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## Hyaluronan Levels are Increased Systemically in Human Type 2 but not Type 1 Diabetes Independently of Glycemic Control

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### Abstract

Hyaluronan (HA), an extracellular matrix glycosaminoglycan, is implicated in the pathogenesis of both type 1 diabetes (T1D) as well as type 2 diabetes (T2D) and has been postulated to be increased in these diseases due to hyperglycemia. We have examined the serum and tissue distribution of HA in human subjects with T1D and T2D and in mouse models of these diseases and evaluated the relationship between HA levels and glycemic control. We found that serum HA levels are increased in T2D but not T1D independently of hemoglobin-A1c, C-peptide, body mass index, or time since diabetes diagnosis. HA is likewise increased in skeletal muscle in T2D

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Author contributions

Conceptualization, NN, PLB; Investigation, NN, VSG, GK, SH, CS; Visualization, NN, PLB; Data/sample requisition, NN, VSG, GK, SH, DNL, CS, SS, TLML, SRL; Writing, NN, PLB; Funding, NN, GK, VGS, PLB; Supervision, NN, TNW, PLB. All authors reviewed the results and approved the final version of the manuscript.

Declaration of interest

PLB and NN are co-founders of Hyalos Therapeutics, a company developing novel small molecules to inhibit HA synthesis.

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subjects relative to non-diabetic controls. Analogous increases in serum and muscle HA are seen in diabetic db/db mice (T2D), but not in diabetic DORMO mice (T1D). Diabetes induced by the  $\beta$ -cell toxin streptozotocin (STZ) lead to an increase in blood glucose but not to an increase in serum HA. These data indicate that HA levels are increased in multiple tissue compartments in T2D but not T1D independently of glycemic control. Given that T2D but not T1D is associated with systemic inflammation, these patterns are consistent with inflammatory factors and not hyperglycemia driving increased HA. Serum HA may have value as a biomarker of systemic inflammation in T2D.

## Keywords

hyaluronan; diabetes; T1D; T2D; biomarker

## Introduction

Hyaluronan (HA) is an extracellular matrix (ECM) polymer and glycosaminoglycan [1]. It is a long, unbranching polymer made of repeating disaccharides (glucuronic acid and N-acetylglucosamine). In healthy tissues, HA is produced by a diverse range of cells and tissues and is incorporated into tissues such as joints [2], basement membranes and the vitreous of the eye [3]. At these sites, it contributes to lubrication, acts as a structural component, and space filler [4]. HA bound within tissues interacts with a number of proteins that modify its function and longevity [1, 5–7].

In inflamed tissues, the amount of HA is greatly increased [3]. Cellular HA production *in vitro* is increased in response to a variety of pro-inflammatory cytokines [8, 9], microbial products [9, 10], hyperglycemia [11, 12], and other triggers [10, 13], and the presumption is that these factors also drive HA synthesis *in vivo*. HA undergoes extensive catabolism mediated by endogenous hyaluronidases, by bacterial hyaluronidases, by mechanical forces, and by oxidative stress [14]. Together, this increased synthesis and catabolism lead to the accumulation of HA fragments within inflamed tissues and tumors [15–19]. Increased HA may contribute to local inflammatory processes. HA fragments promote cellular activation, migration, and proliferation [1, 3, 20–24] via interactions with its receptors [25], including RHAMM [26], CD44 [27], and Toll-like receptors 2 and/or 4 [28].

Upon the resolution of inflammation HA fragments are cleared while in the setting of chronic inflammation, HA accumulates [29]. Abundant local HA characterizes chronic inflammation in many tissues including liver cirrhosis, asthma, and other diseases [30–33]. Reflecting this, serum HA is used as a biomarker of liver cirrhosis [34] and primary sclerosing cholangitis [35]. The amount and size of HA at sites of inflammation may thereby provide contextual information that reflects the stage of injury and its stage of resolution [17, 36].

HA has been implicated in the pathogenesis of autoimmune, type 1 diabetes (T1D). In healthy islets, HA is a normal component of the peri-islet and intra-islet regions adjacent to micro-vessels [37]. The development of autoimmune insulinitis is associated with substantial changes in the islet ECM and deposition of intra-islet HA [38, 39]. In human T1D tissue

samples from cadaveric organ donors obtained through the JDRF nPOD program, HA deposits were found to be present in islets from recent-onset T1D donors but not in non-diabetic controls [40]. Further, HA deposits were both temporally and anatomically associated with autoimmune insulinitis in both T1D and in the DORmO mouse model of the disease. The DORmO mice are the offspring of DO11.10 and RIPmOVA transgenic mice. They carry a T-cell receptor transgene specific for OVA (emulating auto-reactive CD4<sup>+</sup> T-cells) while simultaneously expressing OVA in conjunction with the insulin gene promoter on pancreatic b-cells (emulating the auto-antigen) [41]. In diabetic humans and mice the amount and distribution of HA closely tracked with the infiltration of CD3<sup>+</sup> T-cells and the disappearance of insulin staining. HA was not increased within neighboring islets without active insulinitis [41]. We and others have reported that interventions that clear HA or prevent its synthesis can prevent autoimmune diabetes in mice [41–44].

HA is also implicated in the pathogenesis of type 2 diabetes (T2D). HA deposition in T2D has been noted in skeletal muscle [45], adipose tissue [46, 47], and other tissues of obese and diabetic animals [48–52] as well as at sites of diabetes-associated pathology, such as vascular lesions [51, 53]. The T2D mouse model the db/db mice are leptin receptor deficient and uniformly exhibit severe obesity and hyperglycemia [54]. In humans and mice serum HA levels are likewise increased in T2D [51]. HA in peripheral tissues has been implicated in insulin resistance, as treatment with a recombinant hyaluronidase [45] or anti-CD44 antibodies [46] improve insulin sensitivity.

The finding that HA contributes in different ways to the pathogenesis of both T1D and T2D raises basic questions about the tissue distribution of HA, the role of hyperglycemia in this accumulation, and the specificity of serum HA as a biomarker for T2D versus T1D.

Here, we have asked how T1D and T2D status and glycemic control impact tissue deposition of HA. For these assessments, we have studied serum and skeletal muscle from human subjects with T1D, T2D or non-diabetic controls, as well as the analogous tissues collected from mouse models of these diseases.

## Results

### Serum and muscle HA concentrations are not increased in a mouse model of T1D

We first compared serum HA in the DORmO mouse model of T1D versus healthy, strain matched controls. At 15 weeks of age, DORmO mice had blood glucose levels over 250 mg/dL and were considered diabetic (Fig. 1A). At 10 weeks of age, we observed a significant increase in HA accumulation in the pancreatic islets of DORmO mice compared to BalbC controls (Fig. 1B,C).

We further examined the skeletal muscle of 15 week old mice for HA content and found no significant difference between the DORmO and BalbC mice in histologic muscle sections stained for HA (Fig. 1D,E) or biochemically when determining the HA content of the muscle (Fig. 1F). The muscle HA content of the DORmO mice was 17.3 ± 1.8 ng HA/mg muscle versus 19.6 ± 0.9 ng HA/mg muscle for the BalbC controls (Fig. 1F).

We also collected serum from these animals and examined their HA content. We found that serum HA levels were not significantly elevated in 15 week old DORmO mice (514.0 +/- 71.1 ng/mL) compared to BalbC controls (384.5 +/- 31.6 ng/mL) (Fig. 1G).

These data indicate that serum and muscle HA are not increased in a mouse model of T1D but that HA is increased at the primary site of inflammation in this disease - the pancreatic islet.

### **Serum and muscle HA concentrations are increased in a mouse model of T2D**

We next asked whether serum HA was elevated in the db/db mouse model of T2D compared to strain matched C57B16 controls. By 6 weeks of age db/db mice had blood glucose levels over 250 mg/dL and were considered diabetic (Fig. 2A). We then collected serum from these animals and examined the HA content. Serum HA levels were significantly higher in 16 week old db/db mice (854.3 +/- 37.1 ng/mL) compared to their C57B16 controls (304.8 +/- 68.6 ng/mL) (Fig. 2B).

In skeletal muscle tissues from 16 week old db/db and control mouse muscle sections stained for HA we found that HA was increased in the db/db mice. This was seen by histologic assessments (Fig. 2C,D) as well as by biochemical measurements (Fig. 2E). The muscle HA content of the db/db mice was with 108.1 +/- 14.5 ng HA/mg muscle significantly higher than the muscle HA content of the C57B16 control mice with 48 +/- 7.7 ng HA/mg muscle (Fig. 2E).

Together these data indicate that serum and muscle HA are increased in a mouse model of T2D but not in a mouse model of T1D.

### **STZ treatment induces hyperglycemia in mice but HA serum levels are not significantly elevated**

We also asked whether serum HA was elevated in C57B16 mice after treatment with STZ, a  $\beta$ -cell specific toxin. After administration of two consecutive high doses (200 mg/kg) of STZ, mice were diabetic within one week. Seven days after STZ administration we observed that blood glucose level were significantly increased (307.4 +/- 10.5 mg/dL) in the STZ treated group compared to the untreated controls (91.4 +/- 8.4 mg/dL) (Fig. 3A).

We also saw a significant increase of pancreatic islet HA after STZ treatment compared to the non STZ treated mice (Fig. B,C). Serum HA level on the other hand remained unchanged after STZ treatment (220.7 +/- 18.1 ng/mL for controls vs 252.1 +/- 15.0 ng/mL for STZ treated mice) (Fig. 3D).

These data suggest that hyperglycemia does not immediately result in serum HA elevation.

### **Serum HA concentrations are not elevated in T1D patients**

We next sought to determine whether our findings in mouse models of diabetes would be mirrored in human subjects with T1D or T2D. For these studies, blood samples were drawn from a total of 60 patients (n = 20 T1D patients, n = 20 T2D patients and n = 20 non-diabetic controls). Groups were matched for age (all between 20–45 years), gender (each

group had n = 10 male and n = 10 female patients), and HbA1c (mean 7.5 % (T1D) - 7.1 % (T2D)). Mean C-peptide levels were 3.7 ng/mL for the T2D patients; for the T1D subjects C-peptide data were not available. The body mass index (BMI) from non-diabetic controls (BMI 27.9) was found to be between T1D (BMI 22.8) and T2D (BMI 32.7) patients. None of the patients had any comorbidity. These data are shown in Table 1.

We observed that serum HA levels in T1D subjects (219.4 +/- 18.5 ng/mL) were no different from those in non-diabetic controls (216.6 +/- 17.3 ng/mL) (Fig. 4A)(Table 2). There was no correlation between serum HA levels and HbA1c in T1D patients (Fig. 4B). We did observe a slight decrease of HA with increasing BMI in T1D patients (Fig. 4C). There was no correlation between serum HA levels and duration of T1D (Fig. 4D).

Together these data mirror our findings in the serum of mice with autoimmune diabetes and these data strongly refute the hypothesis that serum HA reflects long-term hyperglycemia. In regards to HA at other sites, we previously reported that islet HA is increased in human subjects with T1D within 1 year of diagnosis with the disease but not in T1D subjects with long standing (> 1 year) disease [40]. Unfortunately, it was not possible to obtain muscle tissue from individuals with T1D.

#### **HA levels are elevated in the serum and muscle of individuals with T2D**

We observed that serum HA levels were significantly higher in T2D subjects (330.6 +/- 24.7 ng/mL) than in non-diabetic controls (216.6 +/- 17.3 ng/mL)(Fig. 5A). However, we found no correlation between HA levels and HbA1c in T2D patients (Fig. 5B). We also found no correlation between HA levels and C-peptide levels (Fig. 5C), BMI (Fig. 5D), or the duration of T2D (Fig. 5E) in these subjects.

We next sought to determine whether HA in muscle tissue was increased in T2D patients. To this end, we examined discarded surgical biopsy specimens collected from individuals with or without T2D following amputations of lower extremity tissues due to peripheral vascular disease. We found that the percentage area positive for HA was significantly increased in T2D patients relative to non-diabetic controls (Fig. 5G).

Together, these data indicate that both serum and muscle HA are elevated in human and mouse T2D (Table 2).

## **Discussion**

We report three notable findings from these studies. First, HA is increased in the serum and muscles of humans with T2D as well as in the db/db mouse model of the disease. This corroborates previous reports linking increased tissue HA to T2D [45, 51, 55] and extends this finding to human skeletal muscle, the major tissue type involved in glucose uptake.

Second, we report that HA is not increased in the serum of individuals with T1D or in the DORmO mouse model of the disease. HA has been implicated in a number of autoimmune diseases like lupus [56], inflammatory bowel disease [57–60], autoimmune hepatitis [61] and others [62–64]. More recent studies show, that the local tissue environment is thought to contribute to immune regulation and the development of T1D [65–67], and that T1D is

associated with substantial changes in the islet ECM and deposition of intra-islet HA [68]. Together with our previous report that autoimmune insulinitis in T1D was associated with islet-specific deposition of HA [40, 41], the data presented here suggest that HA accumulates at the primary site of inflammation in T1D – the pancreatic islet – but not in distant sites, such as the serum or skeletal muscle. This pattern is consistent with T1D being a disease of local, isletspecific inflammation.

Third, we report that HA levels in muscle and tissue are not correlated with hyperglycemia, as measured by HbA1c. Serum HA levels in T2D are also not correlated with insulin levels, as measured by C-peptide, obesity, as measured by BMI, or with age, gender, or duration of disease.

It has been shown, that the total amount of serum HA in individuals with diseases like arthritis [69] and cancer [70, 71] is elevated [72]. We also know, that not only the total amount of HA is important but also its size. HA size has been called a natural biosensor for the state of tissue integrity [14]. For instance, it was discovered, that low molecular mass serum HA can be used to differentiate metastatic and non-metastatic breast cancer [73]. Also, the serum of healthy individuals usually contains low molecular mass between 100–300 kDa [74], and it is reported that even lower molecular mass HA, <50 kDa, is found in the serum of cancer patients [73]. Furthermore, it is known that HA breakdown products predominate during injury and inflammation [20]. These findings are in line with the elevated human serum HA data found in the inflammatory setting of T2D.

Together, these data suggest that serum and tissue HA may be elevated in diabetes in response to inflammation rather than hyperglycemia. In light of this, serum HA levels may have value as a potential biomarker for systemic inflammation in T2D independent of glycemic control.

We and others have previously reported that a great diversity of inflammatory factors, including cytokines and microbial products, drive HA synthesis [8, 9, 75]. The specific factors that promote HA synthesis in T2D and T1D are unknown but will be important to identify given reports linking HA and its receptors in the pathogenesis of these diseases [46, 47, 76].

In conclusion, these data support the use of circulating HA as a biomarker in T2D and suggest that its elevation may reflect inflammatory aspects of the disease that are distinct from glycemic control, insulin production, or BMI.

## Methods

### Human tissue donors and tissue procurement

Serum samples from 20, auto-antibody positive, T1D patients aged between 20 and 45 years, with disease duration of less than 1 year were collected. C-peptide data were not available from those T1D patients. Serum samples were also collected from 20 T2D patients aged between 20 and 45 years, and 20 age-matched autoantibody-negative healthy individuals. These samples were obtained with informed consent, under the auspices of a research

protocol approved by the Benaroya Research Institute institutional review board. Clinical characteristics of donors are presented in Table 1.

Human muscle tissues were obtained from de-identified, discarded surgical specimens collected from T2D subjects or controls. These were provided through the Stanford Pathology Department in the form of formalin-fixed, paraffin-embedded histologic specimens. This study was carried out with the approval of the Institutional Review Board of Stanford University.

## Mice

All animals were bred and maintained under specific pathogen-free conditions, with free access to food and water, in the vivarium at Stanford University. DO11.10 transgenic mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and bred with BalbC mice expressing RIPmOVA (available at the Benaroya Research Institute) to generate the DORmO double-transgenic mice. Both C57Bl6 mice and Leptin receptor-deficient, db/db mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All experiments and animal use procedures were approved by the Animal Care & Use Committee at Stanford University Medical School.

Weight and diabetes monitoring were performed on these mice. DORmO and db/db mice were weighed weekly as well as bled *via* the tail tip for the determination of their blood glucose level (BG) using a Contour blood glucose meter and blood glucose monitoring strips (Bayer Healthcare, Tarrytown, NY). When two consecutive blood glucose readings of 250 mg/dL were recorded, animals were considered diabetic.

C57Bl6 mice were treated two times with STZ at 200 mg/kg intraperitoneally. STZ was administered within 10 min of its dissolution. Mice in the untreated control group received citrate buffer (vehicle) alone. Blood glucose in those experimental groups was monitored daily. Mice were sacrificed following the development of hyperglycemia.

Serum, pancreas and skeletal muscle samples were collected. Serum samples were collected through cardiac puncture from deceased mice immediately after euthanization with CO<sub>2</sub>. Murine skeletal muscle was isolated from the quadriceps tissue and immediately transferred into methyl carnoys (MC) fixation. Tissue for paraffin embedding was processed using a Leica ASP300 Tissue Processor (Leica Microsystems Inc., Buffalo Grove, IL).

## Measurement of serum levels of HA

Both human as well as murine serum samples were thawed and subsequently assayed for HA levels using a modified HA Enzyme-Linked Immunosorbent Assay (ELISA) [77]. Each sample was analyzed in triplicate with a mean value obtained for each individual.

## Histologic staining of skeletal muscle and pancreas tissues for HA

For both human and murine muscle and murine pancreas tissue, 5 µm thick sections were cut on a Leica RM 2255 Microtomes (Leica Microsystems Inc.). All staining steps were performed on a Leica Bond Max™ automated immune histochemistry (IHC) stainer (Leica Microsystems Inc.). For HA affinity histochemistry (AFC) the Bond Intense R Detection kit,



a streptavidin-horse radish peroxidase (HRP) system, (Leica Microsystems, Inc.) was used with 4 µg/mL biotinylated-HABP in 0.1 % BSA-PBS as the primary. The Bond Polymer Detection Kit was used for all other immunohistochemistry. This detection kit contains a goat anti-rabbit conjugated to polymeric HRP and a rabbit anti-mouse post primary reagent for use with mouse primaries.

All images were visualized using a Leica DMIRB inverted fluorescence microscope equipped with a Pursuit 4-megapixel cooled color/monochrome charge-coupled device camera (Diagnostic Instruments, Sterling Heights, MI). Images were acquired using the Spot Pursuit camera and Spot Advance Software (SPOT Imaging Solutions; Diagnostic Instruments). Image analysis was performed using Image J (NIH), as described previously [40].

### Biochemical Quantification of HA in skeletal muscle

Tissues were first lyophilized and weighed, then digested with proteinase K (250 µg/mL) in 100 mM ammonium acetate pH 7.0 overnight at 60°C. After digestion, the enzyme was inactivated by heating to 100°C for 20 minutes. Total amount of HA was determined by a modified competitive ELISA in which the samples to be assayed were first mixed with biotinylated HA-binding protein (b-HABP) and then added to HA-coated microtiter plates, the final signal being inversely proportional to the level of hyaluronan added to the biotinylated HABP [78].

### Statistical analysis

Data are expressed as means  $\pm$  SEM of n independent measurements. Significance of the difference between the means of two or three groups of data was evaluated using the Mann-Whitney U test or one-way Anova, respectively. The statistical significance of differences in serum hyaluronan levels between T1D, T2D and healthy donors was calculated by the Kruskal-Wallis test. Correlation analysis was performed using the non-parametric Spearman correlation test. A p value less than  $<0.05$  was considered statistically significant.

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### Abbreviations

HA                      hyaluronan

<b>ECM</b>	extracellular matrix
<b>JDRF</b>	Juvenile Diabetes Research Foundation
<b>nPOD</b>	National Pancreatic Organ Donor
<b>STZ</b>	streptozotocin
<b>T1D</b>	Type 1 Diabetes
<b>T2D</b>	Type 2 Diabetes

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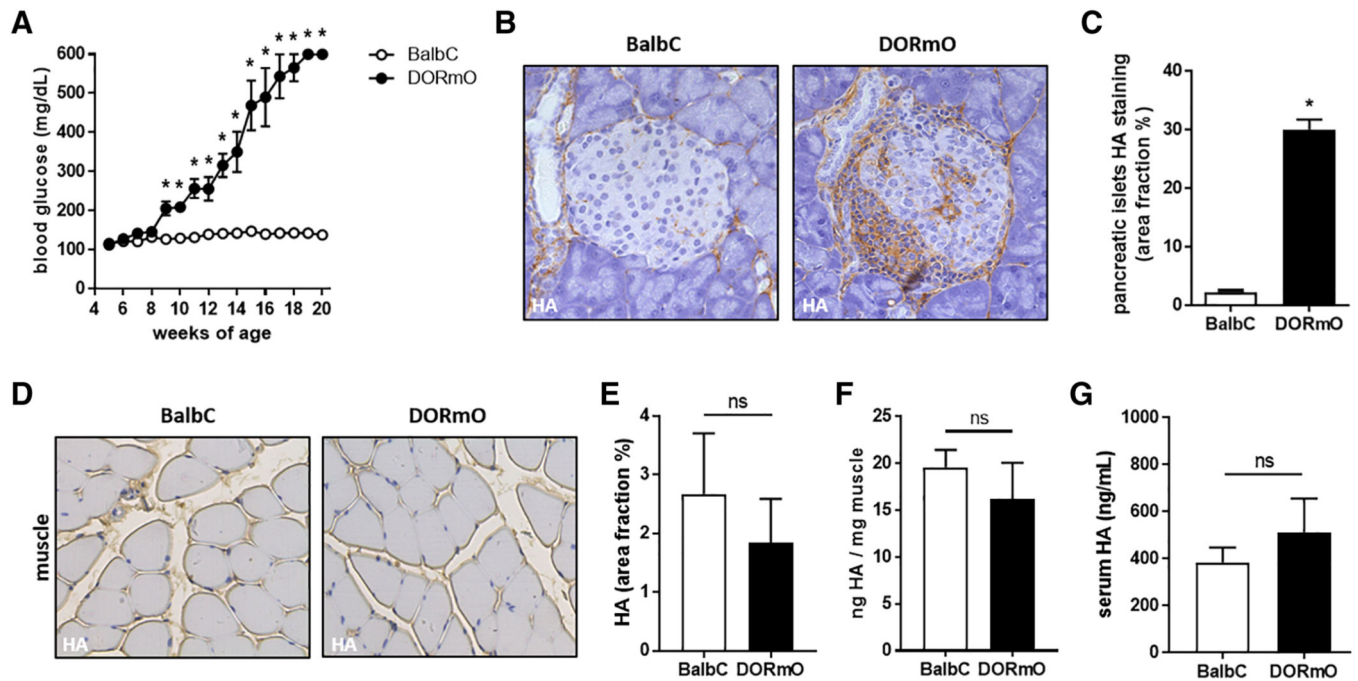
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### Highlights

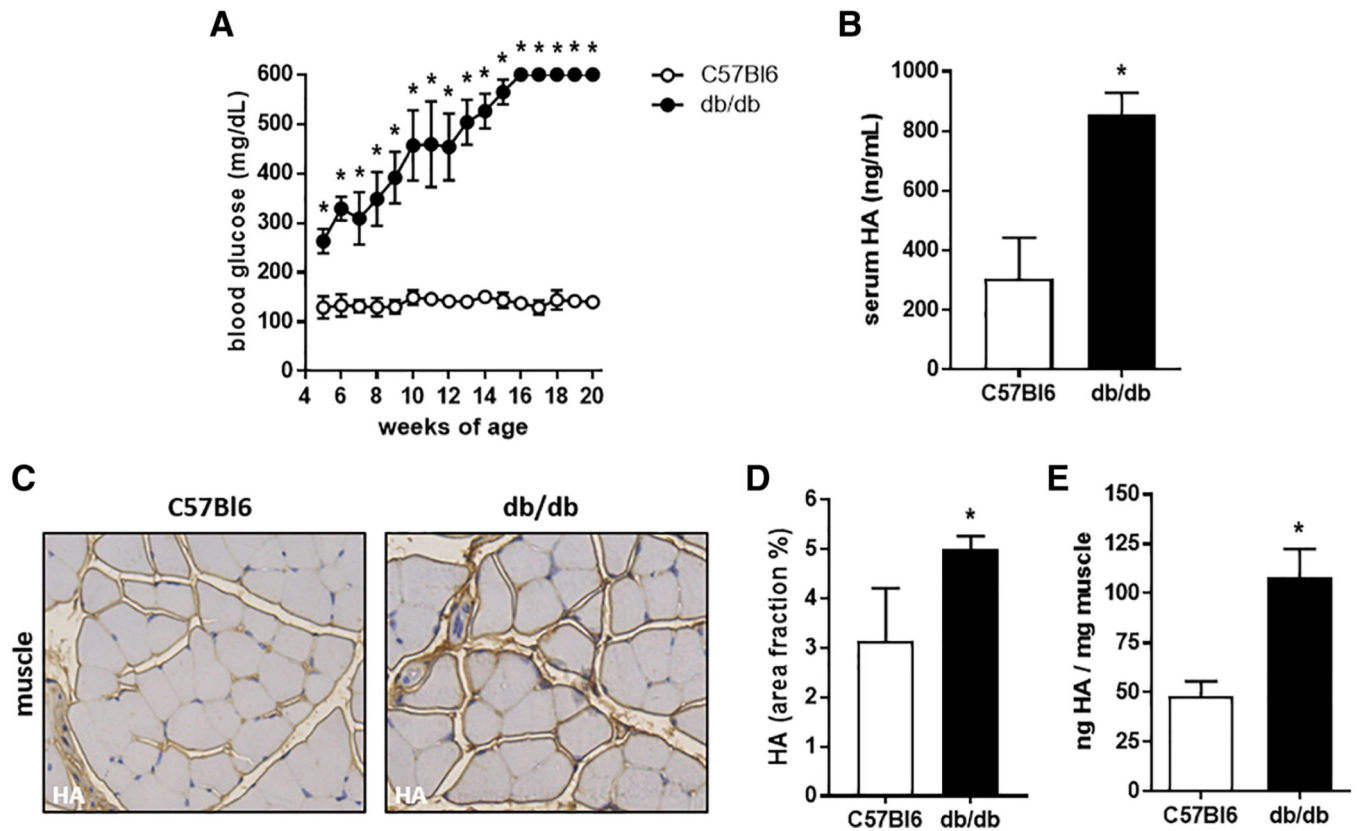
Hyaluronan (HA) is an extracellular matrix polymer that has been implicated in the pathogenesis of both type 1 diabetes (T1D) and type 2 diabetes (T2D). In T1D, HA accumulates at sites of autoimmune insulinitis and has been implicated in local immune dysregulation. In T2D, HA is increased in multiple tissues and has been linked to insulin resistance. *In vitro*, hyperglycemia is one pro-inflammatory factor that drives HA synthesis by multiple cell types raising the possibility that increased tissue HA in these diseases is a consequence of poor glycemic control. We have asked how glycemic control and T1D and T2D status impact tissue deposition of HA. We report that HA is increased in the serum and muscles in T2D but not in T1D. We find no relationship between serum HA and glycemic control, insulin production, or BMI. Together these results suggest that inflammatory factors rather than hyperglycemia drive local HA accumulation in diabetes. These data support the use of circulating HA as a biomarker in T2D and suggest that its elevation occurs independently of glycemic control, insulin production, or BMI.



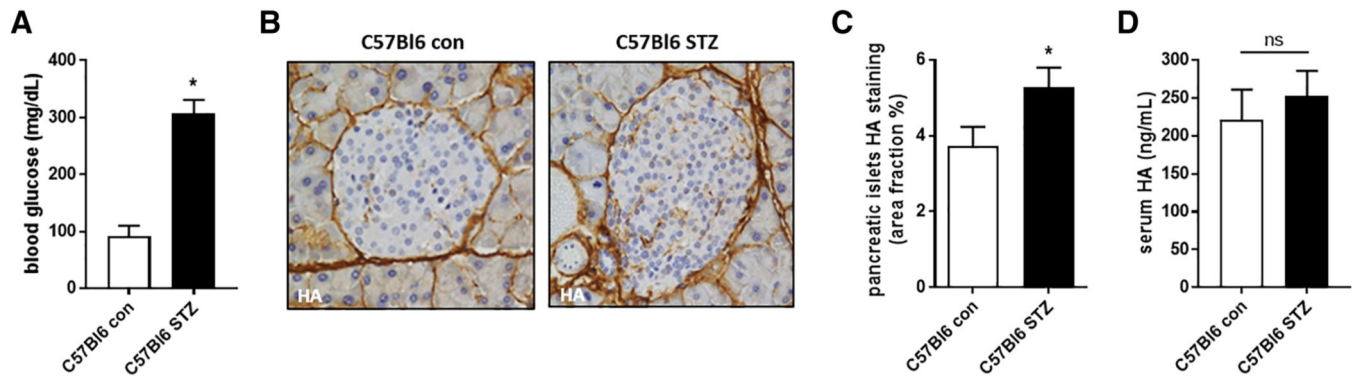
**Figure 1: Serum and muscle HA concentrations are not increased in a T1D mouse model.**

**A:** Blood glucose of DORmO and BalbC mice from 4 to 20 weeks of age. **B:** Representative images of pancreatic islets from DORmO and BalbC mice stained for HA, in brown. **C:** Quantification of HA accumulation in pancreatic islets from IHC images. **D:** Representative images of skeletal muscle images from DORmO and BalbC mice stained for HA, in brown. **E:** Quantification of HA accumulation in skeletal muscle from IHC images. **F:** Quantification of HA content of skeletal muscle from DORmO and BalbC mice. **G:** Serum HA level from DORmO and BalbC mice. N = 5–10 mice per group. Data represent mean +/– SEM, \*P < 0.05 vs the respective control by unpaired t test.



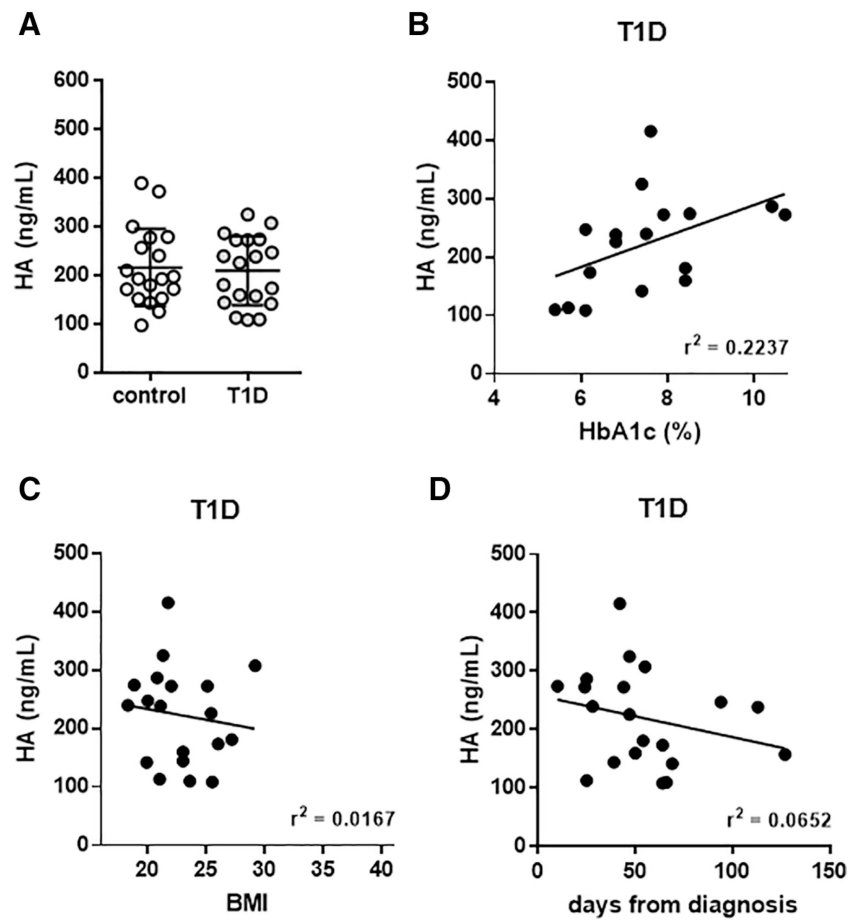


**Figure 2: Serum and muscle HA concentrations are significantly elevated in a T2D mouse model.** **A:** Blood glucose of db/db and C57Bl6 mice from 4 to 20 weeks of age. **B:** Serum HA level from db/db and C57Bl6 mice. **C:** Representative images of skeletal muscle images from db/db and C57Bl6 mice stained for HA, in brown. **D:** Quantification of HA accumulation in skeletal muscle from IHC images. **E:** Quantification of HA content of skeletal muscle from db/db and C57Bl6 mice. N = 5–10 mice per group. Data represent mean  $\pm$  SEM, \*P < 0.05 vs the respective control by unpaired t test.



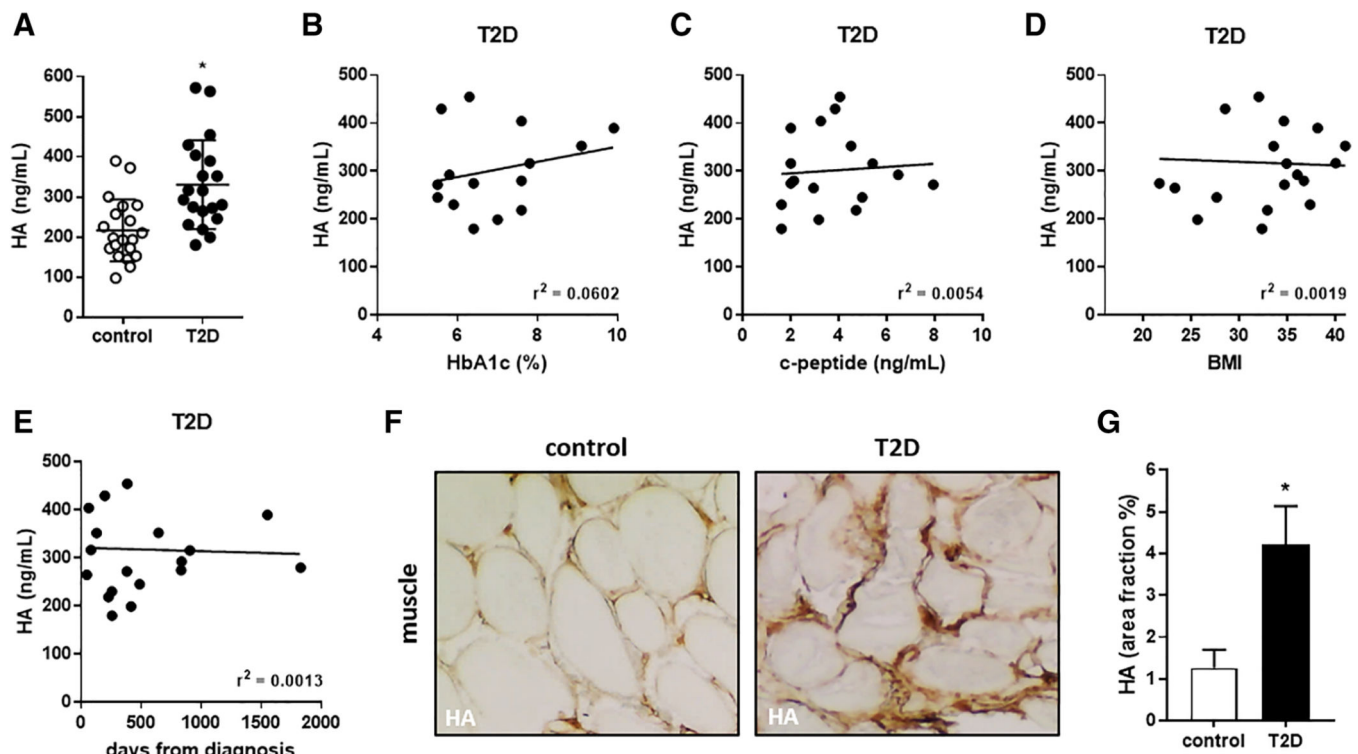
**Figure 3: STZ treatment induces hyperglycemia in mice but HA serum levels are not significantly elevated.**

**A:** Blood glucose of C57Bl6 mice with and without STZ treatment. **B:** Representative images of pancreatic islets from C57Bl6 mice with and without STZ treatment. **C:** Quantification of HA accumulation in pancreatic islets from IHC images. **D:** Serum HA measurements from STZ treated C57Bl6 mice and their untreated controls. N = 5–10 mice per group. Data represent mean  $\pm$  SEM, \*P < 0.05 vs the respective control by unpaired t test.



**Figure 4: Serum and muscle HA concentrations are not elevated in T1D patients.**

**A:** Serum HA measurements in T1D and healthy control subjects. **B:** Relationship between serum HA and HbA1c. Mean serum HA values for each individual in the study are shown in conjunction with the HbA1c level measured for that given individual at the time of blood draw. **C:** BMI and (**D**) days from diagnosis are shown in correlation with HA. N = 1720 per group. Data in A represent mean  $\pm$  SEM, data in B-D are shown as regression plots. \*P < 0.05 vs control by unpaired t test.



**Figure 5: Serum and muscle HA concentrations are elevated in T2D patients.**

**A:** Serum HA measurements in T2D and healthy control subjects. **B:** Relationship between serum HA and HbA1c and **(C)** c-peptide. Mean serum HA values for each individual in the study are shown in conjunction with the HbA1c and c-peptide level measured for that given individual at the time of blood draw. **D:** BMI and **(E)** days from diagnosis are shown in correlation with HA.  $N = 17-20$  per group. **F:** Representative images of muscle sections from healthy donors and T2D patients stained for HA, shown in brown. **G:** Quantification of HA muscle staining shown as % area fraction.  $N = 5$  individuals per group. Data in **A** and **G** represent mean  $\pm$  SEM, data in **B-E** are shown as regression plots. \* $P < 0.05$  vs control by unpaired t test.

**Table 1:**  
**Characteristics of the subjects from whom serum was collected.**

Blood was drawn from a total of 20 patients per group. The group consisted of n = 10 male and n = 10 female patients. The age at time of blood draw was between 20–45 years. For the T1D and T2D patients the days since diagnosis, the HbA1c and C-peptide serum level as well as BMI were reported. None of the patients had any comorbidity. Data are represented as mean  $\pm$  SEM.

	Healthy Controls (n=20)	T1D (n=20)	T2D (n=20)
Sex (M/F)	10/10	10/10	10/10
Age (years)	20–45	20–45	20–45
Days since diagnosis	N/A	60 $\pm$ 32	645 $\pm$ 547
HbA1c (%)	N/A	7.5 $\pm$ 1.5	7.1 $\pm$ 1.3
C-peptide (ng/mL)	N/A	N/A	3.7 $\pm$ 0.4
BMI	27.9 $\pm$ 1.1	22.8 $\pm$ 0.7	32.7 $\pm$ 1.3
Comorbidity (i.e. renal failure, retinopathy)	None	None	None

**Table 2:**  
**Overview of HA accumulation at different diabetic sites in human and mouse.**

The HA content in human and mouse T1D is elevated in pancreatic islets. In T2D HA in human and mouse is elevated in the serum and muscle. STZ treatment in mice increased the islet HA content, but did not significantly change the HA content in serum.

HA content	T1D	T2D	STZ (toxin)
Serum	↔	↑	↔
Muscle	↔	↑	N/A
Islets	↑	N/A	↑

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