# Supplementary Material

# Glycolaldehyde-Derived High-Molecular-Weight Advanced Glycation End-Products Induce Cardiac Dysfunction through Structural and Functional Remodeling of Cardiomyocytes

Dorien Deluyker[a] Lize Evens[a] Sibren Haesen[a] Ronald B. Driesen[a]
Diederik Kuster[b] Maxim Verboven[a] Hanne Beliën[a]
Jolanda van der Velden[b] Ivo Lambrichts[a] Virginie Bito[a]

[a]Biomedical Research Institute, Hasselt University, Hasselt, Belgium, [b]Amsterdam UMC, Vrije Universiteit Amsterdam, Physiology, Amsterdam Cardiovascular Sciences, Amsterdam, The Netherlands

## Supplementary Methods

### Echocardiographic measurements

Just before sacrifice, conventional echocardiographic measurements were conducted as previously described by Deluyker et al. 2016 [15]. Briefly, after 6 weeks of injection, all animals were anesthetized with 3% isoflurane supplemented with oxygen. Echocardiographic measurements were taken with a Vivid i ultrasound machine (GE Vingmed Ultrasound) and a 10 MHz array transducer. Parasternal long axis and short axis views at mid-ventricular level were acquired in B-mode. From parasternal long axis images, LV end-diastolic diameter (LVEDD) and LV end-systolic diameter (LVESD) were obtained. In parasternal short axis views, anterior wall thickness (AWT), posterior wall thickness (PWT), LV inner diastolic diameter (LVIDD) and LV inner systolic diameter (LVISD) were determined. Heart rate (HR) was obtained from parasternal short axis views at mid-ventricular level in M-mode. End-diastolic volume (EDV) and end-systolic volume (ESV) were determined by the formula ($\pi$ * LVIDD2 * LVEDD)/6 and ($\pi$ * LVISD² * LVESD)/6 respectively. Subsequently, stroke volume (SV) was calculated as EDV-ESV and cardiac output (CO) was measured as (SV*HR)/1000. Finally, ejection fraction (EF) was obtained by (EDV-ESV)/EDV and is expressed in %.

### Protein analysis of myosin heavy chain isoforms

Myosin heavy chain-alpha (MHC-α) and myosin heavy chain-beta (MHC-β) was determined by one-dimensional SDS-PAGE [20]. The resolving gel contained 12% acrylamide (pH 9.3), while the stacking gel contained 3.5% acrylamide (pH 6.8). The gels were stained with sypro stain and analyzed by densitometry.

### Enzyme activities

$Ca^{2+}$-ATPase and cytochrome c oxidase (COX) activities were determined using a $Ca^{2+}$-ATPase assay kit (MyBioSource, USA) and a COX assay kit (Sigma Aldrich, Belgium) respectively. For both measurements, experiments were performed following to the manufacturers' protocol.

### AGEs content in heart tissue

Transverse sections of 8 µm thick were obtained from paraffin-embedded tissue. Sections were deparaffinized and antigen retrieval was performed with citrate buffer (pH = 6). The coupes were blocked for 1 hour at room temperature with protein block (0,5% PBS-Triton; Dako). Tissues were incubated with a rabbit anti-rat primary antibody for AGEs (1/250, Abcam, ab23722) for 1 hour. A biotinylated donkey anti-rabbit secondary antibody (1/1000) was used in darkness for 1 hour at room temperature. Envision kit (Dako, K4061, HRP; dual antiRb/anti-mouse) was incubated for 30 minutes at room temperature. DAB solution was added (Dako, K3468) and sections were counterstained with hematoxylin. Coupes were dehydrated and mounted with DPX mounting medium. Negative controls were included in each staining in which the staining procedure was performed with omission of the primary antibody. Images were acquired using a Leica MC170 camera connected to a Leica DM2000 LED microscope. The AGEs

deposition was quantified with ImageJ software in four randomly chosen regions. The AGEs-positive area was normalized to the total cardiomyocyte area and expressed as AGEs content in %.

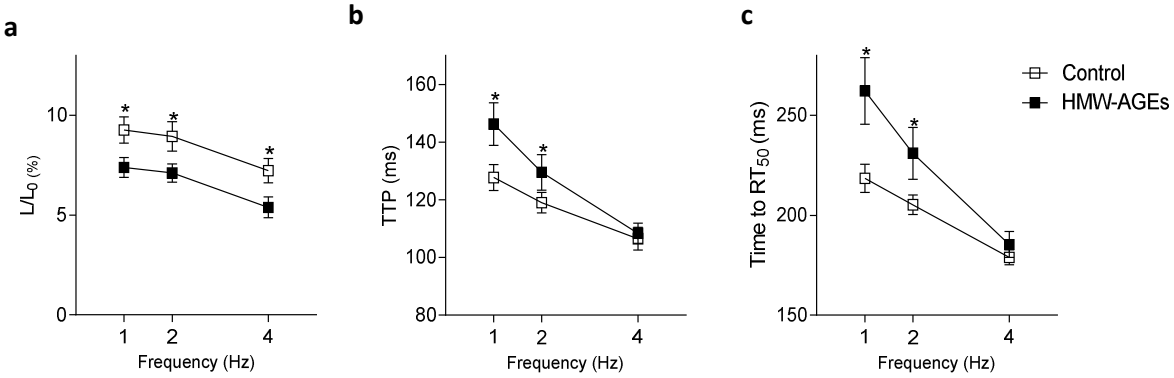
**Supplementary Table**

**Supplementary Table 1: Conventional echocardiographic characteristics.**

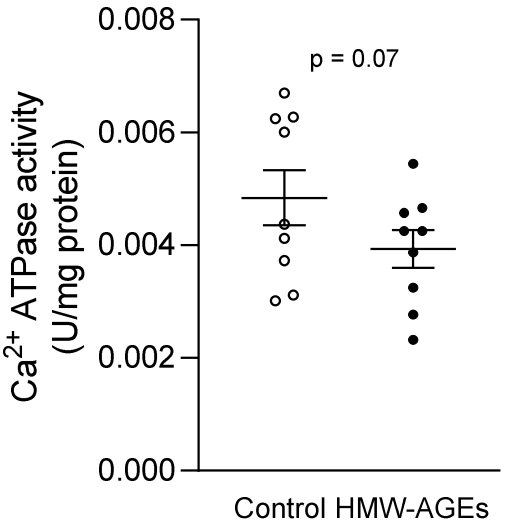
| Parameters | 6 weeks post-injections | |
|---|---|---|
| | Control | HMW-AGEs |
| HR (BPM) | 360 ± 9 | 366 ± 9 |
| AWT (mm) | 1.47 ± 0.02 | 1.58 ± 0.03 ** |
| PWT (mm) | 1.56 ± 0.06 | 1.77 ± 0.04 ** |
| EDV (µl) | 338 ± 18 | 316 ± 13 |
| ESV (µl) | 82 ± 9 | 87 ± 6 |
| SV (µl) | 256 ± 13 | 229 ± 11 |
| CO (ml/min) | 92 ± 5 | 84 ± 4 |
| EF (%) | 76 ± 2 | 73 ± 2 |

Echocardiographic measurement parameters were evaluated 6 weeks post-injections in control (N=18) and HMW-AGEs (N=25) injected animals. Data are presented as mean ± SEM. ** denotes P<0.01, parametric unpaired t-test. HR, heart rate; AWT, anterior wall thickness; PWT, posterior wall thickness; EDV, end-diastolic volume; ESV, end-systolic volume; SV, stroke volume; CO, cardiac output; EF, ejection fraction.

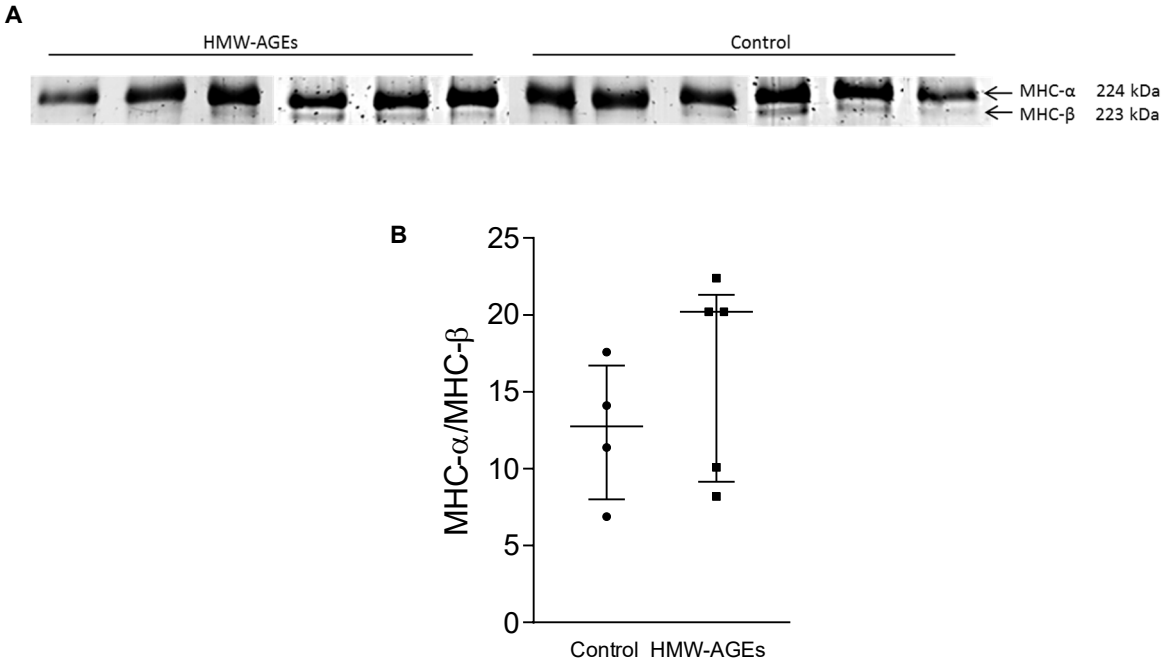
**Supplementary Figures**



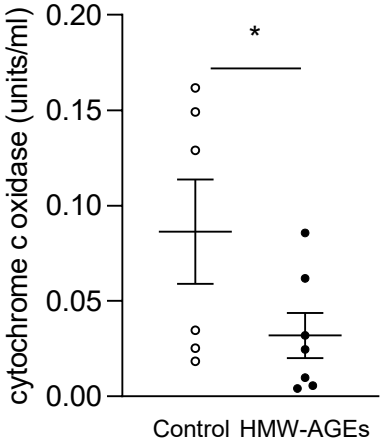

**Supplementary Fig 1. Frequency dependency of cell shortening during field stimulation.** Frequency dependency of fractional cell shortening normalized to cell length ($L/L_0$, %) (left panel, a), time to peak of contraction (TTP, ms) (mid panel, b) and time to half-maximal relaxation ($RT_{50}$, ms) (right panel, c) at 1,2 and 4 Hz in cardiomyocytes from control ($n_{cells}$=64) or HMW-AGEs ($n_{cells}$=104) animals. Data are expressed as mean ± SEM. * denotes P<0.05.



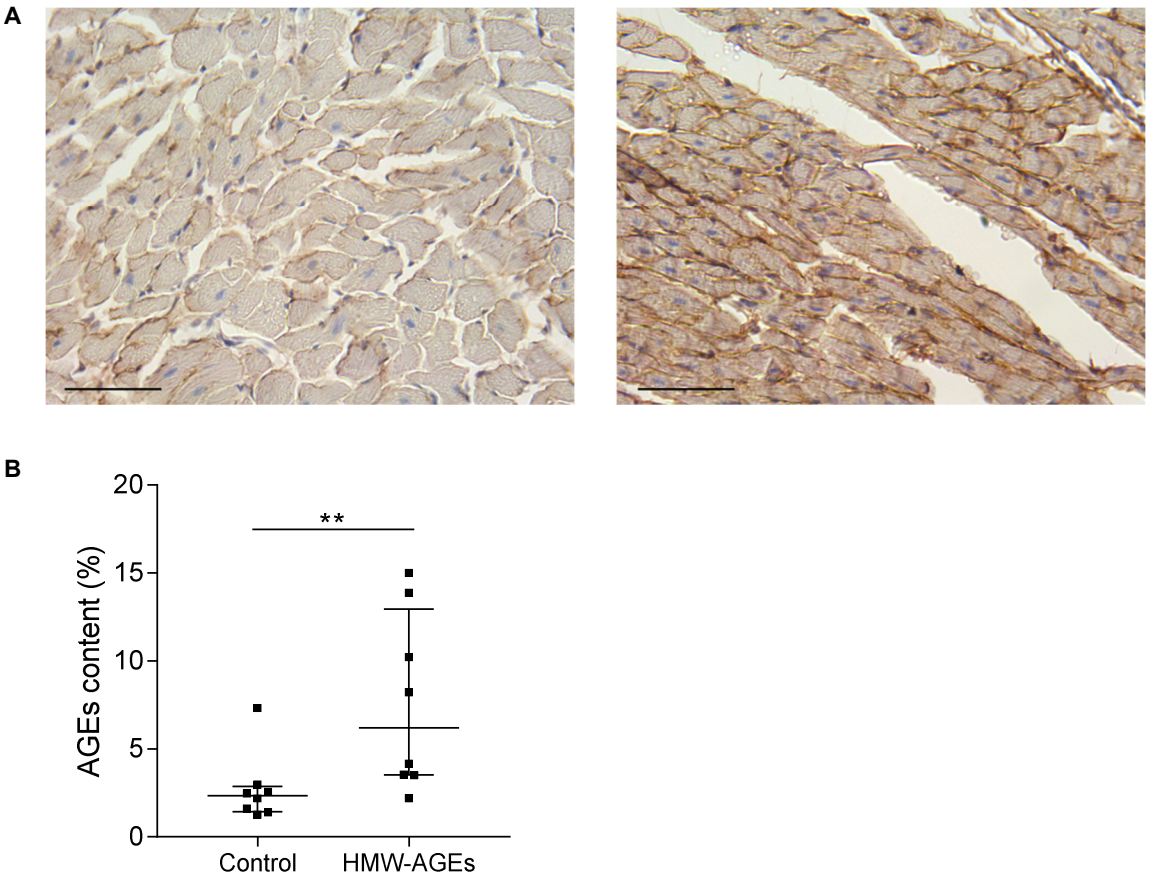

**Supplementary Fig 2. HMW-AGEs tended to decrease the $Ca^{2+}$ ATPase activity.** Determination of $Ca^{2+}$ ATPase activity in control (N=9) and HMW-AGEs (N=9) injected animals (p=0.07, parametric unpaired t-test). Data are expressed as mean ± SEM.




**Supplementary Fig 3. Protein analysis of myosin heavy chain isoforms.** (A) Representative figure, not continuous blot, for different myosin heavy chain (MHC) isoforms in cardiomyocytes derived from control (N=6) and HMW-AGEs- injected (N=6) animals. Myosin heavy chain α-isoform (MHC-α; 224 kDa) and myosin heavy chain β-isoform (MHC-β; 223 kDa). (B) Ratio MHC-α/MHC-β in control (N=4) and HMW-AGEs (N=5) animals (non-parametric unpaired t-test). Data are expressed as median [75$^{th}$ percentile; 25$^{th}$ percentile].




**Supplementary Fig 4. HMW-AGEs significantly decrease the activity of COX**. Determination of COX activity in control (N=6) and HMW-AGEs (N=7) injected animals (P=0.039, parametric unpaired t-test). Data are expressed as mean ± SEM.




**Supplementary Fig 5. AGEs content is significantly increased in heart tissue derived from HMW-AGEs animals.** (A) Representative examples of transverse heart sections from control (left panel) and HMW-AGEs (right panel) animals. The AGEs content was immunohistologically determined with a DAB staining. Magnification: 40X. Scale bars: 50 µm. (B) Quantification of AGEs content in hearts derived from control (N=8) and HMW-AGEs (N=8) animals (p=0.007, non-parametric unpaired t-test). Data are expressed as median [75th percentile; 25th percentile].