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Cell shortening and calcium dynamics in epicardial and endocardial myocytes from the left ventricle of Goto-Kakizaki type 2 diabetic rats

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Abstract

Diabetic cardiomyopathy is considered to be one of the major diabetes-associated complications, and the pathogenesis of cardiac dysfunction is not well understood. The electromechanical properties of cardiac myocytes vary across the walls of the chambers. The aim of this study was to investigate shortening and Ca^{2+} transport in epicardial (EPI) and endocardial (ENDO) left ventricular myocytes in the Goto-Kakizaki (GK) type 2 diabetic rat heart. Shortening and intracellular Ca^{2+} transients were measured by video edge detection and fluorescence photometry. Myocyte surface area was increased in EPI-GK and ENDO-GK compared with control EPI-CON and ENDO-CON myocytes. Time to peak shortening was prolonged in EPI-GK compared with EPI-CON and in ENDO-CON compared with EPI-CON myocytes. Time to half-relaxation of shortening and time to peak Ca^{2+} transient were prolonged in EPI-GK compared with EPI-CON myocytes. Time to half-decay of the Ca^{2+} transient was prolonged in EPI-CON compared with EPI-GK and in EPI-CON compared with ENDO-CON myocytes. The amplitude of shortening and the Ca^{2+} transient were unaltered in EPI-GK and ENDO-GK compared with their respective controls. Sarcoplasmic reticulum Ca^{2+} and myofilament sensitivity to Ca^{2+} were unaltered in EPI-GK and ENDO-GK compared with their respective controls. Regional differences in Ca^{2+} signalling in healthy and diabetic myocytes might account for variation in the dynamics of myocyte shortening. Further studies will be required to clarify the mechanisms underlying regional differences in the time course of shortening and the Ca^{2+} transient in EPI and ENDO myocytes from diabetic and control hearts.

KEYWORDS

calcium transport, epicardial and endocardial myocytes, type 2 diabetes

1 | INTRODUCTION

The electrical and mechanical properties of cardiac myocytes vary across the walls of the heart. The characteristic shape of the action potential changes significantly across the myocardial wall from the epicardial (EPI), to mid-myocardial (MID) to endocardial (ENDO) region, which can be attributed to the differential expression of ion channels. Changes in repolarizing ionic currents, such as a reduction in the transient outward (I_{to}) and delayed rectifier (I_K) currents and an increase in the inward rectifier current (I_{K1}) in ENDO compared with EPI rat myocytes have been reported previously (Bryant, Shipsey, & Hart, 1999; Shimoni, Severson, & Giles, 1995; Volk, Nguyen, Schultz, & Ehmke, 1999; Wang et al., 2010; Yao, Jiang, Fan, Zhou, & Tseng, 1999). Mathematical modelling suggests that a lower density and slower reactivation kinetics of I_{to} in ENDO myocytes might account for the longer action potential duration (Pandit, Clark, Giles, & Demir, 2001).

In terms of ventricular myocyte size, White, Witzel, Breisch, Bloor, and Nimmo (1988) reported that the cross-sectional area of ENDO myocytes is greater than that of EPI myocytes and that exercise training increased the size of myocytes in EPI but not ENDO regions in rat hearts. However, a study carried by Natali et al. (2002) demonstrated that length, width, depth, length-to-width ratio, volume and amplitude of shortening were similar in ENDO and EPI myocytes.

The ultrastructure of the heart may alter in several pathological conditions. For example, 14 weeks of hypertension caused an increase in left ventricular weight and wall thickness. Hypertrophy of myocytes was 76% greater in the EPI compared with the ENDO region. *In vivo* echocardiography has confirmed that sub-endocardial layers contract more and faster than EPI layers of rat ventricular myocardium (Ait, Reboul, Andre, Lacampagne, & Cazorla, 2009). In spontaneously hypertensive rats, McCrossan, Billeter, and White (2004) showed that the effect of hypertension on the morphology, mechanical activity and electrical activity of left ventricular myocytes is dependent on their transmural location. Epicardial, MID and ENDO myocytes from the left ventricle had similar volumes. Cell lengths were longer in EPI and MID compared with ENDO, cell widths were similar in EPI, MID and ENDO, and length-to-width ratios were larger in EPI compared with ENDO myocytes. However, myocyte amplitude, time to peak (TPK) shortening and time to half (THALF) relaxation of shortening were similar in EPI, MID and ENDO myocytes (McCrossan et al., 2004). Ageing also seems to play a role in the variability of results. The decay time of the Ca^{2+} transient and the time required for 50% length relaxation increased with age, but not uniformly across the EPI, MID and ENDO rat ventricular myocytes (Haynes et al., 2014).

During the process of excitation–contraction coupling, membrane depolarization opens L-type Ca^{2+} channels. A small amount of Ca^{2+} entering via L-type Ca^{2+} channels triggers a large release of Ca^{2+} from the sarcoplasmic reticulum (SR). The rise in intracellular Ca^{2+} (Ca^{2+} transient) initiates and regulates cardiac muscle contraction. Several studies have shown that L-type Ca^{2+} channels are similar in rat ventricular myocytes from the ENDO compared with EPI region (Bryant et al., 1999; Smail et al., 2016; Volk & Ehmke, 2002). However, results on regional differences in intracellular Ca^{2+} transport in rat ventricular myocytes vary. Fowler et al. (2005) reported a larger amplitude of the Ca^{2+} transient, prolonged TPK of the Ca^{2+} transient and larger amplitude of caffeine-evoked Ca^{2+} transient in rat ENDO compared with EPI ventricular myocytes (Fowler et al., 2005). Natali et al. (2002) reported a similar amplitude of Ca^{2+} transients in EPI and ENDO myocytes, and McCrossan et al. (2004) reported similar amplitudes of the Ca^{2+} transient in EPI, MID and ENDO myocytes.

Diabetic cardiomyopathy is considered to be one of the major diabetes-associated complications reported in human patients with type 2 diabetes mellitus. This condition is characterized by a change in the structure and function of the heart; in other words, a change in electrical and mechanical performance of the heart that is independent of coronary artery disease (Sheikh et al., 2012). A variety of diastolic and systolic dysfunctions have been reported in type 2 diabetic patients, and the severity of abnormalities depends on the patients' age and the duration of diabetes (Chareonthaitawee et al., 2007; Di Bonito et al., 1996; Yasuda et al., 1992). In addition to increased risk of heart attack and stroke, cardiac electrical conduction abnormalities are also frequently observed in diabetic patients (Ewing, Boland, Neilson, Cho, & Clarke, 1991; Lindström, Jorfeldt, Tegler, & Arnqvist, 1992).

Alterations in Ca^{2+}-regulatory mechanisms are a hallmark of cardiomyopathy and heart failure in human patients (Morgan, Erny, Allen, Grossman, & Gwathmey, 1990; Sheikh et al., 2012). Cardiac cell contractility is controlled by intracellular Ca^{2+} cycling, which is initiated by a small Ca^{2+} influx across the cell membrane via L-type Ca^{2+} channels. The activation of these channels stimulates a further and larger release of Ca^{2+} from intracellular stores (SR) by Ca^{2+}-induced Ca^{2+} release. Relaxation occurs as a result of Ca^{2+} removal from the cytoplasm into the intracellular stores and efflux cross the cell membrane, predominantly via Na^{+}–Ca^{2+} exchange (Bers, 1991).

New Findings

- **What is the central question of this study?**
  To investigate haemodynamic dysfunction in the type 2 diabetic Goto-Kakizaki (GK) rat, we measured shortening and Ca^{2+} transport in ventricular myocytes from epicardial (EPI) and endocardial (ENDO) regions.
- **What is the main finding and its importance?**
  EPI and ENDO GK myocytes displayed similar hypertrophy. Time to peak (TPK) and time to half (THALF) relaxation were prolonged in EPI GK myocytes. TPK Ca^{2+} transient was prolonged and THALF decay of the Ca^{2+} transient was shortened in EPI GK myocytes. Amplitude of shortening, Ca^{2+} transient and sarcoplasmic reticulum Ca^{2+} were unaltered in EPI and ENDO myocytes from Goto-Kakizaki compared with control rats. We demostrated regional differences in shortening and Ca^{2+} transport in Goto-Kakizaki rats.

Studies in animal models have shown that diabetes results in abnormal Ca^{2+} homeostasis and alterations in ventricular excitation–contraction coupling (Chattou, Diacono, & Feuvray, 1999; Howarth et al., 2011; Lebeche, Davidoff, & Hajjar, 2008; Morgan et al., 1990; Sheikh et al., 2012). Cardiac myocytes from diabetic hearts have been shown to display reduced Ca^{2+} uptake into intracellular stores (Netticadan, Temsah, Kent, Elimban, & Dhalla, 2001) and impaired Na^{+}–Ca^{2+} exchange (Hattori et al., 2000), which partly underlies the mechanical defects observed in experimental models of diabetes. For example, Howarth et al. (2011) reported decreased L-type Ca^{2+} channel activity in type 2 diabetic hearts. Furthermore, Sheikh et al. (2012) have shown that diabetes caused a significant alteration in SR Ca^{2+}-ATPase (SERCA) and Na^{+}–Ca^{2+} exchange activity.

Previous studies have demonstrated a variety of contractile dysfunctions in Goto-Kakizaki (GK) rat heart, including decreased heart rate, decreased ejection fraction and prolonged time course of shortening and/or relaxation in ventricular myocytes (Howarth & Qureshi, 2008; Howarth, Shafiullah, & Qureshi, 2007; Iltis et al., 2005). In type 2 diabetic GK rats, Salem et al. (2012) reported changes in the expression of genes encoding various excitation–contraction coupling proteins that are associated with disturbances in myocyte shortening and intracellular Ca^{2+} transport. D'Souza et al. (2014) reported that chronic mild hyperglycaemia can produce molecular and structural correlates of hypertrophic myopathy in spontaneously type 2 diabetic rats. Recently published data have shown prolonged TPK shortening in ENDO and EPI myocytes from streptozotocin (STZ)-treated rats. The THALF relaxation of shortening, TPK Ca^{2+} transient and THALF

decay of Ca^{2+} transient were prolonged in ENDO myocytes from STZ-treated rats compared with ENDO control animals. Sarcoplasmic reticulum fractional release was also reduced in EPI myocytes in diabetic rats compared with control animals (Smail et al., 2016).

Despite the available data, very little is known about the regional effects of diabetes mellitus on Ca^{2+} signalling in the ventricular myocardium. Therefore, the aim of this project was to investigate the regional effects of diabetes mellitus on shortening and Ca^{2+} transport in EPI and ENDO myocytes from the left ventricles of GK type 2 diabetic rats.

2 | METHODS

2.1 | Ethical approval

Male GK rats (aged 3 months) were obtained from Taconic (Germantown, NY, USA). Male age-matched control (CON) Wistar rats were bred in our animal house facility. Rats were kept in cages with a 12 h–12 h light–dark cycle and had free access to food and tap water. Room temperature was kept between 21 and 25°C. Experiments commenced when the animals were 12 months of age. Ethical approval for this project was obtained from the UAE University Animal Research Ethics Committee (reference no. A11-15, 2 March 2015). The authors understand and complied with all ethical principles that the journal upholds.

2.2 | Experimental model

Experiments were performed in the male GK rat, a well-characterized experimental model of type 2 diabetes mellitus (Howarth et al., 2007; Howarth, Jacobson, Shafiullah, & Adeghate, 2008; Kristiansen et al., 2004; Liepinsh et al., 2009). Fasting blood glucose and glucose tolerance measurements were made according to previously described methods (D'Souza et al., 2014). In brief, after an overnight fast, animals aged 12 months were injected I.P. with 2 g glucose (kg body weight)$^{-1}$. Glucose was dissolved in water, and animals were injected with 1.15–1.20 ml of the resulting glucose solution in order to achieve the desired dose of 2 g glucose (kg body weight)$^{-1}$. Small drops of blood were collected from a nick applied to the tip of the tail. Blood glucose was measured at time zero (fasting blood glucose), 30, 60, 120 and 180 min after glucose injection. Body weight, non-fasting blood glucose and heart weight were measured before experiments. Experiments were performed on EPI and ENDO myocytes from the left ventricle of GK and age-matched Wistar control rats ≥12 months of age.

2.3 | Isolation of ventricular myocytes

Ventricular myocytes were isolated according to modifications of previously described techniques (Smail et al., 2016). In brief, animals were killed using a guillotine. This approach was used to minimize the risk of damage to the heart and associated blood vessels. Hearts were removed rapidly and mounted for retrograde perfusion on a Langendorff system. Hearts were perfused at a constant flow rate of 8 ml (g heart)$^{-1}$ min^{-1} and at 36–37°C with cell isolation solution (mmol l^{-1}: 130.0 NaCl, 5.4 KCl, 1.4 $MgCl_2$, 0.75 $CaCl_2$, 0.4 NaH_2PO_4, 5.0 Hepes, 10.0 glucose, 20.0 taurine and 10.0 creatine; adjusted to pH 7.3 with 1 M NaOH solution). When heart contraction had stabilized, perfusion was switched for 4 min to Ca^{2+}-free cell isolation solution containing 0.1 mmol l^{-1} EGTA, and then for 6 min to cell isolation solution containing 0.05 mmol l^{-1} Ca^{2+}, 0.60 mg ml^{-1} type 1 collagenase (Worthington Biochemical Corp., Lakewood, NJ, USA) and 0.075 mg ml^{-1} type XIV protease (Sigma, Taufkirchen, Germany). Left ventricle tissue was excised from the heart. A section of tissue was carefully dissected from ENDO and EPI regions, minced and gently shaken in collagenase-containing isolation solution supplemented with 1% bovine serum albumin. Cells were filtered from this solution at 4 min intervals and resuspended in cell isolation solution containing 0.75 mmol l^{-1} Ca^{2+}.

2.4 | Measurement of ventricular myocyte shortening

Ventricular myocytes were isolated and shortening was measured according to modifications of previously described techniques (Smail et al., 2016). In brief, cells were superfused (3–5 ml min^{-1}) with normal Tyrode solution (mmol l^{-1}: 140.0 NaCl, 5.0 KCl, 1.0 $MgCl_2$, 10.0 glucose, 5.0 Hepes, 1.8 $CaCl_2$; adjusted to pH 7.4 with 1 M NaOH solution). Unloaded EPI and ENDO myocyte shortening were recorded using a video edge-detection system (VED-114; Crystal Biotech, Northborough, MA, USA). Resting cell length, TPK shortening, THALF relaxation and amplitude of shortening (expressed as a percentage of resting cell length) were measured in electrically stimulated (1 Hz) myocytes maintained at 35–36°C. Data were acquired and analysed with Signal Averager software version 6.37 (Cambridge Electronic Design, Cambridge, UK).

2.5 | Measurement of intracellular Ca^{2+}

Myocyte fura-2 ratio (intracellular Ca^{2+}) was measured in fura-2 AM-loaded myocytes according to previously described techniques (Smail et al., 2016). In brief, cells were alternately illuminated by 340 and 380 nm light using a monochromator (Cairn Research, Faversham, UK), which changed the excitation light every 2 ms. The resulting fluorescence emitted at 510 nm was recorded by a photomultiplier tube, and the ratio of the emitted fluorescence at the two excitation wavelengths (340/380 ratio) provided an index of intracellular Ca^{2+} concentration. Resting fura-2 ratio, TPK Ca^{2+} transient, THALF decay of the Ca^{2+} transient and the amplitude of the Ca^{2+} transient were measured in electrically stimulated (1 Hz) myocytes maintained at 35–36°C. Data were acquired and analysed with Signal Averager software v 6.37 (Cambridge Electronic Design, Cambridge, UK).

2.6 | Measurement of SR Ca^{2+} transport

Sarcoplasmic reticulum Ca^{2+} was assessed using previously described techniques (Smail et al., 2016). The protocol used in the present study is illustrated in Figure 5a. In brief, after establishing steady-state Ca^{2+}

transients in electrically stimulated (1 Hz) myocytes maintained at 35–36°C and loaded with fura-2 AM, stimulation was paused for a period of 5 s. Caffeine (20 mM) was then applied for 10 s using a solution-switching device customized for rapid solution exchange (Levi, Hancox, Howarth, Croker, & Vinnicombe, 1996). Electrical stimulation was then resumed and the Ca^{2+} transients allowed to recover to steady state. Fractional release of SR Ca^{2+} was calculated by comparing the amplitude of the electrically evoked steady-state Ca^{2+} transients with that of the caffeine-evoked Ca^{2+} transient.

2.7 | Measurement of myofilament sensitivity to Ca^{2+}

In some cells, shortening and fura-2 ratio were recorded simultaneously (Hamouda et al., 2015). Myofilament sensitivity to Ca^{2+} was assessed from phase-plane diagrams of fura-2 ratio *versus* cell length by measuring the gradient of the fura-2–cell length trajectory during the late relaxation of the twitch contraction. The position of the trajectory reflects the relative myofilament response to Ca^{2+}; hence, it can be used as a measure of myofilament sensitivity to Ca^{2+} (Spurgeon et al., 1992).

2.8 | Statistics

The results were expressed as the means ± SD of *n* observations. Statistical comparisons were performed using either Student's unpaired *t* test or one-way ANOVA followed by Bonferroni-corrected *t* tests for multiple comparisons, as appropriate. A value of $P < 0.05$ was considered significant.

3 | RESULTS

3.1 | General characteristics

The general characteristics of GK rats compared with age-matched control Wistar rats are shown in Table 1. The GK rats displayed significantly ($P < 0.01$) increased heart weight, heart weight-to-body weight ratio and non-fasting blood glucose compared with control animals.

Glucose clearance capacity was significantly reduced in GK animals. Blood glucose concentrations after glucose tolerance tests were higher at all the time points measured ($P < 0.01$) and did not return to the baseline level within 3 h (Figure 1).

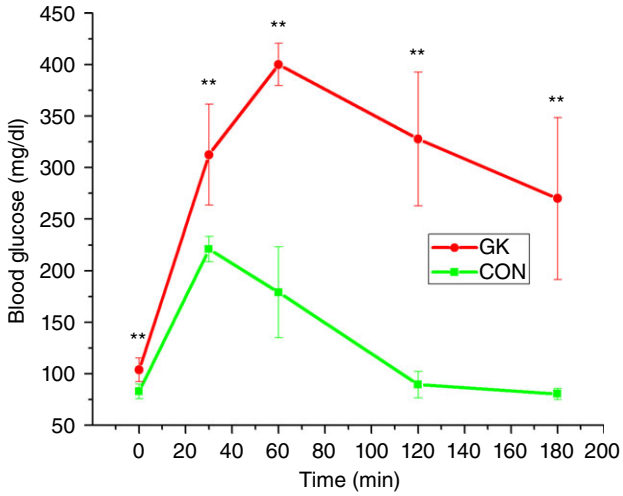

FIGURE 1 Graph showing blood glucose after an overnight fast and an I.P. injection of glucose [2 g glucose (kg body weight)$^{-1}$]. Data are means ± SD. $^{**}P < 0.01$. $n = 10$ hearts

3.2 | Ventricular myocyte shortening

The length, width and surface area of myocytes are shown in Figure 2a–c. The length, width and calculated surface area were significantly ($P < 0.05$) increased, and to similar extents, in EPI and ENDO myocytes from GK compared with control rats (Figure 2a–c). Typical records of shortening in EPI-CON and EPI-GK myocytes are shown in Figure 3a. The TPK shortening was significantly prolonged in EPI-GK (91 ± 20 ms) compared with EPI-CON (78 ± 17 ms) myocytes but was not altered in ENDO-GK compared with ENDO-CON myocytes (Figure 3b). Regionally, in control myocytes there was also a significant prolongation in ENDO-CON (88 ± 20 ms) compared with EPI-CON (78 ± 17 ms) myocytes (Figure 3b). When TPK shortening was normalized to the amplitude (AMP) of shortening, there were no significant differences between EPI-CON (17.45 ± 6.69 ms/AMP), EPI-GK (19.22 ± 8.82 ms/AMP), ENDO-CON (18.96 ± 7.08 ms/AMP) and ENDO-GK (20.47 ± 7.71 ms/AMP). The THALF relaxation of shortening was also prolonged in EPI-GK (60 ± 27 ms) compared with EPI-CON (47 ± 18 ms) myocytes (Figure 3c). The amplitude of shortening was not significantly altered in EPI-GK or ENDO-GK compared with respective controls (Figure 3d).

3.3 | Intracellular Ca^{2+}

Typical records of Ca^{2+} transients in EPI-CON and EPI-GK myocytes are shown in Figure 4a. The resting fura-2 ratio was not significantly

TABLE 1 General characteristics of the rats

Characteristic	Control rats	Goto-Kakizaki rats
Body weight (g)	457.75 ± 50.24 (12)	432.70 ± 25.64 (10)
Heart weight (g)	1.39 ± 0.18 (12)	1.72 ± 0.22 (10)**
Heart weight/body weight (mg g^{-1})	3.01 ± 0.39 (12)	4.00 ± 0.65 (10)**
Blood glucose (mg dl^{-1})	86.08 ± 9.05 (12)	182.70 ± 31.90 (10)**

Data are means ± SD. Numbers in parentheses are the numbers of animals. $^{**}P < 0.01$.

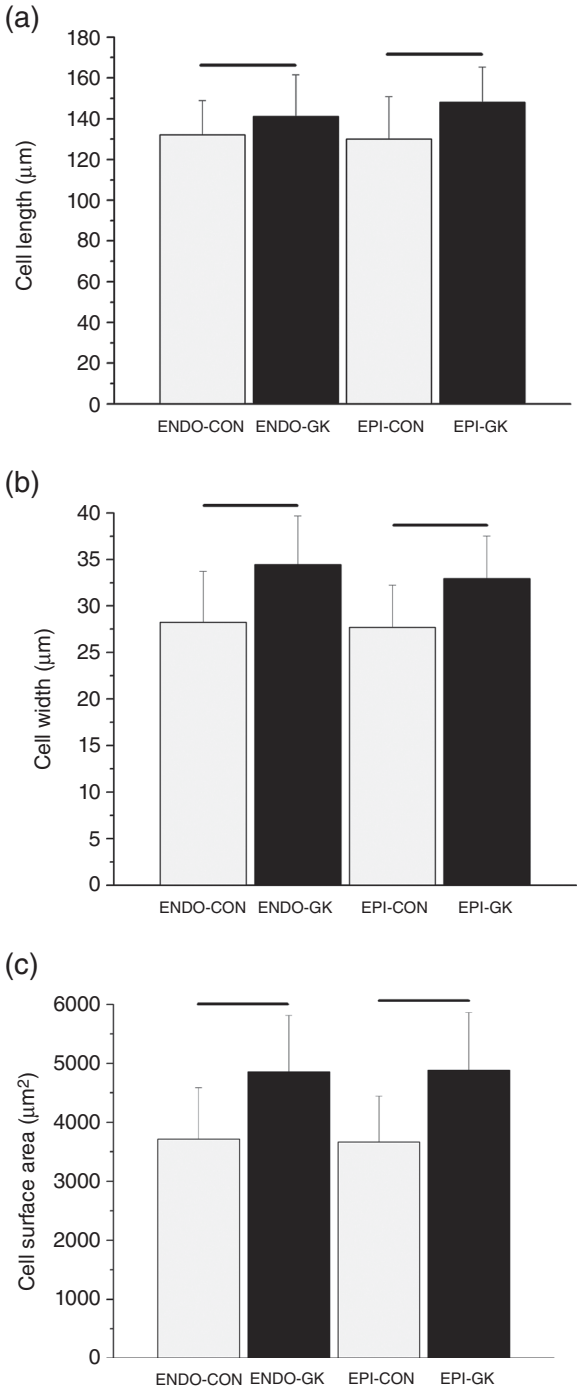

FIGURE 2 Bar graphs showing the length (a), width (b) and surface area (c) of epicardial (EPI) and endocardial (ENDO) ventricular myocytes from Goto-Kakizaki (GK) and Wistar control (CON) rats. The length and width were measured with a ruler, and the size (in micrometres) was calibrated with a standard graticule. Data are shown as means + SD. n = 57–88 cells from 10–12 GK and CON hearts. Horizontal lines above the bars represent significant differences at the level of $P < 0.05$

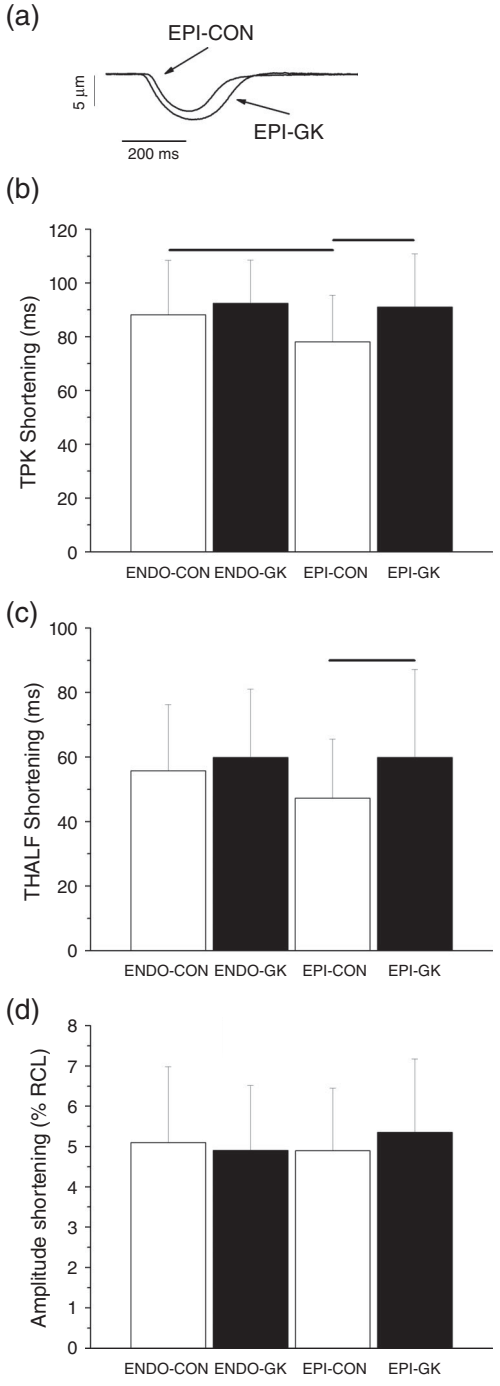

FIGURE 3 Ventricular myocyte shortening. (a)Typical records of shortening in EPI-CON and EPI-GK myocytes. (b–d) Bar graphs showing the mean time to peak (TPK) shortening (b), time to half (THALF) relaxation of shortening (c) and amplitude of shortening (d). Data are shown as means + SD. n = 57–58 cells from 10–12 GK and CON hearts. RCL = Resting cell length. Horizontal lines above the bars represent significant differences at the level of $P < 0.05$

altered in EPI-GK compared with EPI-CON or in ENDO-GK compared with ENDO-CON myocytes (Figure 4b). The TPK Ca^{2+} transient was significantly ($P < 0.05$) prolonged in EPI-GK (55 ± 13 ms) compared with EPI-CON (50 ± 9 ms) myocytes (Figure 4c). When the TPK Ca^{2+} transient was normalized to AMP Ca^{2+} transient there were no significant differences between EPI-CON [339 ± 165 ms/ratio units (RU)], EPI-GK (334 ± 240 ms/RU), ENDO-CON (347 ± 200 ms/RU) and ENDO-GK (346 ± 215 ms/RU). The THALF decay of the Ca^{2+} transient was significantly ($P < 0.05$) prolonged in EPI-CON (149 ± 52 ms) compared with EPI-GK (121 ± 6 ms) myocytes and was

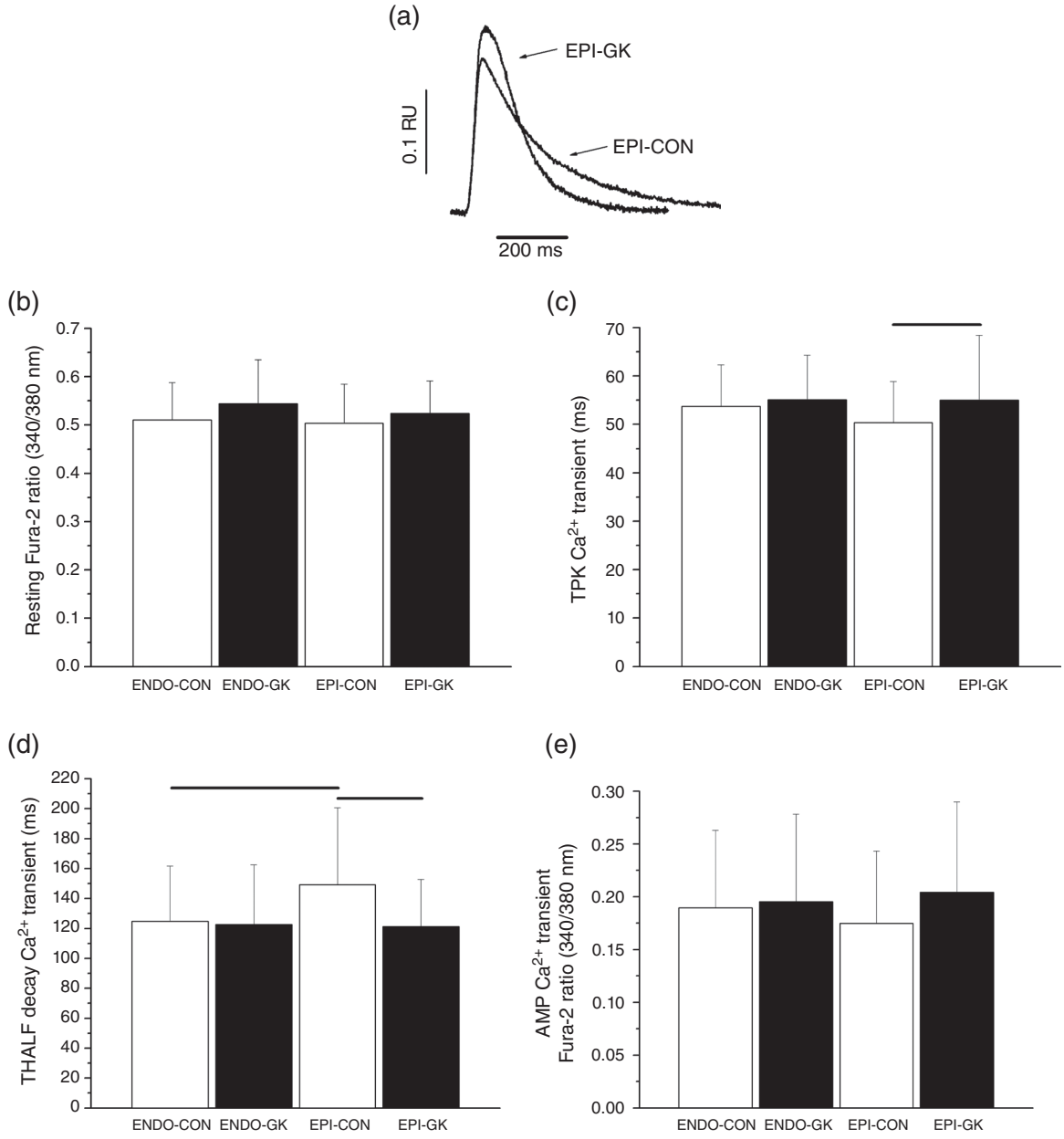

FIGURE 4 Ventricular myocyte Ca^{2+} transient. (a) Typical records of Ca^{2+} transients in EPI-CON and EPI-GK myocytes. (b–e) Bar graphs showing the mean resting fura-2 ratio (b), time to peak (TPK) Ca^{2+} transient (c), time to half (THALF) decay of the Ca^{2+} transient (d) and amplitude (AMP) of the Ca^{2+} transient (e). Data are shown as means + SD. $n = 57–86$ cells from 10–12 GK and CON hearts. RU = Ratio units. Horizontal lines above the bars represent significant differences at the level of $P < 0.05$

also significantly prolonged in EPI-CON (149 ± 6 ms) compared with ENDO-CON (123 ± 40 ms) myocytes (Figure 4d). The amplitude of the Ca^{2+} transient was not significantly altered in EPI-GK or ENDO-GK myocytes compared with respective controls (Figure 4e). (calculated by comparing the amplitude of the electrically evoked steady-state Ca^{2+} transients with that of the caffeine-evoked Ca^{2+} transient; Figure 5d) were not significantly altered in EPI-GK and ENDO-GK compared with respective controls.

3.4 | Sarcoplasmic reticulum Ca^{2+}

Figure 5a is a typical record illustrating the protocol used in these experiments. The amplitudes of caffeine-evoked Ca^{2+} transients (Figure 5b) were not significantly altered in EPI-GK or ENDO-GK compared with respective controls. The area under the caffeine-evoked Ca^{2+} transient curve (Figure 5c) and fractional release of Ca^{2+}

3.5 | Myofilament sensitivity to Ca^{2+}

Typical recordings of myocyte shortening, the Ca^{2+} transient and a phase-plane diagram of fura-2 ratio *versus* cell length are shown in Figure 6a. Myofilament sensitivity to Ca^{2+} was assessed by measuring the gradient of the fura-2–cell length trajectory during late relaxation of the twitch contraction. The position of the trajectory reflects the

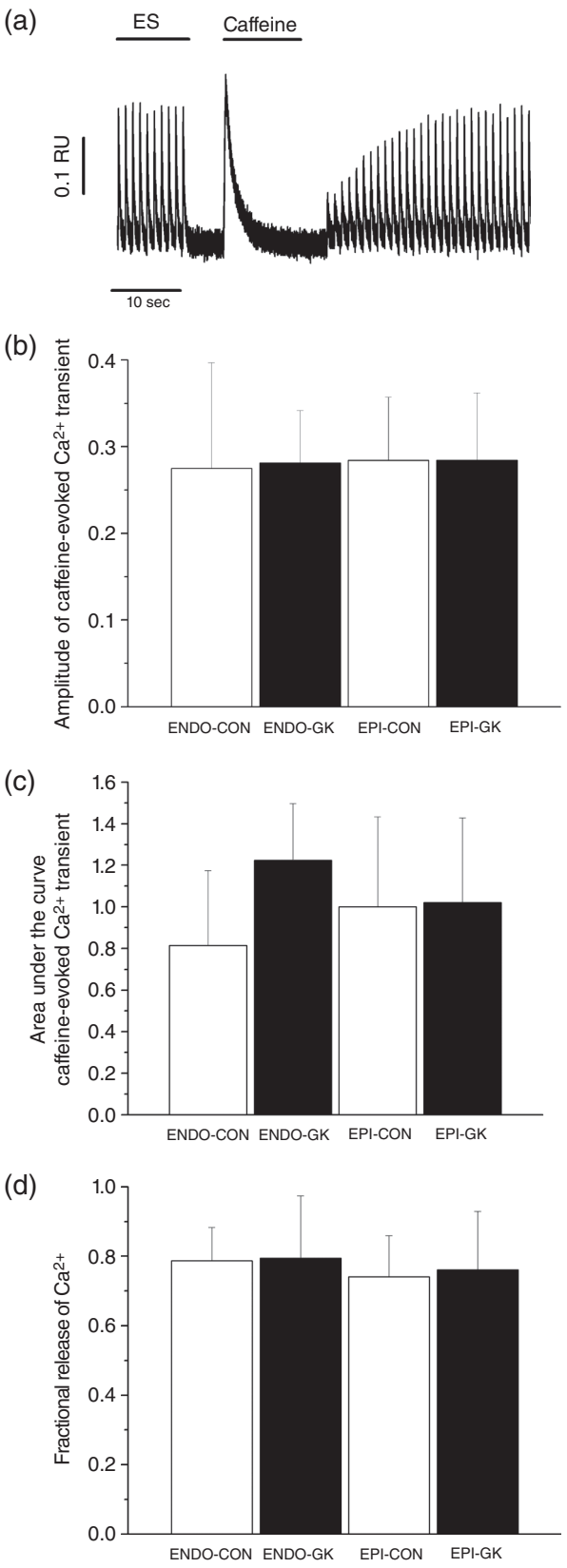

FIGURE 5 Sarcoplasmic reticulum (SR) Ca^{2+}. (a) Typical record showing the protocol used in a control ventricular myocyte during SR Ca^{2+} experiments. Initially, Ca^{2+} transients were recorded during electrical stimulation (ES). Electrical stimulation was then paused for 5 s, and 20 mM caffeine was rapidly applied for 10 s. After application of caffeine, electrical stimulation was resumed. (b–d) Bar graphs showing mean amplitude of caffeine-evoked Ca^{2+} transients (b), the area under the curve of the caffeine-evoked Ca^{2+} transients (c) and fractional release of Ca^{2+} transients (d). Data are shown as means + SD. $n = 22$–31 cells from five or six GK and CON hearts. RU = Ratio units

relative myofilament response to Ca^{2+}; hence, it can be used as a measure of myofilament sensitivity to Ca^{2+} (Spurgeon et al., 1992). The gradient of the trajectory during the period 500–800 ms was not significantly altered in EPI-GK and ENDO-GK compared with respective controls (Figure 6b).

4 | DISCUSSION

In this study, experiments were performed in EPI and ENDO myocytes from the left ventricle of GK and age-matched Wistar control rats ≥12 months of age. The major findings of this study ware as follows: (i) myocyte length, width and surface area were increased to similar extents in EPI-GK compared with EPI-CON and in ENDO-GK compared with ENDO-CON myocytes; (ii) kinetic properties of shortening and Ca^{2+} transients were different in EPI-GK compared with EPI-CON and in ENDO-CON compared with EPI-CON myocytes; (iii) amplitudes of cell shortening and Ca^{2+} transients were similar in EPI-GK compared with EPI-CON and in ENDO-CON compared with EPI-CON myocytes; (iv) the area under the curve of the caffeine-evoked Ca^{2+} transient and SR fractional release of Ca^{2+} were unaltered in EPI and ENDO from GK and CON myocytes; and (v) myofilament sensitivity to Ca^{2+} was unaltered in EPI and ENDO from GK and CON myocytes.

The results of this work show that the length, width and calculated surface area were increased in EPI-GK compared with EPI-CON and in ENDO-GK compared with ENDO-CON myocytes. These findings are consistent with the results of a study conducted by D'Souza et al. (2014), who showed that chronic mild hyperglycaemia causes structural remodelling of the left ventricle and cardiomyocyte hypertrophy in type 2 diabetic GK rats. Differences have also been reported in other regional cell measurements. For example, White et al. (1988) reported that the cross-sectional area is greater in ENDO compared with EPI myocytes, whereas De Clerck et al. (1984) reported no significant difference between resting cell dimensions and sarcomere length in EPI and ENDO. The heart weight-to-body weight ratio was significantly increased in GK rats compared with control rats, a finding that is consistent with previous studies (D'Souza et al., 2014; El Omar, Yang, Phillips, & Shah, 2004; Salem et al., 2013). Absolute values of the TPK shortening were prolonged in EPI-GK compared with EPI-CON and in ENDO-CON compared with EPI-CON myocytes. In our recent study using the STZ model of diabetes, we reported prolongation of TPK shortening in EPI-STZ and also in ENDO-STZ compared with respective controls (Smail et al., 2016). In the present study, however, there was no statistically significant difference between GK and control groups when TPK shortening was normalized to peak shortening. Interestingly, in the type 1 STZ diabetic model there was no change in EPI-STZ; however, THALF relaxation was

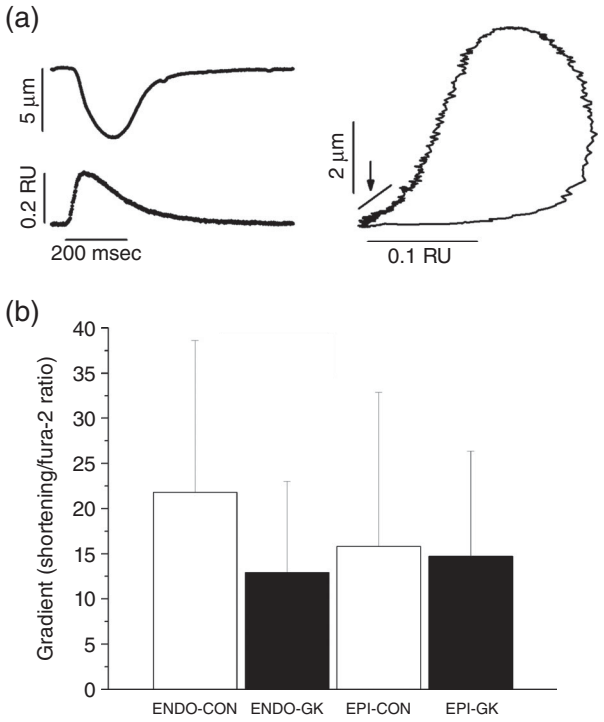

FIGURE 6 Ca^{2+} sensitivity of cardiac myofilaments. (a) Typical record of shortening and Ca^{2+} transient (left panel) and a phase-plane diagram of fura-2 ratio *versus* cell length (right panel) in an EPI-CON myocyte. (b) Mean gradient of the fura-2–cell length trajectory during late relaxation (500–800 ms) of the twitch contraction. Data are shown as means + SD. $n = 18–23$ cells from four or five GK and CON hearts. RU = Ratio units

prolonged in ENDO-STZ compared with respective controls (Smail et al., 2016). The amplitude of shortening was similar in EPI and ENDO myocytes from GK and control rats; a finding that is consistent with previous regional myocyte results in myocytes from the STZ rat (Smail et al., 2016) and generally in a single population of ventricular myocytes in the GK rat (Howarth et al., 2007; Salem et al., 2012). The lack of change in the parameters of cell shortening between the GK and control groups is in stark contrast to the results obtained in multicellular preparations of trabecular muscle from STZ diabetic animals (Zhang, Cannell, Phillips, Cooper, & Ward, 2008). The authors of that study observed a substantial reduction in peak isometric force, decreased rate of force development and decreased rate of relaxation. Although we cannot rule out potential differences between the GK and STZ models of diabetes, the most likely reason for the discrepancies lies in the differences between experimental approaches in these two studies, namely single-cell unloaded shortening *versus* isometric contraction of multicellular tissue.

Indeed, previous studies have variously demonstrated increased collagen deposition and increased ventricular stiffness in different experimental models of type 2 diabetes, which in turn were associated with altered kinetics of myocardial contraction (Patel, Iyer, & Brown, 2009; Rickman, Iyer, Chan, & Brown, 2010).

Resting values of fura-2 ratio were similar in EPI-GK and ENDO-GK compared with respective controls, suggesting that the resting Ca^{2+} concentration was unaltered by diabetes. This was a similar result to that previously reported in EPI and ENDO myocytes and in trabecular preparations from the STZ rat (Smail et al., 2016; Zhang et al., 2008). The TPK Ca^{2+} transient was similar in control EPI and ENDO myocytes. However, the TPK Ca^{2+} transient was prolonged in EPI-GK compared with EPI-CON myocytes. In the STZ rat, there were no changes in TPK Ca^{2+} transient in EPI-STZ compared with EPI-CON; however, ENDO-STZ was prolonged compared with ENDO-CON myocytes (Smail et al., 2016). Previous studies have also reported prolongation of the TPK Ca^{2+} transient in a single population of myocytes from GK hearts (Gaber et al., 2014). This might be explained by an altered flux of Ca^{2+} through L-type Ca^{2+} channels or release of Ca^{2+} from the SR. The THALF decay of the Ca^{2+} transient was also prolonged in EPI-CON compared with EPI-GK and in EPI-CON compared with ENDO-CON myocytes. The decay of the Ca^{2+} transient is partly dependent on efflux of Ca^{2+}, primarily on the Na^{+}–Ca^{2+} exchange and uptake of Ca^{2+} by the SR. In the STZ rat, the THALF decay of the Ca^{2+} transient was prolonged in ENDO-STZ compared with ENDO-CON and in EPI-CON compared with ENDO-CON myocytes (Smail et al., 2016). Consistent with the shortening results, there was no significant variation in the amplitude of the Ca^{2+} transient in ENDO and EPI from control and GK ventricular myocytes. Collectively, the results of intracellular Ca^{2+} dynamics demonstrate regional differences in the time course of the Ca^{2+} transient in healthy ventricle, in addition to differences that can be attributed to diabetes.

The SR Ca^{2+} content was similar in EPI and ENDO myocytes from GK and control rats. This is evident from SR fractional release of Ca^{2+} and the area under the curve of the caffeine-stimulated Ca^{2+} transient, which were unaltered in EPI and ENDO from GK and CON myocytes.

Na^{+}–Ca^{2+} exchange is a major pathway for Ca^{2+} extrusion during relaxation of cardiac muscle, and therefore, it is an important mediator for Ca^{2+} homeostasis (Hattori et al., 2000). Therefore, it is possible that the decrease in Ca^{2+} transients observed in EPI-GK and ENDO-GK might be attributed to altered activity of Na^{+}–Ca^{2+} exchange during Ca^{2+} efflux. A study conducted by Sheikh et al. (2012) demonstrated that cardiac endothelial cells from diabetic rats treated with a Na^{+}–Ca^{2+} exchange inhibitor had a higher intracellular Ca^{2+} transient peak compared with no-inhibitor controls (Sheikh et al., 2012).

In view of the ability of mitochondria to accumulate large amounts of Ca^{2+}, these organelles are known to prevent and/or delay the occurrence of intracellular Ca^{2+} overload in cardiomyocytes in different pathological conditions. For example, during the development of cardiac dysfunction and intracellular Ca^{2+} overload in chronic diabetes, mitochondria are believed to continue accumulating Ca^{2+}, serving as a protective mechanism (Dhalla, Liu, Panagia, & Takeda, 1998; Dhalla, Rangi, Zieroth, & Xu, 2012). Therefore, it is possible that altered mitochondrial uptake of Ca^{2+} during diabetes is responsible for decreased Ca^{2+} transients observed here. Although mitochondria are being established as a contributor to cellular Ca^{2+} signalling, the exact role of changes in these mechanisms in diabetic cardiomyopathy remains to be investigated.

Myofilament sensitivity to Ca^{2+} was unaltered at this stage of diabetes. This result is consistent with an earlier study that showed no effect of type 2 diabetes on myofilament sensitivity in ventricular myocytes from young GK rats (Howarth et al., 2011). However, a previous

study in 18-month-old, type 2 diabetic GK rats reported an increase in the gradient of the fura-2–cell length trajectory, suggesting that myofilament sensitivity to Ca^{2+} might alter during the later stages of type 2 diabetes (Howarth & Qureshi, 2008).

The results of the present study have demonstrated differences in the time course of shortening and the Ca^{2+} transient in EPI compared with ENDO myocytes from GK compared with Wistar rat hearts. A prolonged time course of the Ca^{2+} transient might partly underlie the prolonged time course of shortening in EPI myocytes from GK compared with control rat hearts. Further studies will be required to clarify the mechanisms underlying the changes in the time course of shortening and the Ca^{2+} transient in EPI and ENDO myocytes from diabetic and control hearts.

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COMPETING INTERESTS

None declared.

AUTHOR CONTRIBUTIONS

M.S. was involved in data analysis and critical revision. L.A.K., A.S., M.O. and J.S. were involved in conception, design and critical revision. M.A.Q. was involved in data acquisition and critical revision. F.C.H. was involved in conception, design, interpretation of data, drafting the manuscript and critical revision. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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