Original Article

Glycogen and the Propensity for Atrial Fibrillation: Intrinsic Anatomic Differences in Glycogen in the Left and Right Atria in the Goat Heart

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Abstract

Background: Previous experimental studies have demonstrated electrophysiological and structural remodeling in pacing induced atrial fibrillation. The latter has been characterized by glycogen accumulation but no connection to atrial fibrillation induction and maintenance has as yet been proposed. **Aims:** We determined the presence of glycogen in the right and left atrial appendages in the goat heart, in order to find any intrinsic disparity in distribution and concentration between these sites. **Materials and Methods:** Atrial appendages from 5 goats were stained by the Periodic acid Schiff method to determine the presence of glycogen and the concentration of glycogen by morphometric analysis. **Results:** We are reporting for the first time that the right atrial appendage showed scattered glycogen granules throughout the atrial myocytes which delineated the intercalated discs; whereas glycogen in the left atrial appendage was more dense within cells and coalesced against the intercalated discs and side to side junctions between myocytes. Also, morphometric analysis determined that the stained regions of the right atrial appendages averaged, $0.8 \pm 1.3\ \mu m^2$ compared to the left atrial appendage sections, $2.6 \pm 3\ \mu m^2$, $p = 0.02$. We show that glycogen is heterogeneously distributed in both atria in the normal goat heart; however, the density of glycogen deposits concentrating against the intercalated discs and side to side connections in the left atrial appendage is a critically distinct difference. Impediment of cell to cell conduction could result in a non-uniform wavefront of activation, with areas of slowed conduction, predisposing the left atrium to reentrant based atrial fibrillation. **Conclusion:** These findings provide a basis for the well-known greater propensity for atrial fibrillation in the left versus the right atrium.

Keywords: Atrial appendages, glycogen concentration, glycogen distribution, atrial fibrillation, atrial conduction, activation wavefront

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Introduction

Under various experimental conditions the onset of atrial fibrillation (AF) has been attributed to aberrant electrical impulses originating in the area of the left atrium (LA) providing a left to right atrial (RA) gradient for the initiation and perpetuation of reentry circuits.[1-3] In turn these multiple circuits constitute the electrophysiological substrate for the disorganized and chaotic rhythm in the atrium known as AF. Animal models have been developed to study this phenomenon.[4] A seminal report in goats generated a new understanding of an underlying mechanism for AF and coined the phrase "Atrial Fibrillation begets Atrial Fibrillation".[5] After several weeks of pacing induced AF it was noted that longer and longer episodes were occurring without pacing until AF became continuous. Electrophysiological remodeling, in the form of a consistent shortening of atrial refractoriness provided the substrate for triggers to initiate AF. Perpetuation of AF has been attributed to the establishment of multiple reentry circuits which

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became the hallmark of the of the multiple wavelet theory of AF.[6,7] Surgical procedures to treat patients with AF by physically interrupting the reentrant circuits are now performed in patients worldwide based on the multiple wavelet concept for AF.[8]

It is important to note that the investigators of these initial experimental studies also found structural remodeling four weeks after the onset of AF in the form of changes such as increased myocyte size, myolysis and marked glycogen accumulation within the atrial myocytes. Admittedly they stated that, "It is not easy to understand how certain changes in cellular structure, such as glycogen accumulation can play a role in the perpetuation of AF".[9]

The present study describes the differences in glycogen content and distribution between the right atrial appendage (RAA) and left atrial appendage (LAA) of five normal goats. We hypothesized that glycogen content and distribution found in the LAA would induce an favorable substrate for impulse conduction and thus explain the more frequent AF origin in this chamber.

Materials and Methods

Ethical Statement: Goat tissue samples were obtained at a local federally inspected slaughter house. Permission to harvest these tissues was granted by a public health veterinarian, on site, representing the United States Department of Agriculture (USDA).

RAA and LAA were freshly harvested from five goats immediately after slaughtering. The tissue samples were separately preserved in 10% buffered formalin (N = 3) and in 95% ethanol N = 2).

Histochemical procedures

Fixed tissue samples were routinely processed, paraffin-embedded and sectioned at 4 μm. Following sectioning, the tissues were stained separately with either Hematoxylin and Eosin (H and E) or Periodic acid-Schiff (PAS) stain performed with or without diastase digestion for demonstration of glycogen.

Morphometric analysis

Digital images of the PAS-stained sections were acquired by Olympus DP25 digital photomicroscopy. Five random, color photomicrographs were obtained from all regions of the myocardium (subendocardial, subepicardial and intervening myocardium). The digital images were transferred to NIH Image J[10] and the total area of the image calculated (μm^2) by setting the scale to scale bar acquired with the image. The color threshold was set to identify only glycogen (magenta stain from PAS), which was converted to a black and white image and then set to binary for measurement. The binary image was regularly compared to the original to confirm scope and intensity of glycogen staining. Glycogen was reported as area covered (μm^2) by staining in the binary image, which could then be compared to the total area of the image.

Statistical analysis

Data are expressed as mean ± standard deviation (STD). A standard two tailed T-test was used to compare equal numbers of the 20 sections, taken from four goats, of the LAA and RAA samples. A p-value ≤ 0.05 was considered significant.

Results

Glycogen could be demonstrated in all LAAs and RAAs; however, there was marked variability in the intensity and distribution of glycogen deposition from animal to animal. Glycogen distribution was similar in both formalin-fixed and ethanol-fixed tissue samples. The formalin fixed tissue samples provided for more crisp images for photography and morphometric analysis (because of superiority of formalin for fixation). Glycogen deposition in both RAAs and LAAs was most consistently located in the subepicardial and subendocardial myocardium [Figures 1 and 2 respectively]. Glycogen was also present in the mid-myocardium; however, this was most conspicuous in LAAs (only) and in goats with the highest levels of myocardial glycogen. Although the glycogen content was markedly variable from animal to animal, the LAAs consistently had higher glycogen levels based upon PAS staining.

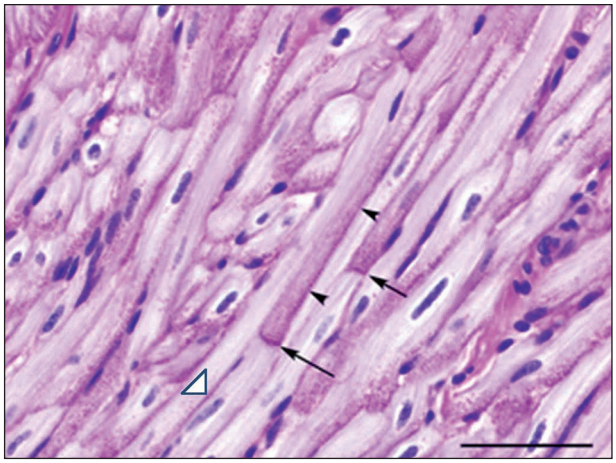

Figure 1: Heart, goat. In the RAAs glycogen deposition delicately highlighted the intercalated discs (arrows). Also glycogen deposition was seen along and just beneath the sarcolemmal membrane (solid arrowhead). Some cells showed little Periodic-acid Schiff (PAS) stain without diastase treatment (open arrowhead). Diastase treatment cleared magenta PAS staining (notshown). Bar = 50 μm

At the cellular level, glycogen in the RAAs occasionally highlighted the intercalated discs [arrows, Figures 1 and 2] and was distributed along and just beneath the sarcolemmal membrane [solid arrowhead, Figures 1 and 2]. This was not observed at other myocyte to myocyte connections [Figure 1, open arrowhead].

In the LAAs, the glycogen often concentrated against the intercalated disc [arrows, Figure 2], particularly in regions of heavy glycogen accumulation. Furthermore, not only was the glycogen found just beneath the sarcoplasmic membrane, but extended into and occupied the sarcoplasm [Figure 2, arrowhead]. In heavily stained regions, the entire sarcoplasm was diffusely occupied by PAS-stained glycogen.

Morphometric analysis [Figure 3] shows that PAS stained regions of the LAAs averaged 2.6 ± 3 μm^2 compared to RAAs, 0.8 ± 1.3 μm^2, p = 0.02. LAAs glycogen levels (per μm^2) always exceeded RAAs levels in each individual animal. It should be noted that besides the three fold greater prevalence of the mean glycogen concentration in the LAA compared to the RAA, the larger standard deviation in the LAA indicates the greater heterogeneity of glycogen in the LAA. The significance of these finding will be discussed below.

Discussion

Major findings of the present study

Histological sections made from tissues in normal goats showed a differential distribution and concentration of glycogen in left versus right atrial tissues. The concentration of glycogen in the LAA was not only greater than in the RAA but the density and location of the glycogen was critically distinct [Figures 1 and 2].

We suggest that the potential arrhythmogenic aspect of these glycogen differences are a potential contributory mechanism for the initiation and maintenance for AF and in particular the greater propensity for the development of an AF substrate in the left than in the right atrium.[11]

The present study demonstrates that in the normal caprine heart there are intrinsic quantitative differences in glycogen concentrations between the LAA and RAA. Both sides showed subepicardial presence of glycogen [Figures 4 and 5] that is notably displayed in a greater concentration in the LAA. The significantly greater glycogen in the LAA is heterogeneously found as high-density depositions against the intercalated discs and extending into the myocytes. Also condensed glycogen was observed at the sided to side junction of adjacent myocytes. In the RAA granules of glycogen were scattered throughout the cells. Although there were individual myocytes in which the glycogen granules outlined the intercalated disc and side

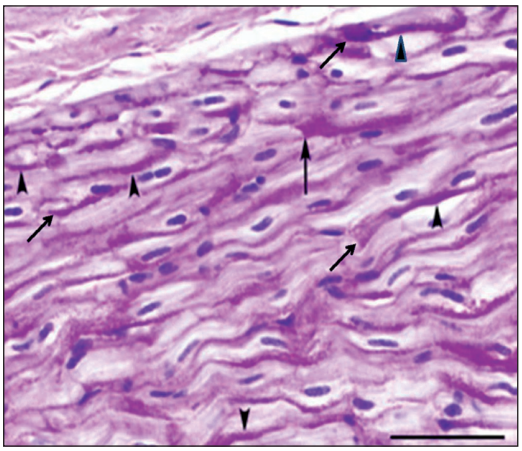

Figure 2: Heart, goat. In contrast to the RAAs, in the LAAs glycogen often concentrated against intercalated discs (arrows) with dense tails of glycogen extending into the cell along the lateral wall at the myocyte-myocyte junction (solid arrowhead). Periodic-acid Schiff (PAS) stain without diastase treatment. Diastase treatment cleared magenta PAS staining (not shown). Bar = 50 μm

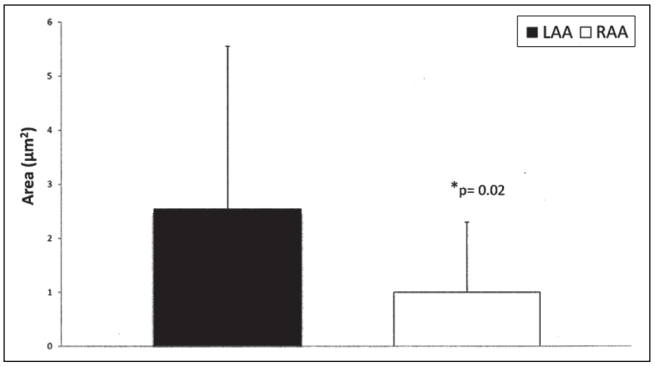

Figure 3: Morphometric quantitation of glycogen content of LAAs compared to RAAs. Glycogen was quantitated based upon area covered by PAS-stained glycogen as a percent of the area of the total image. Most variability is secondary to animal-to-animal variation. LAAs glycogen levels always exceeded RAAs levels. The PAS-stained area of LAAs (2.6 ± 3.0 μm^2) was significantly greater than RAAs (0.8 ± 1.3μm^2), p = 0.02

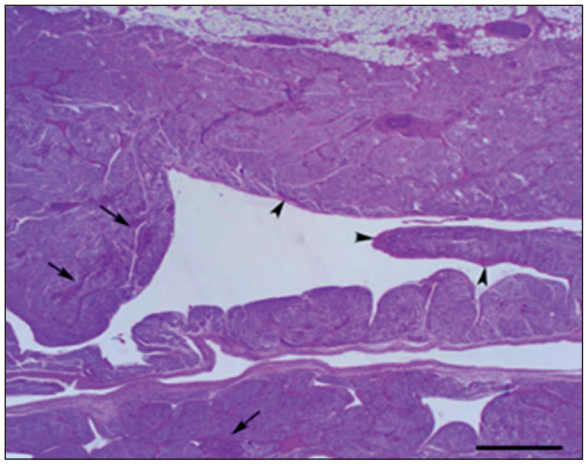

Figure 4: RAAs myocardium showing PAS staining (never as conspicuous as the LAA) Bar 1 mm. Please note that there is subepicardial glycogen concentration

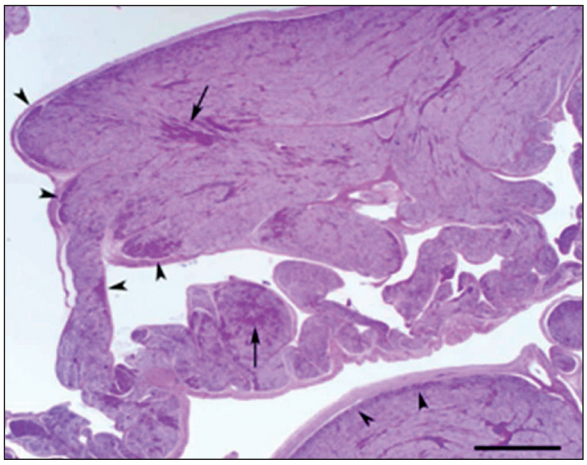

Figure 5: LAAs myocardium also exhibited consistent regions of subepicardial and subendocardial PAS-positive glycogen staining (arrowheads) however, the intensity of RAAs staining was never as conspicuous or as widely distributed as LAAs glycogen particularly within the mid-myocardium (arrows). Periodic-acid Schiff (PAS) stain/diastase treatment. Diastase treatment cleared magenta PAS staining (not shown). Bar = 1 mm

to side myocyte junctions, many other cells did not show this pattern [Figure 1, open arrow head]. Moreover, there was no presence of dense glycogen accumulation at cell connections.

Of interest, one of the striking characteristic structural changes noted in the studies of the Allessie group in the goat[12,13] and also observed by others clinically[14] was the accumulation of glycogen in the atrial myocytes associated with myolysis and a loss of contractile elements. It has been suggested that as a results of reduced contractility during AF, "lowered oxygen supply-demand ratio switches the energy metabolism of atrial cardiomyocytes from the use of fatty acids to the use of glucose…" leading to the accumulation of glycogen.[15] However, this would not explain the presence and differential concentrations of glycogen in normal atria as shown in the present study.

Intrinsic factors predisposing the normal atria for atrial fibrillation

Early on, studies showed that anisotropic conduction, that is, differential longitudinal versus lateral conduction velocity of atrial myocytes predisposed to microreentry in old compared to young atrial bundles.[16] More recently the contribution of the intrinsic autonomic nervous system innervating the atria appears to play a critical role in the initiation[17] and persistence of AF. We propose that the differential qualitative and quantitative distribution of glycogen in the normal goat heart demonstrated in the present study provides another intrinsic factor in the susceptibility of the atria for AF.

Glycogen/atrial fibrillation hypothesis

In the present study, there was a dense accumulation of glycogen concentrating at the intercalated discs and extending into the myocytes along the side to side connections between myocytes. As noted in [Figures 1 and 2], this effect was notably observed in the LAA albeit in a heterogeneous manner. We propose that the large glycogen molecules provide an impediment to electrical conduction both longitudinally and laterally. These heterogeneously distributed islands of impaired conduction create a non-uniform wavefront of activation with areas of slow conduction predisposing for reentry circuit development. Evidence in support this hypothesis comes from several sources. For example, Muir[18] subjected stands of Purkinje cells to centrifugation and found that "the centrifugal movement of the PAS-positive material, glycogen] appears to be arrested by transverse partitions, which correspond to the intercellular junctions. In uncentrifuged strands there is considerable variation in the concentration of stainable glycogen on the two sides of the intercellular junctions… these observations suggest that the intercellular junctions are impermeable to stainable glycogen particles". More recently, Embi *et al*.,[19] confirmed, *in vivo,* in atrial myocytes, this impermeability to be the result of the selectivity of the gap junction pore to allow particles of a given molecular size to pass from cell to cell. The observations of Muir can then be explained as follows: Glycogen particles have been reported to be 24.8 ± 1.8 nm in diameter,[20] whereas the atrial myocyte gap junction pore has a reported radius of 0.6-1.5 nm.[21] As the intracellular glycogen level increases during AF, it could cause fractionated intercellular communication, thus disrupting the stability of the atrial syncytium. Dhillon *et al*.,[22] studied gap junction resistivity and measured conduction velocity in guinea pig atria and found significant differences between the left and right atria. They found that the left atrial gap junction resistivity was significantly higher and conduction velocity significantly lower compared to the right atrium. Moreover, addition of a gap junction uncoupling agent slowed atrial conduction more in the left than in the right atrial appendage.

In another report from Tai *et al*.,[23] in a model of AF caused by ventricular pacing induced heart failure, they compared activation of the left and right atria in control and heart failure dogs. They designated 3 different forms of activation: Type 1, a single broad wavefront; Type 2, a non-uniform wavefront associated with conduction block and/or slow conduction or the presence of two wavelets; Type 3, the presence of 3 or more wavelets associated with areas of slow conduction and multiple arcs of conduction block. In the right atrium only 2 of the 5 control dogs had Type 2 activation whereas in the left atrium, all five control dogs had Type 2 activation.

Furthermore, the left atrium had a greater dispersion of refractoriness (a biomarker for AF susceptibility) than the right atrium.

Evidence supporting glycogen as a contributing factor promoting AF

Other evidence supporting glycogen as a contributing factor promoting AF relates to the finding that angiotensin II receptor blockers (ARBs) have had significant improvement in reducing the metabolic parameters such as glucose levels in large human clinical trials.[24] The drug irbesartan is such a blocker and has been known to reduce glucose levels amongst other effects such as attenuating atrial stunning after cardioversion,[25] and most important for our hypothesis it has been used as a stand alone or adjunctive therapy in controlling AF.[26] ARBs have also been used as intensive therapy to treat patients with glycogen storage disease.[27]

Myocardial sites with intrinsic glycogen concentrations

It is interesting to note that other areas of the normal heart have appreciable amount of glycogen. Indeed, the Purkinje system is known to contain a high glycogen content yet conduction velocity in Purkinje fibers is on the order of 2-4 times greater than in the atrium. Here again, it is not the concentration but the localization of the glycogen in relation to the myocytes that appears to determine conduction velocity and the activation wavefront. In an electron microscopic study of the Purkinje system, Legato[28] clearly showed that the strands of Purkinje cell were separated by "pools of glycogen". This arrangement would favor rapid longitudinal conduction through unimpeded intercalated discs without loss by side to side cellular connections. In fact, this arrangement is associated with low internal resistance in these Purkinje strands.

Limitations

Please note that the evidence obtained in our study and supported findings were restricted to normal atrial tissues taken from goat hearts. The translation of our hypothesis, developed in the normal goat heart to conditions of established AF, is moot since we do not know the role of glycogen in perpetuating AF. However, many studies in different species of normal animals have shown the inducibility for atrial fibrillation[4] and the greater propensity of the left rather than the right atrium to initiate and maintain AF.[1,22] Indeed in humans (with valvular disease and AF), the presence of myocytes with the total intercellular space filled with glycogen has been documented.[29]

Conclusions

The present study demonstrates that glycogen is heterogeneously distributed in both atria in the normal goat heart. Glycogen granules were observed scattered throughout the RAA myocytes and at the intercalated discs as well as the side to side connections between some but not all myocytes. In contrast, in the LAA dense glycogen deposits were coalesced against the intercalated discs and side to side connections between myocytes with "tailing" into the cell interior. For the first time, evidence is presented suggesting that this results in an impediment of cell to cell conduction leading to a non-uniform wavefront of activation. In conjunction with areas of slowed conduction, these conditions predispose the LA, when vulnerable, that is, in response to premature beats, for reentrant based AF.

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