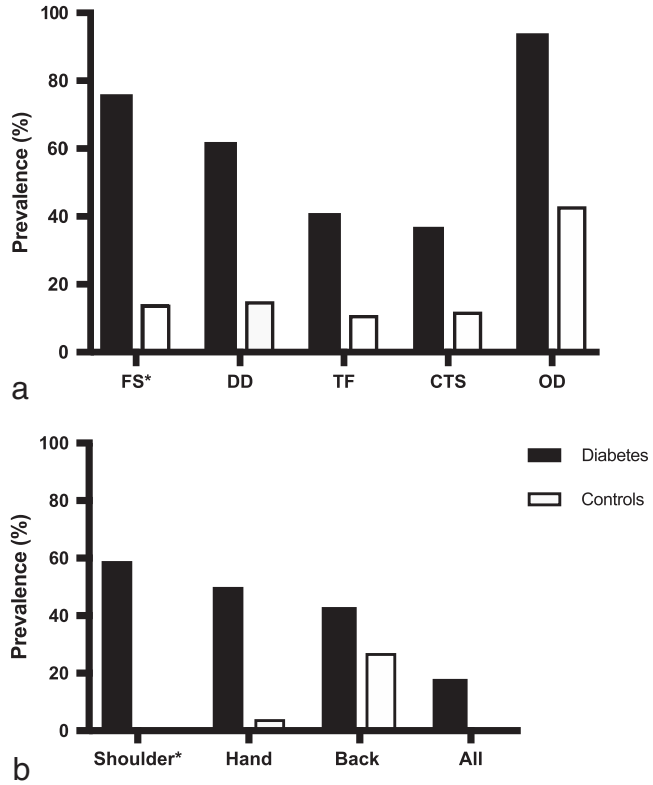


**Fig. 1.** a: Lifetime prevalence of hand and shoulder disorders in the diabetes group versus in controls. All p-values < 0.001 for the group difference. FS = frozen shoulder; DD = Dupuytren's disease; TF = trigger finger; CTS = carpal tunnel syndrome; OD = one or more of the hand/shoulder diagnoses. b: Point prevalence of shoulder stiffness, hand stiffness, back stiffness and stiffness in all three joints (all) in the diabetes group versus in controls. There were no cases of shoulder stiffness or "All" in the control group. p-values < 0.001 for the group difference, except for back stiffness where the p-value was 0.035. *The lifetime and point prevalence of frozen shoulder has previously been reported (15).

We did not find any significant associations with age, diabetes duration or gender. Apart from the association between neuropathy and back stiffness in the univariate analysis, we did not find any associations between microvascular or macrovascular complications and stiffness. Estimated Full Duration $HbA_{1c}$ was associated with full stiffness in the full model ($p = 0.023$), along with the AGEs MOLD ($p = 0.039$) and pentosidine ($p = 0.037$). We did a separate multiple regression analysis with a composite variable of CML, glucosepane, MOLD, pentosidine and LW-1 and full stiffness as the outcome in the diabetes group. The composite variable had an odds ratio of 3.4 (95% CI 1.3–8.9, $p = 0.013$). No significant associations were found between AGEs and shoulder stiffness in univariate or multiple regression analyses. LW-1 was associated with hand stiffness in univariate analysis ($p = 0.048$), but did not quite reach statistical significance when adjusted for Estimated Full Duration $HbA_{1c}$ ($p = 0.054$). MOLD ($p = 0.005$), LW-1 ($p = 0.029$) and Estimated Full Duration $HbA_{1c}$ ($p = 0.01$) were associated with back stiffness in univariate/adjusted analyses. However, MOLD ($p = 0.002$) pentosidine ($p = 0.002$) and Estimated Full Duration $HbA_{1c}$ ($p = 0.006$) were positively associated with back stiffness in the full model, while CEL was inversely associated with back stiffness ($p = 0.005$) (Fig. 2). In the control group, the multiple stepwise backward regression analysis with back stiffness as the outcome, revealed MOLD (OR 2.56, 95% CI 1.26–5.19, $p = 0.009$) CEL (OR 0.30, 95% CI 0.11–0.85, $p = 0.023$) and Current $HbA_{1c}$ (OR 62.3, 95% CI 3.10–1253.8, $p = 0.007$) to be significantly associated with back stiffness. No further analyses were carried out on the control group due to a low prevalence of other forms of stiffness.

When controlling for multiple tests with the Benjamini-Hochberg procedure, Estimated Full Duration $HbA_{1c}$ and MOLD remained significant in the univariate back stiffness model, whereas MOLD, pentosidine, CEL and Estimated Full Duration $HbA_{1c}$ remained significant in the full back stiffness model.

## 4. Discussion

Our main findings were: (i) that participants with type 1 diabetes for >45 years have a very high lifetime prevalence of DD, CTS and trigger finger as well as frozen shoulder compared to controls; (ii) that the diabetes participants also had higher point prevalence of shoulder, hand and back stiffness than the controls: and lastly, (iii) that there was an association between joint stiffness and selected skin collagen AGEs and HbA1c.

There are few studies on type 1 diabetes and musculoskeletal complications. While we report both lifetime and point prevalence of the hand and shoulder diagnoses, previous studies only report lifetime prevalence. Larkin et al. studied the Diabetes Control and Complications Trial and Follow-up study (DCCT/EDIC) cohort (mean age 52 years, mean diabetes duration of 31 years) regarding hand and shoulder diagnoses.[3] They did not include a control group and the diagnostic criteria were not as comprehensive as the current study. They found a lifetime prevalence of frozen shoulder of 31%, DD 9%, trigger finger 28% and CTS 30%. Other studies of participants with type 1 diabetes of 22–29-year duration demonstrated a prevalence of frozen shoulder of 10–16%, 14%–30% for DD, 12% for trigger finger and 12% for CTS.[2,4] The current study revealed a higher prevalence of all diagnoses compared to these studies. The higher age profile, longer duration of diabetes, collection of historical information and differences in diagnostic definitions in this study, may contribute to these differences. Larkin et al. found a clear, but small, association between duration of diabetes, $HbA_{1c}$ and hand and shoulder diagnoses. Our cohort was selected based on a diabetes duration >45 years with a resulting interquartile range of only 7 years and it had a limited $HbA_{1c}$ range. This makes it difficult to draw any conclusion about the association between duration, $HbA_{1c}$ and hand and shoulder diagnoses.

There are several important clinical findings in our study. Hand and shoulder diagnoses are very common in long term type 1 diabetes and might be considered another long-term complication of type 1 diabetes. The high QuickDash score in the diabetes group mirrors the considerable clinical burden they place on these patients. Frozen shoulder can be very disabling and our recent data suggests that the condition lasts longer in patients with diabetes shown by a lifetime prevalence of 76% and point prevalence of 59%. In addition, these conditions lead to the use of extra health care resources as 44% had surgical intervention of these hand and shoulder disorders.

In the current study the point prevalence of shoulder stiffness (frozen shoulder) was 59%, hand stiffness (positive prayer sign) 50% and back stiffness 43% in the diabetes group. Previous studies have found a prevalence of hand stiffness of 20% and 60%.[3,4] Whereas the difference between the diabetes and control groups was substantial with regard to hand and shoulder stiffness, the diabetes group only experienced slightly more back stiffness compared to the controls. Diffuse idiopathic skeletal hyperostosis (DISH) which stiffens the spine through diffuse calcification and ossification of the ligaments and entheses has been suggested as a possible explanation of spinal stiffness in patients with diabetes,[1] but this was not examined in the present study. The fingertip-to-floor distance used as a measure for back stiffness reflects flexibility of several entities, including both the back and hips as well as the hamstring muscles and the minor difference observed between the patients with diabetes and controls should be interpreted with caution.

The present study is to our knowledge the first study examining the association between AGEs and joint stiffness with such an

**Table 2**
Logistic regression analyses with back stiffness, hand stiffness, shoulder stiffness and full stiffness as outcomes in the diabetes group.

| Variable | Back | | | Hand | | Shoulder | | Full stiffness | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Univariate | Adjusted | Full model | Univariate | Adjusted[b] | Univariate | Adjusted[b] | Univariate | Adjusted | Full model |
| Age | 1.0 (0.95–1.1) | | | 1.0 (0.9–1.0) | | 1.0 (0.9–1.05) | | 1.0 (0.9–1.1) | | |
| Duration | 1.0 (0.95–1.1) | | | 1.1 (0.99–1.2) | | 1.0 (0.9–1.1) | | 1.0 (0.9–1.2) | | |
| Male sex | 0.7 (0.3–1.5) | | | 0.8 (0.4–1.7) | | 1.9 (0.9–4.3) | | 0.6 (0.2–1.6) | | |
| BMI | 1,2 (1,0–1,3)*,[a] | | | 1.0 (0.9–1.1) | | 1.1 (0.98–1.2) | | 1.1 (0.9–1.2) | | |
| Alb.uria | 1.4 (0.5–4.1) | | | 1.5 (0.5–4.2) | | 1.0 (0.3–2.9) | | 1.6 (0.5–5.8) | | |
| Retinop | 1.5 (0.7–3.1) | | | 1.8 (0.9–3.7) | | 1.1 (0.6–2.3) | | 1.2 (0.5–3.1) | | |
| Neurop | 2,6 (1,1–6.2)* | | | 1.0 (0.4–2.2) | | 1.5 (0.7–3.4) | | 1.3 (0.4–3.8) | | |
| CVD | 1.2 (0.5–3.4) | | | 0.9 (0.3–2.3) | | 1.5 (0.6–4.2) | | 1.3 (0.4–4.4) | | |
| $HbA_{1c}$ | 1.8 (1.1–2.7)^,[a] | | 2.1 (1.2–3.6)^,[a] | 1.2 (0.8–1.8) | | 1.3 (0.8–1.9) | | 1.8 (1.0–3.0)* | | 2.0 (1.1–3.7)* |
| AGEread | 2.0 (0.9–4.7) | | | 1.4 (0.7–3.0) | | 0.8 (0.4–1.7) | | 1.2 (0.4–3.3) | | |
| CML | 1.4 (0.8–2.2) | | | 1.3 (0.8–2.1) | | 1.1 (0.7–1.8) | | 1.9 (0.99–3.5) | 1.9 (0.97–3.6) | |
| CEL | 0.8 (0.5–1.2) | 0.7 (0.5–1.1) | 0.4 (0.2–0.8)^,[a] | 1.0 (0.7–1.5) | | 1.3 (0.9–2.0) | | 0.8 (0.4–1.4) | | |
| MG-H1 | 0.8 (0.6–1.2) | | | 1.0 (0.7–1.4) | | 1.2 (0.8–1.8) | | 1.1 (0.7–1.8) | | |
| Fruc-lys | 1.7 (0.9–3.1) | | | 1.2 (0.7–2.1) | | 1.7 (0.9–3.1) | 1.6 (0.8–3.2) | 1.7 (0.8–3.7) | | |
| Glucosep | 1.4 (0.7–2.5) | | | 1.5 (0.8–2.6) | | 0.9 (0.5–1.6) | | 2.2 (0.9–5.1) | 1.8 (0.7–4.3) | |
| MOLD | 1,9 (1,2–3,1)^,[a] | 1.9 (1.2–3.1)^ | 2.2 (1.3–3.6))^,[a] | 1.1 (0.8–1.6) | | 1.1 (0.8–1.7) | | 1.6 (0.98–2.5) | 1.5 (0.95–2.5) | 1.7(1.0–2.7)* |
| Pentosid | 1.5 (0.97–2.2) | 1.5 (0.96–2.3) | 2.6 (1.4–4.7))^,[a] | 1.3 (0.9–1.9) | | 1.0 (0.7–1.5) | | 1.6 (0.97–2.7) | 1.7 (0.96–2.8) | 1.8 (1.0–3.2)* |
| LW-1 | 1.6 (1.1–2.4)* | 1.6 (1.0–2.4)* | | 1.5 (1.0–2.2)* | 1.5 (0.99–2.2) | 0.9 (0.6–1.3) | | 1.5 (0.9–2.4) | 1.5 (0.9–2.5) | |
| Comp. score[e] | 3.3 (1.6–6.8)^ | 3.3 (1.6–6.8)^ | 5.5 (2.3–13.0)^ | | | | | 3.3 (1.3–8.2)* | 3.4 (1.3–8.9)* | 3.4 (1.3–8.9)* |
| CEL | | | 0.4 (0.2–0.8)^ | | | | | | | |
| $HbA_{1c}$ | | | 2.1 (1.3–3.6)^ | | | | | | | 1. (1.0–3.5)* |

Results reported as OR (95% C.I.). In the adjusted analyses, only AGEs with $p < 0.2$ are shown. $HbA_{1c}$ = Estimated Full Duration $HbA_{1c}$. CVD = Cardiovascular disease. $HbA_{1c}$ and AGEs were analyzed as standardized values and the OR represents 1SD change.

[a] Still significant when applying Benjamini-Hochberg procedure.

[b] Full model for Hand and Shoulder stiffness: No significant findings.

[c] Composite score in the back analysis calculated from standardized values of MOLD, pentosidine, and LW-1. Composite score in the full model calculated from standardized values of CML, glucosepane, MOLD, pentosidine and LW-1.

* $p < 0.05$.

^ $p < 0.01$.

extensive panel of AGEs. We studied a total of 8 different AGEs, some of which are known to be cross-linkers of collagen. Apart from MOLD, all AGEs were about 1.5 to twice the level in the diabetes group compared to controls. Glucosepane is the most abundant AGE in collagen and its concentration has previously been measured at up to 2000 pmol/mg in controls at 100 years of age and up to 4000 pmol/mg in patients with diabetes at age 30–40 years.[26] AGEs have previously been shown to increase with age and duration.[23] Our diabetes group had a mean glucosepane level of 6480 pmol/mg of collagen, confirming the increase with age and diabetes duration.

Surprisingly, glucosepane, which is to date known as the main AGE cross-linker,[26,27] was not associated with stiffness in contrast to pentosidine and MOLD which are also cross-linkers, but present in small amounts. We found that MOLD, pentosidine and LW-1 were positively associated with joint stiffness. Although this was a cross-sectional study, the results support the hypothesis that AGE collagen cross-linking leads to structural and functional change of the peri-articular connective tissues with resulting joint stiffness.[28] Previous studies examining AGEs and joint stiffness have been small and analyzed only a few markers. Although Lyons et al. did not find a correlation between non-enzymatic glycation in skin collagen and limited joint mobility in 1985, Monnier et al. identified a correlation between collagen-linked fluorescence, pentosidine and limited joint mobility.[10,12,29] More precisely, McCance et al. found a tendency for the age-corrected pentosidine and fluorescence to increase with the severity of limited joint mobility, but the increase was significant only for grades 0 and 2 limited joint mobility, and not after control for diabetes duration.[11] In contrast to this study, they were unable to find a correlation between joint stiffness and serial $HbA_1$ measurements over a 6-year period. While our results could indicate that collagen AGE cross-linking leads to joint stiffness, a small ($n = 28$) study of patients with type 1 diabetes with and without DD did not show a major difference in the concentration of biochemical markers of synthesis and degradation of collagen type I and type III between the two groups.[30] This may point to other pathophysiological processes

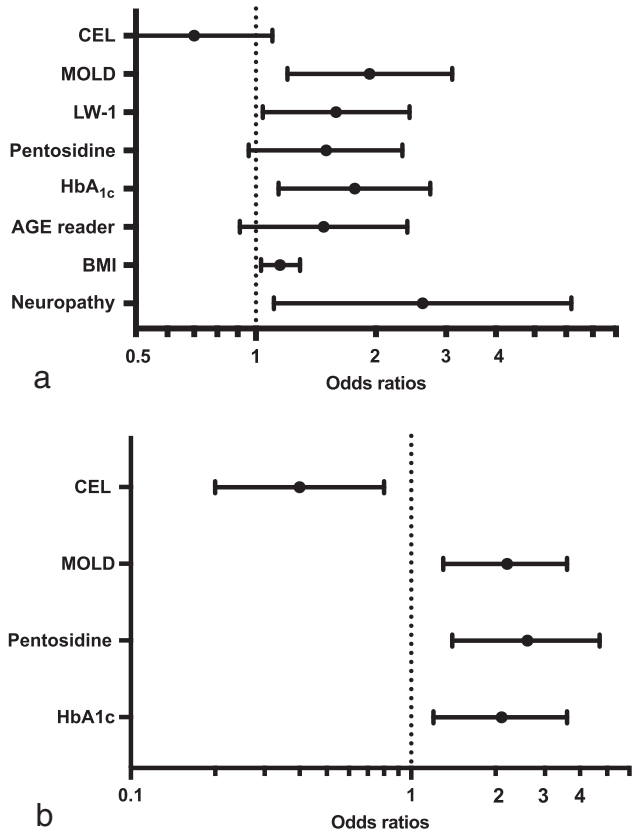


**Fig. 2.** Forest plots of the odds ratios of back stiffness in the diabetes group (and 95% CIs) for a 1SD change in collagen AGEs and Estimated Full Duration $HbA_{1c}$, and other relevant variables, (a) from the univariate/adjusted logistic regression model and (b) from the full model.

being important in patients with type 1 diabetes and joint stiffness, and further work is needed to clarify the mechanisms behind joint stiffness in type 1 diabetes.

In the present study, MOLD, pentosidine and Estimated Full Duration $HbA_{1c}$ were associated with full stiffness in the full model. However, an interesting finding was the strong association between back stiffness and AGEs, particularly with MOLD, both in the diabetes group and the control group (Fig. 2). This should be interpreted cautiously, as the difference in back stiffness between the groups was not as striking as with hand and shoulder stiffness, which did not correlate with AGEs in a similar way. In fact, we did not find any associations between AGEs and shoulder stiffness and only between LW-1 and hand stiffness in the univariate analysis. In addition, the concentration of MOLD was similar in patients with diabetes and controls. Stiffening joints is a known complication of ageing, and AGE cross-linking of collagen is thought to contribute to this process independently of diabetes.[31] As such, our findings could be relevant to the general population.

Another interesting finding is the inverse association between CEL and back stiffness. As CEL can be formed through the reaction between methylglyoxal and lysine residues,[32] a possible explanation is the blockage of lysine residues by CEL preventing the formation of deleterious lys-lys MOLD crosslinks. Nevertheless, MOLD is present in low amounts and its formation is unlikely to fully explain the increased joint stiffness seen in diabetes. Methylglyoxal, whose formation is enhanced in diabetes,[33] and is considered to be important in other long-term complications,[34] is the precursor for MOLD formation. There may exist other, so far unidentified, AGE cross-linkers formed in a similar manner which contribute to stiffness. On the other hand, the association of back stiffness with pentosidine could suggest that increased ascorbic acid oxidation correlates with stiffness.[35] Estimated Full Duration $HbA_{1c}$ was associated with full stiffness and back stiffness in the diabetes group and cross sectional $HbA_{1c}$ with back stiffness in the control group. Accordingly, hyperglycemia may be an important part of the pathological process. However, the fact that neither glucosepane, fructose-lysine or microvascular complications were associated with stiffness, suggests that the pathogenesis of stiffness is different from microvascular complications.[14]

Our study has several limitations. It is a cross-sectional study and can therefore not give causal answers with regard to underlying mechanisms. Secondly, the control group was similar to the diabetes group with regard to distribution of age, gender and education level, but it was relatively small. Most members of the control group were spouses who were likely to have a similar lifestyle to their husband/wife. A similar intake of exogenous AGEs cannot be ruled out which could possibly have some influence on the total collagen AGEs level.[36] Nevertheless, the regression analyses were done separately for the two groups. Our exposure of interest was chronic hyperglycemia and the endogenous formation of AGEs. While the similar levels of MOLD may theoretically be explained by a similar intake of exogenous AGEs, all other AGEs were much higher in the diabetes group emphasizing the importance of chronic hyperglycemia and the endogenous production of AGEs (Table 1). Thirdly, we do not know whether the concentration of AGEs in skin collagen is an accurate marker for the concentration in other tissues more relevant to joint mobility, such as tendons, ligaments, muscles and cartilage. The rate of turnover of the protein is an important factor for AGE synthesis and whereas the half-life of skin collagen is about 15 years, both tendon collagen and cartilage collagen (117 years) half-lives are much longer.[37,38] The concentration of pentosidine has indeed been shown to be higher in tendon collagen than skin collagen.[39] There is some conflicting evidence that AGE collagen crosslinking of different tissues lead to increased stiffness.[39–42] Strengths of this study are extensive examination methodology, the presence of a control group, availability of $HbA_{1c}$ measurements for 30 years for most participants and the assessment of 8 different collagen AGEs.

## 5. Conclusions

In conclusion, we found a very high lifetime prevalence of musculoskeletal hand and shoulder diagnoses in patients with type 1 diabetes of a long duration versus in controls, and a high point prevalence of stiffness of the hand, shoulder and back. These diagnoses are associated with increased symptoms and disability of the upper limbs. This study also shows an association between both $HbA_{1c}$ and AGEs with joint stiffness, in particular MOLD and pentosidine with back stiffness. This should be interpreted cautiously, as the difference in back stiffness between the groups was not as striking as with hand and shoulder stiffness, which did not correlate with AGEs in a similar way. Nevertheless, our results suggest that the pathogenesis of stiffening joints is different from the cause of microvascular complications in diabetes. The precise role of AGEs in tissue stiffening is still not settled and more research is needed to fully understand the pathogenesis of matrix stiffening in limited joint mobility in type 1 diabetes.

## Acknowledgements

The authors thank Anne Karin Molvær, research nurse and the other staff at the Norwegian Diabetics Center for administrative help. The authors also thank Morten Valberg and Cathrine Brunborg for help with statistical analyses (University of Oslo, Center for biostatistics and epidemiology).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jdiacomp.2017.06.007.

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