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# Arterial heparan sulfate is negatively associated with hyperglycemia and atherosclerosis in diabetic monkeys

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## Abstract

**Background:** Arterial proteoglycans are implicated in the pathogenesis of atherosclerosis by their ability to trap plasma lipoproteins in the arterial wall and by their influence on cellular migration, adhesion and proliferation. In addition, data have suggested an anti-atherogenic role for heparan sulfate proteoglycans and a pro-atherogenic role for dermatan sulfate proteoglycans. Using a non-human primate model for human diabetes, studies examined diabetes-induced changes in arterial proteoglycans that may increase susceptibility to atherosclerosis.

**Methods:** Control (n = 7) and streptozotocin-induced diabetic (n = 8) cynomolgous monkeys were assessed for hyperglycemia by measurement of plasma glycated hemoglobin (GHb). Thoracic aortas obtained at necropsy, were extracted with 4 M guanidine HCL and proteoglycans were measured as hexuronic acid. Atherosclerosis was measured by enzymatic analysis of extracted tissue cholesterol. Glycosaminoglycan chains of arterial proteoglycans were released with papain, separated by agarose electrophoresis and analysed by scanning densitometry.

**Results:** Tissue cholesterol was positively associated with hexuronic acid content in diabetic arteries ($r = .82$, $p < .025$) but not in control arteries. Glycosaminoglycan chain analysis demonstrated that dermatan sulfate was associated with increased tissue cholesterol in both control ($r = .8$, $p < 0.05$) and diabetic ($r = .8$, $p < .025$) arteries, whereas a negative relationship was observed between heparan sulfate and tissue cholesterol in diabetic arteries only ($r = -.7$, $p < .05$). GHb, which was significantly higher in diabetic animals ($8.2 \pm 0.9$ vs $3.8 \pm 0.2\%$, $p < .0005$) was negatively associated with heparan sulfate in diabetic arteries ($r = -.7$, $p < .05$).

**Conclusions:** These data implicate hyperglycemia induced modifications in arterial proteoglycans that may promote atherosclerosis.

## Background

Cardiovascular disease (CVD) is the major complication of diabetes, leading to 70–80% of deaths in diabetic individuals [1-3]. The relative risk of CVD for diabetic versus non-diabetic individuals is 2–3 for men and 3–4 for women [4-9]. This risk is independent of established risk factors such as hypercholesterolemia, hypertension and cigarette smoking [6], indicating that factors unique to the diabetic state may be important contributors to macrovascular processes leading to CVD. As such, hyperglycemia has been postulated to contribute significantly to the increased risk of CVD associated with diabetes, and epidemiological studies have shown that in diabetic patients, the higher the plasma glucose, the higher the incidence of CVD [10-13]. In a recent study, glycated hemoglobin (GHb), a marker of blood glucose concentration, was shown to explain most of the increased mortality risk in men with diabetes [14].

The mechanism is unknown by which hyperglycemia may confer increased risk for CVD but may involve processes operative at the level of the artery wall. Atherosclerosis involves the deposition and accumulation of lipid from apolipoprotein B (apoB)-containing lipoproteins in cells and in the extracellular matrix of large vessels [15,16]. The process of entrapment and retention of LDL in arterial tissue is proposed to be mediated by arterial proteoglycans (PG). This is based on studies showing co-isolation of glycosaminoglycans (GAG), the carbohydrate moieties of PG with lipid from human atherosclerotic lesions [17,18] and immunogold labeling [19] and ultrastructural studies [20] showing LDL associated with extracellular matrix PG in rabbit arteries. In a recent study using transgenic mice expressing recombinant LDL with site specific mutations in apoB, animals with PG-binding-defective LDL developed significantly less atherosclerosis than animals expressing wild type LDL [21].

It has been understood for many years that all proteoglycans are not equally atherogenic, with an atherosclerosis-promoting role associated primarily with PG bearing dermatan sulfate (DS) chains [22]. By contrast, an anti-atherogenic role has been proposed for heparan sulfate (HS) proteoglycans based on their ability to inhibit monocyte binding to the sub-endothelial matrix [23], to regulate fibroblast growth factor [24], and to inhibit arterial smooth muscle cell proliferation [24,25]. Increased risk for atherosclerosis may therefore be conferred by diabetes-associated biochemical processes that produce modifications in arterial PG. An earlier report indicated that in human arteries, an atherosclerosis-associated decrease in the ratio of HS to DS was further decreased with diabetes [26]. Our previous studies using a non-human primate model of streptozotocin-induced diabetes, demonstrated increased atherosclerosis at all arterial sites in the diabetic group [27]. The present studies were conducted to determine whether hyperglycemia in these animals was associated with changes in arterial PG that may promote the development of atherosclerosis.

## Research design and methods

### *Animals*

The animals used as a source of arteries for these studies have been previously described [27-29]. Briefly, they were adult male cynomolgous monkeys (Macaca fascicularis) that received streptozotocin (Zanosar*, The Upjohn Co., Kalamazoo, MI) as an intravenous bolus (30 mg/kg). All animals were fed a moderately atherogenic diet (0.28 mg cholesterol/kcal, 45% calories from fat) for 2 months prior to baseline measurements and throughout the 6 month trial period. Fasting blood glucose measurements were taken routinely following induction of hyperglycemia and exogenous insulin therapy was initiated. Total daily insulin doses were 4 to 26 U (1 to 5 U/kg weight administered intramuscularly twice daily). Control animals were given saline injections on a similar schedule. Intravenous glucose tolerance tests performed prior to and 6 weeks after induction of diabetes, resulted in similar disappearance of plasma glucose in streptozotocin-treated animals to that measured in spontaneously diabetic animals [28]. Other clinical assessments including plasma insulin, GHb and plasma lipids have been fully described [27-29]. All procedures involving animals were conducted in strict accordance with "The Principles of Laboratory Animal Care" (NIH Publication No. 85-23) and in compliance with state and federal laws, standards of the U.S. Department of Health and Human Services and guidelines established by the Institutional Animal Care and Use Committee.

### *Proteoglycan preparation*

At necropsy the arteries were excised, stripped of adventitia, and quick-frozen in liquid nitrogen for storage at -70°C. For PG extraction, tissues were thawed, weighed, minced into 2–3 $mm^2$ pieces, and incubated for 48 hours at 4°C in 4.0 M Guanidine HCL, 0.05 M sodium acetate (pH 4.5, 3 ml/mg wet tissue) containing protease inhibitors (0.1 M 6-aminohexanoic acid, 10 mM disodium EDTA, 5 mM benzamidine hydrochloride, 3 mM phenanthroline, 5 mM tryptamine HCL). Following extraction, fluids were removed, tissues were rinsed with 1 ml guanidine HCL and rinses and extracts were combined. PG remaining in tissues were termed extraction-resistant PG. Based on measurement of purified GAG, similar levels of extractable PG were present in control (70 ± 5% of total PG) and diabetic tissues (74 ± 3% of total PG, mean ± SEM).

### *Purification of GAG*

*From guanidine-extracted PG*

Extracts were dialysed into versene buffer (0.1 M sodium acetate:0.05 M cysteine HCl; 0.01 M disodium EDTA; 7:1:1 v/v/v) pH 6.0, and GAG were released by digestion with 10 μg papain, 5 hours, 65°C, repeating addition of papain after 1 hour. Following papain release, GAG were complexed to a final concentration of 1% with cetylpyridinium chloride (CPC), 24 hours, 26°C, and separated by centrifugation at 1400 × g for 30 minutes. Complexes were dissociated by addition of 2MNaCl: absolute ethanol (100:15, v/v) and GAG were precipitated by addition of 2 volumes absolute ethanol. After incubation for 24 hours at 26°C, GAG were separated by centrifugation at 1400 × g for 30 minutes and pellets were air-dried. Recoveries at all stages of purification were monitored by dimethylene blue assay [30] of discarded fractions. No GAG were detected in these fractions.

### *From guanidine-extracted tissues. (extraction-resistant PG)*

Dried delipidated tissues were rehydrated by incubation for 2 hours in versene buffer followed by addition of 10 ug papain/mg dry tissue. GAG liberation and purification was conducted as described for guanidine-extracted PG with the exception of an additional purification step as follows to remove contaminating DNA in the tissue extracts: GAG-ethanol precipitates were solublized in 500 μl of $dH_2O$, adjusted to a final concentration of 10% trichloroacetic acid, 0.01% BSA and incubated on ice for 1 hour. Precipitated DNA was removed by centrifugation at 1400 × g, 30 minutes and the supernatant containing GAG was dialysed vs $dH_2O$ and dried by speed-vac. GAG pellets were dissolved in $dH_2O$ and hexuronic acid content was measured by the method of Blumenkrantz and Asboe-Hansen [31].

### *Identification of GAG*

GAG were separated by agarose electrophoresis (150 volts, 2 hour, 10°C) using 0.5% agarose gels and a buffer of 0.05 M 1,3, diaminopropane pH to 9.0 with acetic acid [32]. Gels were fixed with 0.1% hexadecyltrimethylammonium bromide for 3 hours, air-dried I hour, stained with 0.1% toluidine blue, 0.1% alcian blue in acetic acid:ethanol:water (0.1:5:5) for 30 minutes and destained for 30 minutes. GAG were identified by comparing their migration with those of authentic commercial GAG. Staining of the individual GAG was linear over the range used (2–10 ug) and all samples were loaded within this range. Stained gels were air-dried overnight, scanned using a UMAX Astra 1220S, and scans were analysed using Scion Image software from Scion Corporation, Frederick, MD. Amounts of individual GAG were calculated based on areas under the curves as a percentage of total GAG loaded. Total GAG were calculated as the sum of GAG from guanidine-extractable and extraction resistant PG.

### *Measurement of tissue cholesterol*

Lipid extraction for measurement of cholesterol was performed with tissues following guanidine HCL extraction to remove extracellular matrix PG and before papain digestion to isolate GAG from membrane associated PG. After guanidine extraction, tissues were rinsed with distilled water, air-dried, and delipidated by extraction with 1:1 (v/v) absolute ethanol:acetone (10 volumes × tissue weight) at 50°C for 1 hour. Extracts were removed and extractions were repeated with additional ethanol:acetone overnight at 26°C and then with acetone alone for I hour at 26°C. A pool of extracts for each sample was evaporated to dryness, resolublized in 1 ml chloroform, 0.5% Triton X-100 and again evaporated to dryness [33]. The dried extracts were dissolved in 0.5 ml of 0.15 M NaCl and assayed for total cholesterol using an enzymatic assay reagent (Cat. No. 23669, Boehringer Mannheim Diagnostics, Indianapolis, IN, USA). No GAG were detected in the extract by dimethylene blue assay.

### *Immunostaining*

Slides prepared from paraffin sections of grossly normal thoracic aortas were deparaffinized, hydrated, blocked with 1% BSA in phosphate buffered saline and incubated overnight at 4°C with a 1:1000 dilution of primary antibody to HS GAG chains (HepSS-1, Seikagaku America, Inc., Ijamsville, MD). A horseradish peroxidase system was used for detection and cells were stained with hematoxylin.

### *Data analysis*

Values are presented as mean ± SEM. Statistically significant differences were determined by Student's t-test. *P* values less than 0.05 were considered significant. Relationship between variables was determined by linear regression analysis.

## Results

Group measures comparing control and diabetic monkeys at time of necropsy are shown in table 1. GHb was the only parameter measured that was significantly higher in the plasma of diabetic animals compared to control. Mean total plasma cholesterol was 30% higher for the diabetic group but this did not reach statistical significance. Likewise, slightly higher tissue cholesterol measurements in both the thoracic aortas (TA) and carotid artery (CA) segments from diabetic animals were not significantly different from control. A strong positive relationship was observed between TA cholesterol and CA cholesterol ($r = 0.86$, $p < 0.0005$ for both groups combined). In the same artery segments used to measure cholesterol, total GAG, expressed as hexuronic acid (UA) was also similar

**Table 1: Group differences between control and diabetic monkeys**

| | **TPC** mg/dl | **GHb** % | **TA Chol** mg/g dlt | **CA Chol** mg/g dlt | **TA UA** mg/g dlt | **CA UA** mg/g dlt |
|---|---|---|---|---|---|---|
| **C (7)** | 343 ± 47 | 3.8 ± 0.3 | 31.6 ± 11.3 | 42.4 ± 14.1 | 3.4 ± 0.4 | 7.4 ± 0.6 |
| **D (8)** | 456 ± 43 | 8.2 ± 0.8* | 40.6 ± 12.6 | 54.1 ± 19.6 | 4.1 ± 0.9 | 8.7 ± 0.9 |

* $p < 0.0005$ Values are means ± SEM for Control (C, n = 7) and Diabetic (D, n = 8) animals. TPC, Total Plasma Cholesterol; GHb, glycated hemoglobin; TA, thoracic aorta; CA, carotid artery; Chol, tissue cholesterol; UA, hexuronic acid; dlt, delipidated tissue

between control and diabetic groups for both TA and CA (table 1).

Further analyses were conducted to determine the relationship of GAG with tissue cholesterol accumulation in the TA segments. As shown in figure 1, whereas total GAG and cholesterol were not related in tissues from control animals (figure 1A), in tissues from diabetic animals, there was a positive correlation between arterial GAG and cholesterol concentrations (figure 1B). Individual GAG were separated by agarose electrophoresis and gels were stained with a combination of alcian and toluidine blue and then scanned by densitometry. Commercials standards were used to identify the migration positions of the GAG: dermatan sulfate (DS), chondroitin sulfate (CS), and heparan sulfate (HS). Figure 2 shows the staining patterns and densitometric scans of representative standards and samples. The double staining technique resulted in similar staining efficiencies in standards of 2 to 6 μg (lanes 1–3) that were linear up to 10 μg (not shown). Lanes 4–6 show staining patterns and scans of 3 different samples: Lanes 5 and 6 are representative samples of papain-liberated GAG of PG isolated from the tissues by extraction with 4 M guanidine hydrochloride. All three of the major sulfated arterial GAG were identified in these samples. For CS and HS, the migration positions of standards and samples were similar, but for DS the slight difference in migration suggested composition or structural differences in the arterial DS compared to the standard. Lane 4 shows GAG of extraction-resistant PG. No DS was identified in these samples.

Determinations of arterial content of specific GAG were made based on areas under densitometric peaks and conversion to hexuronic acid per mg delipidated tissue (dlt). No group differences were observed between control and diabetic arteries in amounts of any specific GAG (Table 2). Based on hexuronic acid, control arterial tissue contained: 1.8 ± 0.3 μg CS, 0.7 ± 0.2 μg DS and 0.9 ± 0.1 μg HS/mg dlt and diabetic arteries contained: 2.1 ± 0.6 μg CS, 0.8 ± 0.3 μg DS and 1.1 ± 0.2 μg HS/mg dlt. Correlation analyses were performed to identify any relationships that may be associated with tissue cholesterol and specific for the diabetic state. As a percent of total GAG, only DS was positively related to increased tissue cholesterol in both control ($r = 0.8$, $p < 0.05$) and diabetic ($r = 0.8$, $p < 0.025$) arteries (figure 3). A negative association was observed between HS and tissue cholesterol in diabetic arteries only ($r = 0.7$, $p < 0.05$, figure 3B). GHb, which was significantly higher in the plasma of diabetic animals (table 1), was negatively associated with HS in diabetic arteries (figure 4). No associations were observed between arterial CS or DS and GHb. Moreover, no associations were observed between total plasma cholesterol, HDL-C or LDL-C and arterial GAG from either control or diabetic animals (data not shown).

To determine the distribution of HS in the TA of these animals, sections of histologically normal aortic tissue were immunostained with an anti-HS GAG antibody. As shown in Figure 5, distribution of immunoreactive product was primarily sub-endothelial with minimal distribution observed in the media. This is consistent with a major role for endothelial cells in the production of arterial HS.

## Discussion

The goal of these studies was to identify diabetes-induced changes in arterial PG which may enhance the susceptibility of the artery to atherosclerosis or lesion progression. We have previously shown that in these animals, all arterial sites of the diabetic group had more tissue cholesterol than the control group [27]. Moreover, consistent with the increased arterial cholesterol content, the diabetic animals had significantly greater intimal area in both femoral arteries and abdominal aortas. In these animals also, altered measures of glucose tolerance, and increased glycation of hemoglobin and skin have confirmed a state of chronic hyperglycemia in the diabetic group [28]. In the present study, the segments of thoracic aortas used did not show group differences in cholesterol content. However with a range of atherosclerosis in both groups and a range of GHb values in the diabetic group, it was possible to detect associations between GAG and the glycemic state of the animal and also with early cholesterol accumulation in the arteries. These data support the hypothesis that hyperglycemia induces alterations in arterial PG.

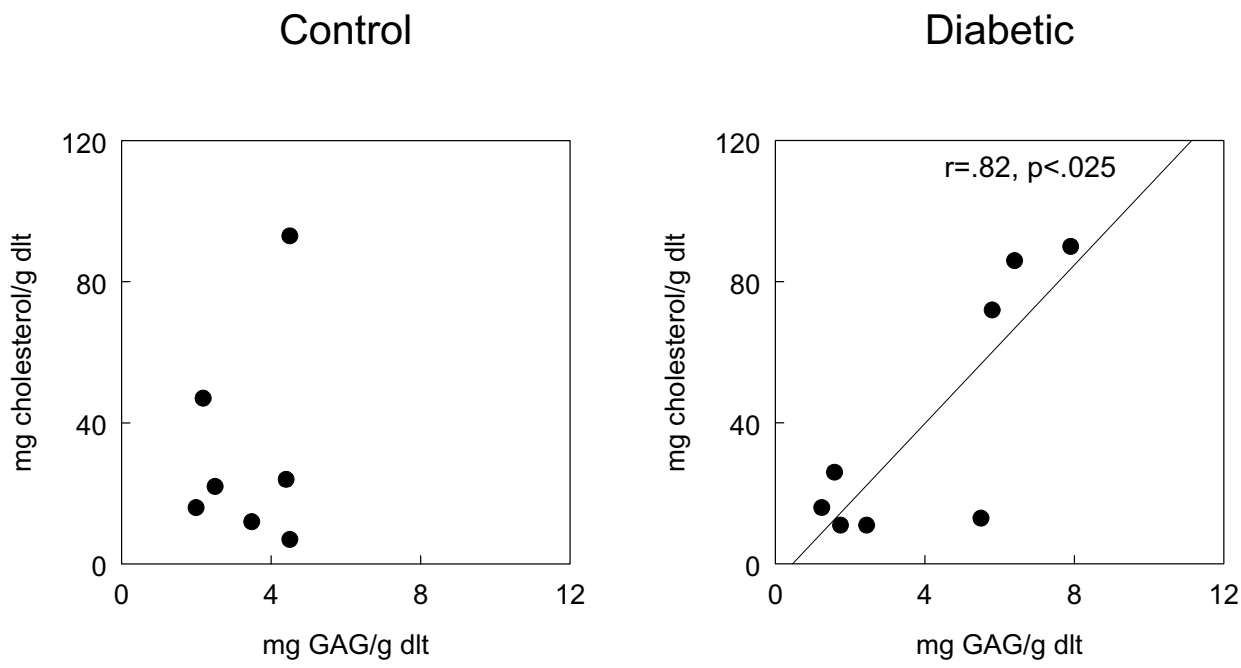


**Figure 1**
Relationship between GAG and tissue cholesterol in segments of thoracic aorta from control and diabetic monkeys. Tissues were collected at necropsy, 6 months after induction of diabetes mellitus. GAG values are total hexuronic acid / mg delipidated tissue. The correlation coefficient and significance is given. Y = 7.33X + 0.78.

A positive relationship was observed between total GAG and cholesterol accumulation in diabetic but not control arteries. In human arteries, the content of sulfated GAG has been shown to increase in early lesions but decrease with lesion development [34,35]. More importantly atherosclerosis-associated changes in specific GAG have been reported. The most consistent findings are an increase in DS and CS and a decrease in HS [36,37]. In rhesus monkeys, total GAG and DS were positively related to aortic cholesterol but, as in control animals in the present study, no relationship was found between arterial HS and tissue cholesterol [38].

Wasty et al. [26] have previously compared the GAG content of normal artery and atherosclerotic lesions from both non-diabetic and Type 2 diabetic individuals. That study was conducted with autopsy specimens, from aged individuals (57–92 years) having a wide range in duration of diabetes (3->15 years) and with significant atherosclerosis. In tissues from non-diabetic subjects, total GAG was lower in plaque compared to normal intima. The most significant change observed was a decrease in the ratio of HS:DS with atherosclerosis in both non-diabetic and diabetic tissues. Comparison of the diabetic and non-diabetic groups showed no difference in total GAG but a significant change in distribution: an increase in DS and a decrease in HS in the diabetic tissues. These data indicated that atherosclerosis-associated changes in GAG were exacerbated in the diabetic state. The findings of the present study using a non-human primate model of chemically-induced diabetes extend those of Wasty et al [26] by examining GAG changes in relation to the glycemic state of the animals. In the present study, the increase in DS was associated with tissue cholesterol content in both groups of animals whereas the negative association in diabetic animals between arterial HS content and plasma GHb suggest that the reduction in HS may be an early event in the pathologic consequences of hyperglycemia.

The idea that some GAG may exert pro-atherogenic and some anti-atherogenic effects has been reinforced by a number of observations. A pro-atherogenic role for DS is supported by studies demonstrating a high affinity of DS for plasma LDL [39] and by the preferential localization of biglycan, a DS-containing proteoglycan, to lesion tissue [22]. Thus DS is proposed to be major factor in retention

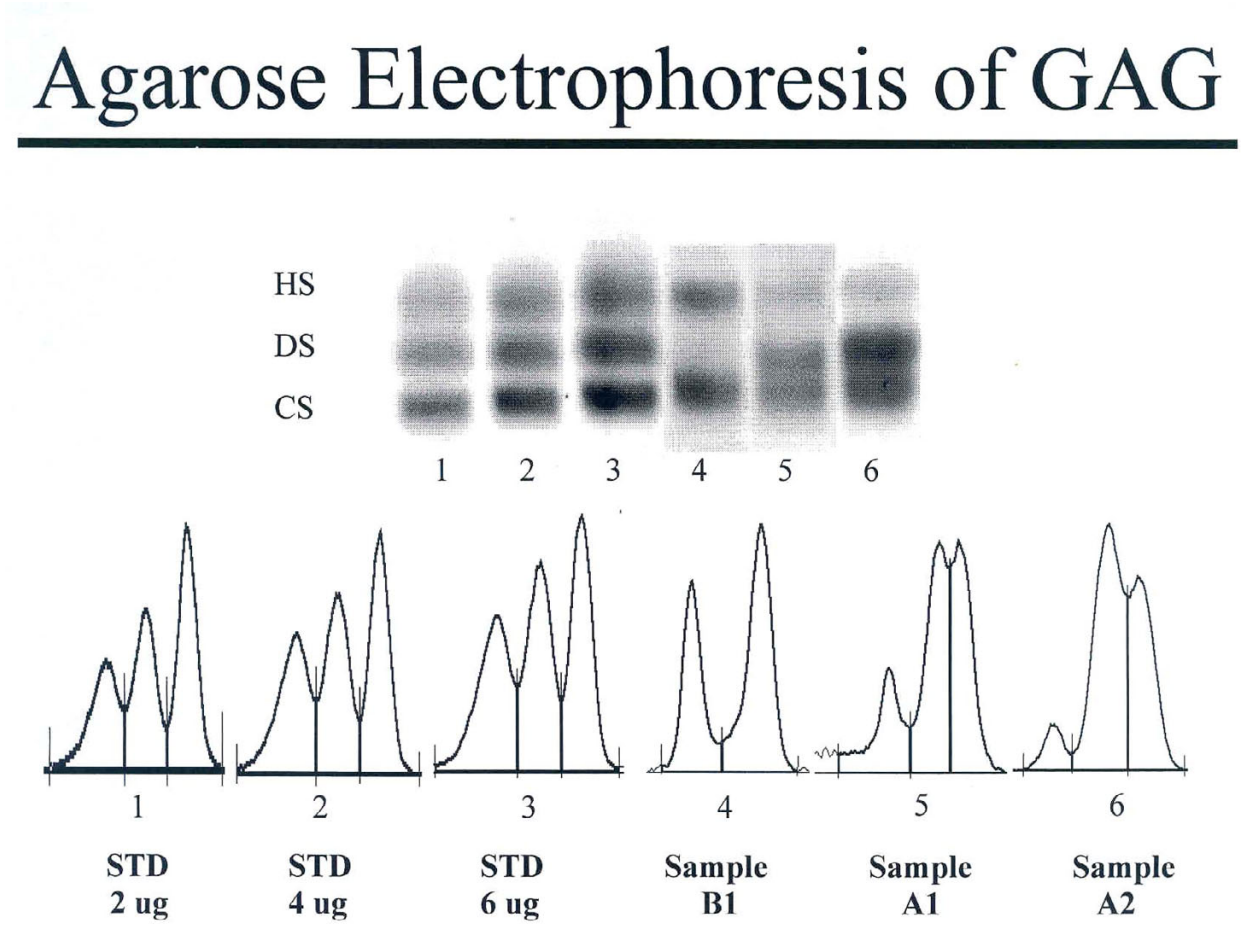


**Figure 2**
Identification of GAG by agarose electrophoresis. Upper panel shows separation of GAG standards and representative samples on 0.5% agarose, at 150 volts, 2 hours in 0.05 M 1,3, diaminopropane, pH 9.0. GAG were stained with 0.1% toluidine blue, 0.1% alcian blue. Lower panel shows densitometric scans of stained GAG bands after electrophoretic separation. Lanes 1–3 contained a mixture of equal amounts as shown of standard GAG. Lanes 4–6 show representative samples. HS, heparan sulfate; DS, dermatan sulfate; CS chondroitin sulfate.

of apo B and apo E-containing lipoproteins in the arterial wall. DS-containing PG in the arterial wall are produced primarily by arterial smooth muscle cells and also by endothelial cells [40,41]. Increased DS production by these cells may result from increased activity of cytokines [42] and growth factors [43] associated with the presence of macrophages in the developing lesion. Enhanced synthesis of CS/DSPG has been observed in cultured cholesterol-enriched arterial smooth muscle cells [44]. In addition, *in vitro* oxidized LDL was found to stimulate DS and CS chain elongation of arterial smooth muscle cell PG and specifically stimulate the expression and secretion of biglycan [45]. Therefore our finding of a positive relationship between DS and tissue cholesterol in both control and diabetic arteries is consistent with an effect of lipids on arterial DS synthesis.

By contrast HS was negatively associated with tissue cholesterol in diabetic arteries only. HS is proposed to be anti-atherogenic by several mechanisms. One is based on studies demonstrating anti-proliferative effects of HS on arterial smooth muscle cells [25]. HSPG are produced by arterial smooth muscle cells but the major contributors of arterial HS are endothelial cells which secrete the

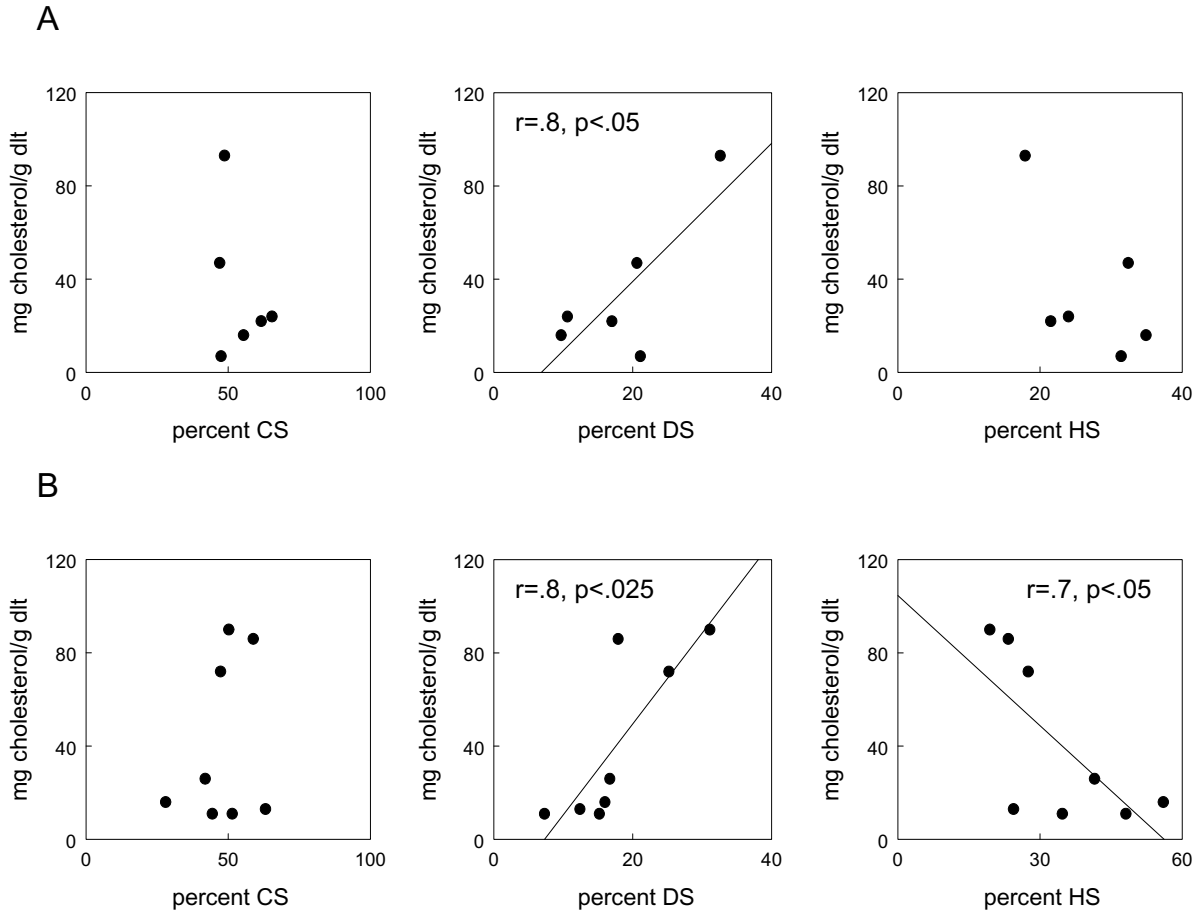


**Figure 3**
Plot of the association of tissue cholesterol and GAG distribution in segments of thoracic aorta from control (A) and diabetic (B) monkeys. Percent GAG were measured as areas under the curves of stained GAG following agarose electrophoresis as shown in figure 2. Values used in the correlational analyses are the sum of GAG of guanidine extracted + non-guanidine extracted PG. Correlation coefficients and significance are shown. In A: percent DS, Y = 2.96X - 20.32. In B: percent DS, Y = 3.90X - 28.51; percent HS, Y = -1.86X + 104.59. HS, heparan sulfate; DS, dermatan sulfate; CS, chondroitin sulfate; dlt, delipidated tissue.

basement membrane HSPG, perlecan, into the subendothelial matrix (Figure 5 and [46]). We have recently shown that in cultured human and bovine aortic endothelial cells, high glucose exposure results in a structural modification of perlecan that is consistent with the loss of an HS GAG chain [46,47]. Studies have shown that subendothelial HS inhibits monocyte binding to matrix proteins such as fibronectin [23]. Thus the reduced arterial HS associated with hyperglycemia may favor monoctye retention in diabetic arteries.

HSPG have been shown to be hyperglycemia-sensitive targets in other tissues, most notably kidney. Several studies of diabetic nephropathy have reported reduced HS in the glomerular basement membrane of diabetic kidneys [48,49]. Loss of HS was shown to be associated with reduced charge permselectivity leading to increased urinary albumin excretion [49]. Moreover, HS is a strong inhibitor of mesangial cell growth and reduced HS in the glomerular basement membrane is proposed result in mesangial expansion and clinical nephropathy [49-51]. In vitro, several lines of kidney cells have been used to demonstrate that exposure to high glucose results in decreased

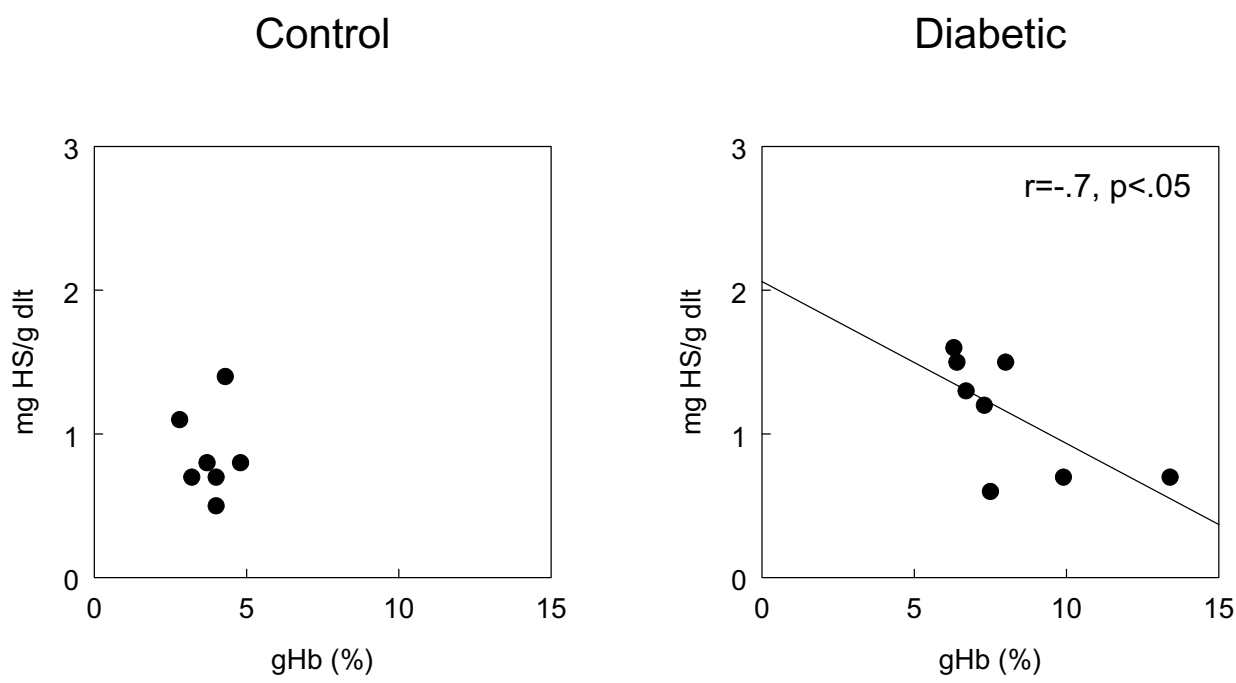


**Figure 4**
Plot of the association of plasma glycated hemoglobin and heparan sulfate content in segments of thoracic aorta from control (A) and diabetic (B) monkeys. Glycated hemoglobin was measured in plasma isolated immediately prior to animal necropsy. HS was measured as percent of total GAG based on areas under the curves of stained GAG following agarose electrophoresis as shown in figure 2. HS mass was calculated from hexuronic acid values. Values used in the correlational analyses are the sum of GAG of guanidine extracted + non-guanidine extracted PG. Correlation coefficient and significance are shown. Y = -0.11X + 2.05. HS, heparan sulfate; dlt, delipidated tissue; gHb, glycated hemoglobin.

**Table 2: Group measurements of arterial GAG in control and diabetic monkeys**

| | **CS** μg/mg dlt | **DS** μg/mg dlt | **HS** μg/mg dlt |
|---|---|---|---|
| **C (7)** | 1.8 ± 0.3* | 0.7 ± 0.2 | 0.9 ± 0.1 |
| **D (8)** | 2.1 ± 0.6 | 0.8 ± 0.3 | 1.1 ± 0.2 |

*Values are mean ± SEM of total CS, DS and HS in segments of thoracic aortas. C, control; D, diabetic; CS, chondroitin sulfate; DS, dermatan sulfate; HS heparan sulfate; dlt, delipidated tissue.

production of HS GAG [52]. HS synthesis was decreased in the retinas of diabetic rats thus providing a mechanism for the reduction in retinal basement membrane anionic sites and increased capillary permeability associated with diabetes [53]. In a recent study, the HSPG, perlecan, was reduced in the livers of diabetic mice [54]. This was proposed to result in impaired remnant clearance from plasma leading to the production of atherogenic lipoproteins. In vitro, high glucose was shown to reduce HS synthesis in HepG2 cells thereby implicating hyperglycemia with the decreased liver HSPG.

## Conclusions

Although no group differences were detected in any of the arterial GAG in these small groups of animals, our study demonstrates that in the diabetic animals there was an association between arterial content of HS and plasma GHb. It suggests that diabetes-associated hyperglycemia

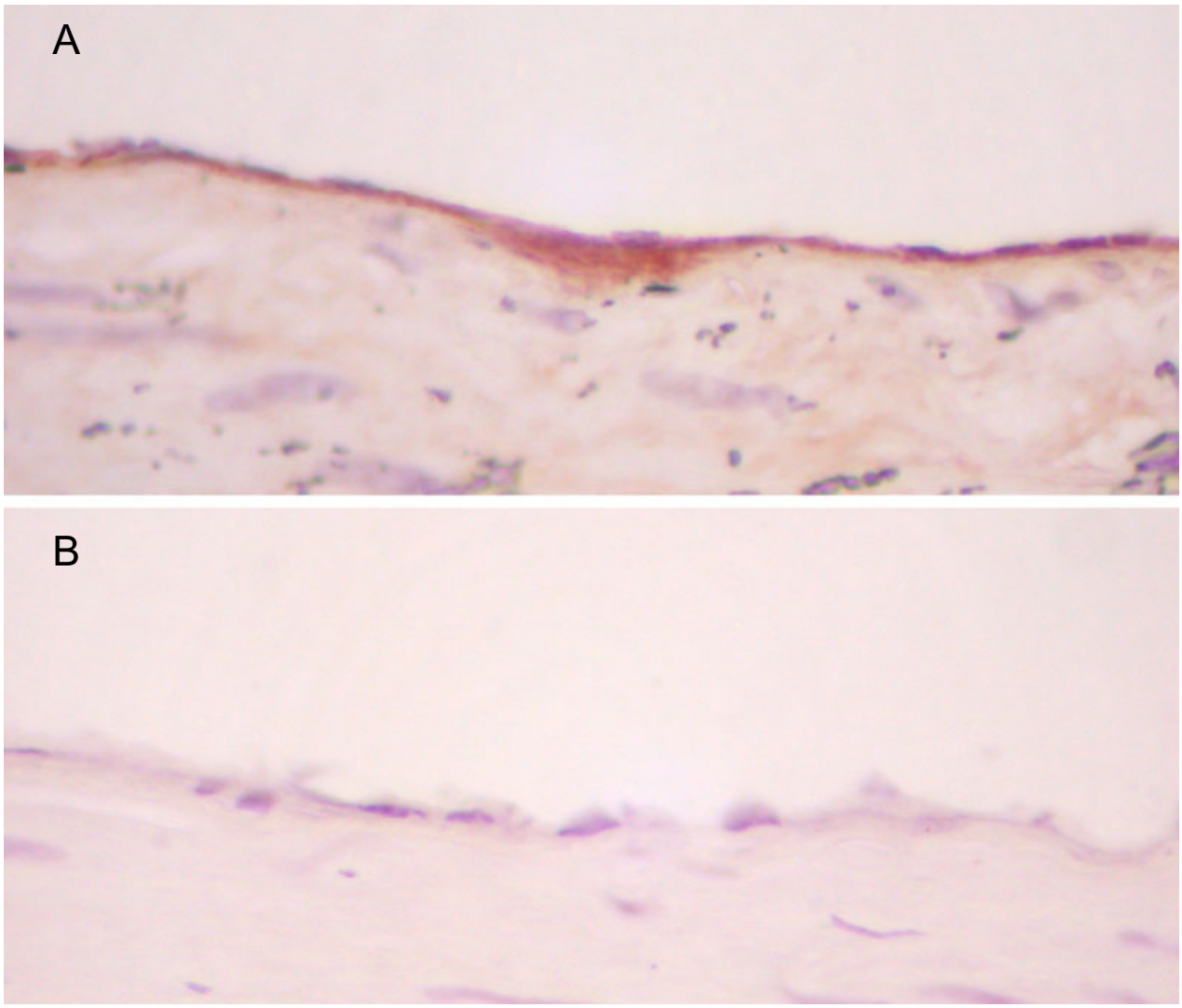


**Figure 5**
Photomicrograph of section of thoracic aorta immunostained for HS GAG. Paraffin sections prepared at necropsy were deparaffinized, blocked and incubated with anti-HS GAG antibody (A) or no primary antibody (B). Cells were stained with hematoxylin.

may affect the synthesis or metabolism of HSPG by arterial cells. This process is a potential target for intervention in diabetes-related atherosclerosis and further studies are needed to identify the biochemical mechanisms of glucose-induced modification of arterial HS.

## List of abbreviations

cardiovascular disease – CVD

glycated hemoglobin – GHb

low density lipoproteins – LDL,

proteoglycan(s) – PG

dermatan sulfate – DS

heparan sulfate – HS

chondroitin sulfate – CS

apolipoprotein B – apo B

glycosaminoglycans – GAG

guanidine HCL – GdnHCL

phospholipid – PL

triglyceride – TG

free cholesterol – FC

cholesteryl ester – CE

protein – Pro

thoracic aortas – TA

carotid artery – CA

hexuronic acid – UA

## Authors' contributions

Animal study and design: JDW, KNL, WTC.

Plasma lipids: JDW, KNL

Glycated hemoglobin: WTC

Artery analyses: IJE

Immunostaining: CAVW.

Drafting of the manuscript: IJE

Critical revision of the manuscript for important intellectual content: All authors

All authors read and approved the final manuscript.

## Competing interests

None declared.

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