Acetyl-L-Carnitine and Oxfenicine on Cardiac Pumping Mechanics in Streptozotocin-Induced Diabetes in Male Wistar Rats

Chih-Hsien Wang¹, Shoei-Shen Wang¹, Wen-Je Ko², Yih-Sharng Chen¹, Chun-Yi Chang³, Ru-Wen Chang², Kuo-Chu Chang⁴*

¹ Department of Surgery, National Taiwan University Hospital, Taipei, Taiwan, ² Department of Surgery and Traumatology, National Taiwan University Hospital, Taipei, Taiwan, ³ Department of Emergency Medicine, National Taiwan University Hospital, Taipei, Taiwan, ⁴ Department of Physiology, College of Medicine, National Taiwan University, Taipei, Taiwan

Abstract

Introduction: In the treatment of patients with diabetes, one objective is an improvement of cardiac metabolism to alleviate the left ventricular (LV) function. For this study, we compared the effects of acetyl-L-carnitine (one of the carnitine derivatives) and of oxfenicine (a carnitine palmitoyltransferase-1 inhibitor) on cardiac pumping mechanics in streptozotocin-induced diabetes in male Wistar rats, with a particular focus on the pressure-flow-volume relationship.

Methods: Diabetes was induced by a single tail vein injection of 55 mg kg⁻¹ streptozotocin. The diabetic animals were treated on a daily basis with either acetyl-L-carnitine (1 g L⁻¹ in drinking water) or oxfenicine (150 mg kg⁻¹ by oral gavage) for 8 wk. They were also compared with untreated age-matched diabetic controls. LV pressure and ascending aortic flow signals were recorded to calculate the maximal systolic elastance (Eₘₚₚₚ) and the theoretical maximum flow (Qₘₚₚ). Physically, Eₘₚₚ reflects the contractility of the myocardium as an intact heart, whereas Qₘₚₚ has an inverse relationship with the LV internal resistance.

Results: When comparing the diabetic rats with their age-matched controls, the cardiodynamic condition was characterized by a decline in Eₘₚₚ associated with the unaltered Qₘₚₚ. Acetyl-L-carnitine (but not oxfenicine) had reduced cardiac levels of malondialdehyde in these insulin-deficient animals. However, treating with acetyl-L-carnitine or oxfenicine resulted in an increase in Eₘₚₚ, which suggests that these 2 drugs may protect the contractile status from deteriorating in the diabetic heart. By contrast, Qₘₚₚ showed a significant fall after administration of oxfenicine, but not with acetyl-L-carnitine. The decrease in Qₘₚₚ corresponded to an increase in total vascular resistance when treated with oxfenicine.

Conclusions: Acetyl-L-carnitine, but not oxfenicine, optimizes the integrative nature of cardiac pumping mechanics by preventing the diabetes-induced deterioration in myocardial intrinsic contractility associated with unaltered LV internal resistance.

Introduction

It has been established that diabetes results in a cardiomyopathy, and increasing evidence suggests that an altered substrate supply and utilization by cardiac myocytes could be the primary injury in the pathogenesis of this specific heart muscle disease [1,2]. For example, patients with diabetes have an impaired cardiac glucose oxidation shifted toward a greater uptake and usage of free fatty acids (FFA) with reduced metabolic efficiency [3]. These alterations in cardiac metabolism may be responsible for both the increased susceptibility of the diabetic heart to myocardial ischemia and a proportionally greater decrease of myocardial performance [4,5]. Thus, in the treatment of patients with diabetes, one objective is an improvement of cardiac carbohydrate metabolism to alleviate myocardial ischemia and left ventricular (LV) dysfunction [6]. The major strategies of the treatment are either reducing the circulating levels of FFA through carnitine supplementation or inhibiting the mitochondrial uptake of FFA through suppression of carnitine palmitoyltransferase-1 (CPT-1).

CPT-1, located in the outer mitochondrial membrane, is a key enzyme in FFA oxidation, and is the rate-limiting step involved in the transfer of fatty acyl groups into the mitochondria [7]. Carnitine is the essential cofactor of CPT-1, acting as the acceptor of fatty acyl groups to transport long-chain fatty acids across mitochondrial membranes for β-oxidation [8,9]. Carnitine also
Acetyl-L-Carnitine Improves Diabetic Heart

reduces the intramitochondrial ratio of acetyl-CoA to free CoA, which stimulates the activity of the pyruvate dehydrogenase complex to facilitate glucose oxidation. An alternative approach to achieve a switch in energy substrate preference, away from FFA metabolism and toward glucose metabolism, is to inhibit FFA uptake by the mitochondria using CPT-1 inhibitors.

Carnitine derivatives are potent antiarrhythmic agents and may protect tissues from oxidative damage [10,11]. Acetyl-L-carnitine (ALC) (a carnitine derivative) possesses similar physiological functions but better bioavailability and antioxidant capacity compared with carnitine [12]. The more effective action of ALC compared with t-carnitine on oxidative stress may be attributed to the acetyl group [13]. ALC was reported to have protective action on NADPH-induced lipid peroxidation of rat cardiac microsomes [14]. Moreover, long-term treatment with ALC may be of potential value in preventing the progressive loss of myocardial sympathetic nervous function in patients with diabetes [15]. Conversely, oxfenicine (OXF) is a well-characterized CPT-1 inhibitor that can reduce the accumulation of long-chain acyl-carnitine to enhance glucose metabolism [16]. Our team demonstrated in the past that ALC, but not OXF, attenuated arterial stiffening by reducing aorta levels of malondialdehyde (MDA) in insulin-deficient rats [17]. MDA is a highly toxic byproduct formed by lipid oxidation-derived free radicals, which can react with collagen to form MDA-collagen cross-links with profound cardiovascular risk [18,19]. Thus, the crucial question yet to be answered is whether the impaired cardiac function in diabetes can be improved by OXF therapy associated with high MDA content in the diabetic heart.

The myocardium of the left ventricle is a viscoelastic material whose mechanical properties are reflected in the behavior of the ventricular chamber (i.e. the relationships among chamber pressure, volume, and flow) [20,21]. For this study, we compared the effects of ALC and of OXF on cardiac pumping mechanics in streptozotocin (STZ)-induced diabetes in male Wistar rats, with a particular focus on the pressure-flow-volume relationship. LV pressure and ascending aortic flow signals were measured to evaluate the systolic mechanical behavior of the ventricular pump, by making use of the elastance-resistance model [22,23]. Cardiac levels of MDA were also detected in the diabetic rats after administration of ALC or OXF.

Methods

Animals and Catheterization

Two-month-old male Wistar rats were randomly divided into 6 groups: (i) normal controls (NC) (n = 16); (ii) NC+ALC (n = 16); (iii) NC+OXF (n = 16); (iv) STZ-induced diabetic rats (DM) (n = 16); (v) DM+ALC (n = 16); and (vi) DM+OXF (n = 16). Diabetes was induced in animals by a single tail vein injection with 55 mg kg\(^{-1}\) STZ in 0.1M citrate buffer (pH 4.5) (Sigma Chemical Co., St. Louis, MO, USA). Blood glucose levels were determined using SURESTEP Test Strips (Lifescan Inc., Milpitas, CA, USA) for confirming developments of hyperglycemia. Two wk later, rats with stable hyperglycemia were daily treated with either ALC (Sigma Chemical Co., St. Louis, MO, USA) or OXF (Sigma Chemical Co., St. Louis, MO, USA). It has been suggested that treatment with t-carnitine (1 g L\(^{-1}\) in drinking water) could exert cardio-protective effects in STZ-induced diabetic rats [24]. In this study, the insulin-deficient animals were administered ALC at a dose of 1 g L\(^{-1}\), which was added to the drinking water for the duration of the study. Considering that the NC had higher body weight and lower drinking amount than the DM, we treated the NC with ALC at a dose of 3 g L\(^{-1}\) in drinking water. Two to 3 animals were housed per cage in a 12-h light/dark cycled animal room with free access to Purina chow and water. We measured the water amount the animals daily consumed per cage and calculated the water consumption per rat in average. At the end of the experiment, the DM drank 48.2±0.5 mL d\(^{-1}\), and the NC drank 23.1±0.4 mL d\(^{-1}\). In average, the dosage of ALC per rat was ~140 mg kg\(^{-1}\) in the DM and ~146 mg kg\(^{-1}\) in the NC. Conversely, OXF was dissolved in carboxymethylcellulose sodium salt (Sigma Chemical Co., St Louis, MO, USA) because of its poor water solubility. OXF was then delivered to rats by gavage at the doses of 150 mg kg\(^{-1}\) d\(^{-1}\). Rats were studied 8 wk after exposure to ALC or OXF to determine the drug’s effects on their systolic mechanical behavior of their ventricular pump. All animal experiments were approved by National Taiwan University’s Animal Care and Use Committee and conducted according to the Guide for the Care and Use of Laboratory Animals.

General surgical procedures and measurements of cardio-dynamic variables in anesthetized rats have been previously described [25]. Animals were anesthetized with intraperitoneal sodium pentobarbital (50 mg kg\(^{-1}\)), placed on a heating pad, intubated, and ventilated with a rodent respirator (model 131, New England Medical Instruments, Medway, MA, USA). The chest was opened through the second intercostal space of the right side. An electromagnetic flow probe (model 100 series, internal circumference 8 mm; Carolina Medical Electronics, King, NC, USA) was positioned around the ascending aorta to record the pulsatile aortic flow. A high-fidelity pressure catheter (model SPC 320, size 2F; Millar Instruments, Houston, TX, USA) was inserted via the isolated right carotid artery into the LV to measure LV pressure. The electrocardiogram (ECG) of lead II was recorded with an ECG/Biotach amplifier (Gould, Cleveland, OH, USA). The selective LV pressure and aortic flow signals averaged 5–10 beats in the time domain using the peak R wave of ECG as a fiducial point. A single-beat estimation technique was performed to calculate the systolic elastance and resistance, which characterize the pumping mechanics of diabetic hearts [26,27].

Prediction of the LV Pressure Using the Elastance-resistance Model

Model-derived pressure of the LV \(\hat{P}(t)\) can be predicted using the elastance-resistance model if the model parameters are previously identified [22,23]. The relationship among instantaneous LV pressure, isovolumic pressure, and aortic flow can be written as follows:

\[
P(t) = P_{iso}(t)\left[1 - \frac{V_{ch}(t)}{V_{ref}}\right]\left[1 - \frac{Q(t)}{Q_{max}}\right]
\]

where \(V_{ch}(t)\) is the instantaneously ejected volume computed by numerically calculating the running integral of the aortic flow signal \(Q(t)\), \(Q_{max}\) is the theoretical maximum flow, and \(V_{ref}\) is the effective LV end-diastolic volume, which is the volume difference between LV end-diastolic volume and the zero-pressure volume-axis intercept. \(P_{iso}(t)\) is the isovolumic pressure obtained by occluding the ascending aorta near the sinuses of the Valsalva at the end of the diastole. In this study, \(P_{iso}(t)\) was derived from the measured pressure of an ejection contraction by using a nonlinear least-squares approximation technique [28]:

\[
P_{iso}(t) = \frac{1}{2}P_{ld\ max}[1 - \cos(\omega t + \phi)] + P_d
\]

where \(P_{ld\ max}\) is a peak-developed isovolumic pressure, \(\phi\) is an
Figure 1. The solid lines of A and B show the measured ascending aortic flow signal and the LV pressure waveform, respectively, of one control rat. In Graph B, the dashed line represents the isovolumic pressure curve at an end-diastolic volume, which is estimated by fitting a sinusoidal function to the isovolumic portions of the measured LV pressure. Graphs C and D show the measured data and model-generated data when the elastance-resistance model is fit over $t_{e}<t<t_{p_{iso}}$. $t_{e}$ is the onset of ventricular ejection and $t_{p_{iso}}$ is the time of peak isovolumic pressure. In Graph C, the solid line represents measured data, and dashed lines represent model-derived data. In Graph D, the dashed line has the
angular frequency, $c$ is a phase shift angle of the sinusoidal curve, and $P_d$ is the LV end-diastolic pressure. $P_{iso}(t)$ in Figure 1B is obtained by fitting the measured LV pressure curve segments from the end-diastolic pressure point to the peak $+dP/dt$ and from the pressure point of the peak $-dP/dt$ to the same level as the end-diastolic pressure of the preceding beat [29]. The peak of the ECG R wave is used to identify the LV end-diastolic point. The estimated peak isovolumic pressure $P_{iso}$ is the pressure sum of $P_{\text{ad max}}$ and $P_{\text{g}}$.

Both $V_{\text{ad}}$ and $Q_{\text{max}}$ are the model parameters that remain to be determined by curve-fitting techniques. Campbell et al. [22] found that Equation 1 can be used to fit the measured LV pressure of an ejecting beat effectively, if the fitting interval is $t_{\text{ej}} < t < t_{\text{ej max}}$; $t_{\text{ej}}$ is the onset of ventricular ejection and $t_{\text{ej max}}$ is the time of peak isovolumic pressure. Initial values of $V_{\text{ad}}$ and $Q_{\text{max}}$ are chosen first. Thereafter, the Nelder-Mead simplex algorithm is used to adjust $V_{\text{ad}}$ and $Q_{\text{max}}$ iteratively to minimize the root-mean-square error ($Q_{\text{g}}$) [30]. Parameters coinciding with the minimum objective function are recorded as the model estimates of the systolic pumping mechanics of the LV (Figure 1C). Thus, the LV systolic elastance can be calculated by using $E(t) = P_{iso}/V_{\text{ad}}$. Its maximal value is the maximal systolic elastance ($E_{\text{max}} = P_{\text{max}}/V_{\text{ad}}$). The internal resistance of the LV can be expressed as $R(P_{iso}) = P_{iso}(t)/Q_{\text{max}}$ [23]. In addition, the total vascular resistance of the systemic circulation ($R_s$) was calculated as the mean aortic pressure/mean aortic flow.

**LV End-systolic Equilibrium Point**

The LV end-systolic equilibrium point could be identified as follows. The peak LV isovolumic pressure at the end-diastolic volume ($P_{\text{max}}$) was estimated by the equation (2). The pressure-ejected volume loop was obtained by the time integration of aortic flow and the measured LV pressure. Thus, drawing a tangential line from the estimated $P_{\text{max}}$ to the right corner of the pressure-ejected volume loop yielded a point referred to as the end-systolic equilibrium point [27,31].

**Cardiac $dP/dt_{\text{max}}$, $dP/dt_{\text{min}}$, and Time Constant of LV Isovolumic Pressure Decline**

Readings of pulsatile LV pressure waveform yielded cardiac $dP/dt_{\text{max}}$, $dP/dt_{\text{min}}$, and time constant of LV isovolumic pressure decline ($\tau$). The LV end-diastolic point was identified as the peak of the ECG R wave. The time constant of LV pressure decay during the isovolumic relaxation period was calculated using the method proposed by Weiss et al. [32]; $\tau$ was calculated as the negative inverse slope of the lnP versus t relationship. Since the LV isovolumic pressure decline was assumed to be monoexponential, we examined the linearity of the lnP versus t relation and calculated LV $\tau$ only when the relation between lnP and t yielded a high linear correlation coefficient [33].

**Estimate of MDA Content in the LV by the use of Thiobarbituric Acid (TBA) Assay**

Although MDA is not the only physiological molecule that can react with TBA [34], the TBA assay is still the most frequently used assay for MDA. Based on this method, results are “TBA reactive substances” (TBARS) instead of MDA. Hence, TBARS is used as an estimate of MDA herein.

At the end of catheterization, the rat heart was perfused with phosphate buffered saline (PBS). Thereafter, the LV was dissected, washed quickly with ice-cold PBS, and immediately frozen with liquid nitrogen. The frozen tissues were stored at $-80^\circ$C until analysis. All tissues were homogenized in the RIPA buffer (Sigma Chemical Co., St Louis, MO, USA) with a 1% protease inhibitor cocktail (Sigma Chemical Co., St Louis, MO, USA) and centrifuged at 1600 g for 4°C for 10 min to obtain supernatants for MDA measurement. LV MDA contents were estimated by TBARS using a commercial kit (Cayman, U.S.A.) [35]. Protein concentrations of the LV were assayed using the Bradford method (DCProtein Assay, Bio-Rad) [36].

**Statistics**

Results are expressed as means ± SE. Two-way ANOVA was used to assess the cardiodynamic and metabolic effects of ALC and of OXF in the STZ-induced diabetic rats. Simple effect analysis was implemented when a significant interaction between diabetes and ALC or OXF occurred. Differences among means within levels of a factor were determined using Tukey’s honestly significant difference (HSD) method. Statistical significance is defined as $p<0.05$.

**Results**

Table 1 shows the effects of either ALC or OXF on blood glucose level, body weight (BW), and left ventricular weight (LVW) in the DM. The high glucose level in the DM did not change in response to either ALC or OXF treatment. After exposure to

| Variable | NC | NC+ALC | NC+OXF | DM | DM+ALC | DM+OXF |
|----------|----|--------|--------|----|--------|--------|
| BS       | 98.8±1.5 | 105.8±2.9 | 104.6±2.0 | 468.3±16.8 | 462.0±8.0 | 458.8±9.8 |
| BW       | 451.9±9.1 | 477.5±10.9 | 471.3±8.4 | 292.1±8.1 | 326.6±10.1 | 309.7±11.7 |
| LVW      | 0.839±0.025 | 0.829±0.023 | 0.916±0.020 | 0.689±0.027 | 0.635±0.022 | 0.746±0.024 |
| LVW/BW   | 1.852±0.030 | 1.736±0.024 | 1.950±0.041 | 2.359±0.060 | 1.944±0.031 | 2.409±0.068 |

All values are expressed as means ± SEM. BS, blood sugar (mg Dl $^{-1}$); BW, body weight (g); LVW, left ventricular weight (g); LVW/BW, ratio of the LVW to BW (mg g $^{-1}$). NC, normal controls; NC+ALC, NC treated with ALC; NC+OXF, NC treated with OXF; DM, STZ-diabetic rats; DM+ALC, DM treated with ALC; DM+OXF, DM treated with OXF; ALC, acetyl-L-carnitine; OXF, oxfenicine.

Statistical difference (P<0.05) from the NC.

Statistical difference (P<0.05) from the DM.

doi:10.1371/journal.pone.0069977.t001
Table 2. Effects of either ALC or OXF on hemodynamic parameters in the STZ-diabetic rats.

|         | NC   | NC+ALC | NC+OXF | DM    | DM+ALC | DM+OXF |
|---------|------|--------|--------|-------|--------|--------|
| HR      | 407.8± 406.3± | 398.0± 373.5± | 354.1± 331.9± | 9.9 | 8.3 | 7.6 |
| CO      | 1.92± 1.95± | 2.01± 1.92± | 1.89± 1.70± | 0.09 | 0.06 | 0.09 |
| CI      | 2.04± 1.97± | 2.09± 2.63± | 2.47± 2.27± | 0.10 | 0.06 | 0.08 |
| MAP     | 100.5± 98.4± | 105.2± 95.1± | 88.8± 108.9± | 1.9 | 2.5 | 1.8 |

All values are expressed as means ± SE. HR, basal heart rate (beats min⁻¹); CO, cardiac output (mL min⁻¹); CI, cardiac index (L min⁻¹ m⁻²); MAP, mean aortic pressure (mmHg); NC, normal controls; NC+ALC, NC treated with ALC; NC+OXF, NC treated with OXF; DM, STZ-diabetic rats; DM+ALC, DM treated with ALC; DM+OXF, DM treated with OXF; ALC, acetyl-L-carnitine; OXF, oxfenicine.

Table 3. Effects of either ALC or OXF on cardiac function in the STZ-diabetic rats.

|         | NC   | NC+ALC | NC+OXF | DM    | DM+ALC | DM+OXF |
|---------|------|--------|--------|-------|--------|--------|
| Pcor     | 105.8± 102.9± | 105.7± 107.2± | 96.2± 3.1± | 2.49± 3.09 | 2.58± 3.01 | 9.77± 1.18± | 0.006± 0.070 | 5.16± 1.08± | 112.5± 3.0±|
| Pcor     | 1021.3± 1023.1± | 1023.1± 1035.6± | 10207.1± 443.5± | 6918.1± 209.7± | 6815.7± 353.2± | 50.9368± 0.3126± | 0.3909± 5.0393± | 8229.9± 317.9± |
| dP/dtmax | -726.9± 299.5± | -694.0± 287.2± | -699.0± 190.2± | -508.5± 198.5± | -592.9± 225.4± | -4936.6± 193.5± | 8.54± 0.21 | 9.38± 0.26 | 9.35± 0.24 | 14.81± 0.65± | 11.13± 0.38± | 14.46± 0.55± |

All values are expressed as means ± SE. Pcorr, LV end-systolic pressure (mmHg); Pmax, LV end-diastolic pressure (mmHg); dP/dtmax, mmHg s⁻¹; t, time constant of LV isovolumic pressure decay (ms); NC, normal controls; NC+ALC, NC treated with ALC; NC+OXF, NC treated with OXF; DM, STZ-diabetic rats; DM+ALC, DM treated with ALC; DM+OXF, DM treated with OXF; ALC, acetyl-L-carnitine; OXF, oxfenicine.

Table 2. Effects of either ALC or OXF on hemodynamic parameters in the STZ-diabetic rats.

Table 3. Effects of either ALC or OXF on cardiac function in the STZ-diabetic rats.
levels of MDA/TBARS (Figure 5). The diabetes-related increase in plasma levels of FFA and cardiac levels of MDA/TBARS were attenuated by the administration of ALC to the diabetic rats. By contrast, treatment of the DM with OXF enhanced their already high FFA plasma levels (shown in Ref. 17). Moreover, OXF therapy produced no beneficial effects on lipid oxidation-derived MDA/TBARS of the diabetic rat heart. Neither ALC nor OXF therapy exerted effects on those metabolic factors in the NC.
Discussion

Previous work from our laboratory demonstrated that OXF, but not ALC, elevates $R_p$ in the diabetic peripheral arteries, which parallels its increase in plasma levels of FFA [17]. By contrast, ALC attenuates the diabetes-related arterial stiffening and cardiac hypertrophy through its ability to reduce aorta levels of MDA/TABRS in the DM.

In this study, we compared the effects of ALC and OXF on cardiac pumping mechanics in insulin-deficient rats. The systolic
mechanical behavior of the ventricular pump could be characterized by both $E_{\text{max}}$ and $Q_{\text{max}}$ [22,23]. Physically, $E_{\text{max}}$ is an indicator of elasticity, which reflects subtle changes in contractile status and is independent of preload, afterload, and heart rate in a given contractile state of the ventricle [20,37]. Therefore, $E_{\text{max}}$ represents the contractility of the myocardium as an intact heart. However, $Q_{\text{max}}$ has an inverse relationship with LV internal resistance and is the amount of outflow generated by the ventricle if it were to eject under zero load condition [23]. Results from this study suggest that either ALC or OXF increases $E_{\text{max}}$ to protect
the contractile status from deteriorating in the diabetic heart. However, OXF diminishes $Q_{\text{max}}$ so that the LV internal resistance rose, impairing the ventricular outflow in the DM. The novelty of this study is that one can distinguish the effect of $Q_{\text{max}}$ from that of $E_{\text{max}}$ on the pumping function of the diabetic heart administered either ALC or OXF.

Abnormalities of insulin regulation in the diabetic heart may cause disturbances in calcium homeostasis and the myosin isoenzyme profile [38–40], which is responsible for the defects of cardiac pumping mechanics. As mentioned, LV $E_{\text{max}}$ can be determined by the ratio of $P_{\text{iso}}$ to $V_{\text{eed}}$. In this study, a significant decrease in $P_{\text{iso}}$ implied that the diabetic myocardium was incapable of producing enough pressure to support $E_{\text{max}}$ along with the increased $V_{\text{eed}}$. The worsened $P_{\text{iso}}-V_{\text{eed}}$ relation in the diabetic heart suggested that the underlying cooperative mechanisms in the cardiac muscle, such as length-sensitivity [41], may be impaired. Meanwhile, the shift of the myosin isoenzyme profile from the fast V1 isoform toward the slow V3 isoform has been noticed in the diabetic heart [38–40]. Although Shroff et al. [23] reported that $Q_{\text{max}}$ has an inverse relationship with the percentage of slow V3 isoform, no significant alteration in $Q_{\text{max}}$ was observed in the DM in this study.

In experimental animals, it has been shown that carnitine levels are depressed in the diabetic cardiomyopathic heart [42]. In this study, we found that treatment with ALC significantly affected the STZ-derived impairment in $V_{\text{eed}}$, leading to an increase in $E_{\text{max}}$. Neely and Morgan [43] suggested that higher plasma levels of FFA and their fatty acyl-CoA esters may be detrimental to the myocardial structure and function. Folden et al. [44] also demonstrated a novel role of MDA in lipid peroxidation and oxidative stress-associated cardiac dysfunction. Thus, the reduced plasma FFA and cardiac MDA levels from ALC may be responsible for the prevention of diabetes-related damage in the myocardial contractility. By contrast, in the absence of any significant changes in cardiac MDA content, the already high plasma levels of FFA augmented when the DM was treated with OXF [17]. OXF might accordingly be expected to exert no benefit to the diabetic heart and even worsen the contractile status of the LV. However, we found that treating the diabetic rats with OXF showed a decrease in $V_{\text{eed}}$ with an accompanied increase in $P_{\text{iso}}$, which significantly increased $E_{\text{max}}$. Zarain-Herzberg and Rupp [45] reported that CPT-1 inhibitor has effects on LV function, which can be attributed to selective changes in the dysregulated gene expression of cardiomyocytes whereby the structure of several proteins are modified. Thus, its ability to increase the sarcoplasmic reticulum (SR) Ca$^{2+}$-ATPase-2 protein expression and the SR Ca$^{2+}$-ATPase activity may allow OXF to improve the contractile state of the diabetic heart.

Another aspect of cardiac mechanics is $Q_{\text{max}}$, which remained unchanged in diabetic rats when compared with their age-matched controls. Rupp et al. [16] reported that the decrease in myosin ATPase activity may be prevented by treating the CPT-1 inhibitor. Hence, the isoenzyme shift by OXF toward fast myosin V1 might be expected to raise $Q_{\text{max}}$ and thus, decrease LV internal resistance. However, $Q_{\text{max}}$ did not increase, but decreased with OXF administration to the DM. This result could be explained by the finding that arterial load is also an important factor that inversely affects $Q_{\text{max}}$ [25,46]. Our previous study showed that treating with OXF enhanced the already high plasma levels of FFA, which increased $R_p$ in the diabetic peripheral circulation [17]. This elevated $R_p$ may prevail over the isoenzyme shift toward fast V1, resulting in a decline in $Q_{\text{max}}$ (Figure 4B). The diminished $Q_{\text{max}}$ may augment LV internal resistance and impair the ventricular outflow in insulin-deficient rats. Thus, OXF could

---

**Figure 5. Effects of either ALC or OXF on cardiac levels of MDA/TBARS in DM.** NC, normal controls; NC+ALC, NC treated with ALC; NC+OXF, NC treated with OXF; DM, STZ-induced diabetic rats; DM+ALC, DM treated with ALC; DM+OXF, DM treated with OXF; ALC, acetyl-L-carnitine; OXF, oxfenicine.

doi:10.1371/journal.pone.0069977.g005
not optimize the integrative nature of the cardiac pumping mechanics because the reduced $Q_{\text{max}}$ counteracted the enhanced $E_{\text{max}}$ in the DM. By contrast, ALC supplementation did not modify $Q_{\text{max}}$ in the diabetic animals (Figure 3B). Thus, the enhanced $E_{\text{max}}$ and unaltered $Q_{\text{max}}$ from ALC treatment may maintain the optimality of energy transferred from the LV to the arterial system, which is essential for the metabolic needs of tissues and/or organs in diabetes.

As for the diastolic properties of the LV, ALC treatment of the diabetic rats improved LV relaxation in terms of $dP/dt_{\text{max}}$ and $\tau$ (Table 3). The similar finding of a significant improvement of cardiac diastolic function has been observed in STZ-diabetic rats administered l-carnitine [47]. Although etomoxir (another CPT-1 inhibitor) was reported to have a selective influence on the rate of relaxation of pressure-overloaded rat heart [48], OXF treatment in this study produced no beneficial effects on either $dP/dt_{\text{max}}$ or $\tau$ in the DM.

Based on the findings in this study, there is a good possibility to use ALC to treat patients with metabolic disturbances in diabetic cardiomyopathy. That is because ALC therapy may target those metabolic aberrations in the heart and exert a great benefit to cardiovascular performance. Treatment with ALC significantly reduced abnormalities in lipid profiles, attenuated arterial stiffening and cardiac hypertrophy, and improved myocardial contractility and ventricular relaxation. As for the clinical study with the CPT-1 inhibitor OXF, it was reported to significantly increase the time to onset of angina in patients subjected to progressive pacing stress [49]. However, the drug was shown to damage mitochondrial metabolism, reducing oxygen consumption and uncoupling oxidative phosphorylation in the rat heart [50]. Its development in clinical study was discontinued [51]. Herein, although OXF improved myocardial contractility in diabetes, it exerted biochemical toxicity to the heart, i.e. accumulation of FFA and MDA. Treatment with OXF had no effect on cardiac mass and relaxation function, even deteriorated cardiac output, mean aortic pressure and LV end-systolic pressure. In this informative manner, we presented the clinical differences between the effects of OXF and ALC, suggesting that ALC may be a potential candidate for the treatment of patients with metabolic disturbances.

Certain limitations of this study need to be addressed. Our approach is highly dependent on the elastance-resistance model, which is not a perfect model for the evaluation of LV systolic mechanics. Hunter et al. [20] demonstrated that in addition to elastance and resistance, at least 2 or more processes are involved that determine systolic mechanical behavior of the ventricular pump. These processes include the effects of the volume influence factor and the deactivation factor. However, Campbell et al. [22] showed that the elastance-resistance model could be used to effectively fit the measured LV pressure of an ejecting beat if the fitting interval is $t_{\text{p}}<t<t_{\text{p}}w_{\text{max}}$. Furthermore, Shroff et al. [23] believed that the elastance-resistance model is useful for quantifying the systolic pumping mechanics of the LV if one clearly understands its limitations.

Our contribution in this endeavor was to provide a path to consider the clinical application of an elastance-resistance model in the study of cardiodynamics. From the technical point of view, the indispensable isovolumic signals must be obtained by occluding the ascending aorta at the end of diastole, and this measuring technique is not permitted in human subjects. To unravel this serious issue, the isovolumic pressure curve was obtained from the instantaneous pressure of an ejecting contraction by a curve-fitting technique, proposed by Sunagawa et al. [28]. Our data showed good quality of the model fit when this elastance-resistance model with the estimated isovolumic pressure was applied. The practical advantage of such an approach was that one could compute the ventricular elastance and resistance without any measurements of isovolumic contraction. Moreover, these two cardiac systolic parameters could be calculated from the pulsatile LV pressure and ascending aortic flow signals obtained over a single cardiac cycle without any perturbations of the loading conditions.

Overall, alterations that occurred in the LV included a decline in $E_{\text{max}}$ in the absence of any significant changes in $Q_{\text{max}}$ in the STZ-induced diabetic rats. An increase in $P_{\text{voll}}$ might act in concert with the decreased $P_{\text{vomax}}$ and reduce $E_{\text{max}}$ so that the contractile status of the diabetic heart was impaired. ALC (but not OXF) had reduced plasma FFA levels and cardiac MDA contents in diabetes. However, treating with either ALC or OXF resulted in $E_{\text{max}}$ increases, which suggests that these 2 drugs may protect the myocardial contractility from deteriorating in rats with insulin deficiency. By contrast, $Q_{\text{max}}$ decreased with OXF, but not with ALC, augmenting LV internal resistance in diabetes. Moreover, treating the diabetic rats with ALC, but not with OXF, produced a benefit on LV relaxation in terms of $dP/dt_{\text{max}}$ and $\tau$. Thus, its ability to prevent the cardiovascular dysfunction of the diabetic rats allowed ALC, but not OXF, to maintain the optimality of energy transferred from the LV to the arterial system.

**Author Contributions**

Conceived and designed the experiments: KCC CHW. Performed the experiments: CHW RWG. Analyzed the data: CYC RWG CHW. Contributed reagents/materials/analysis tools: SSW WJK YSC. Wrote the paper: KCC CHW.

**References**

1. Rodrigues B, McNeill JH (1992) The diabetic heart: metabolic causes for the development of a cardiomyopathy. Cardiovasc Res 26: 913–992.

2. Rodrigues B, Cann MC, McNeill JH (1995) Myocardial substrate metabolism: implications for diabetic cardiomyopathy. J Mol Cell Cardiol 27: 169–179.

3. Vitale C, Collins P (2008) Optimization of cardiac metabolism in diabetes mellitus. Curr Pharm Des 14: 2537–2550.

4. Stanley WC, Lopaschuk GD, McCormack JG (1997) Regulation of energy substrate metabolism in the diabetic heart. Cardiovasc Res 34(1): 25–33.

5. Young ME, McNulty P, Taegtmeyer H (2002) Adaptation and maladaptation of substrate metabolism in the diabetic heart. Cardiovasc Res 34(1): 25–33.

6. Rodrigues B, Cam MC, McNeill JH (1995) Myocardial substrate metabolism: implications for diabetic cardiomyopathy. J Mol Cell Cardiol 27: 169–179.

7. Vitale C, Collins P (2008) Optimization of cardiac metabolism in diabetes mellitus. Curr Pharm Des 14: 2537–2550.

8. Stanley WC, Lopaschuk GD, McCormack JG (1997) Regulation of energy substrate metabolism in the diabetic heart. Cardiovasc Res 34(1): 25–33.

9. Young ME, McNulty P, Taegtmeyer H (2002) Adaptation and maladaptation of the heart in diabetes: Part II: potential mechanisms. Circulation 105(15): 1061–1070.

10. Rodrigues B, Cann MC, McNeill JH (1998) Metabolic disturbances in diabetic cardiomyopathy. Mol Cell Biochem 180: 53–57.

11. McGarry JD, Mills SE, Long CS, Foster DW (1983) Observations on the affinity for carnitine, and malonyl-CoA sensitivity, of carnitine palmitoyltransferase I in animal and human tissues. Demonstration of the presence of malonyl-CoA in non-hepatic tissues of the rat. Biochem J 214(1): 21–28.

12. Lee I, Horozov J, Frenneaux M (2004) Metabolic manipulation in ischaemic heart disease, a novel approach to treatment. Eur Heart J 25: 634–641.

13. Steiber A, Kerner J, Hoppel CL (2004) Carnitine: a nutritional, biosynthetic, and functional perspective. Mol Aspects Med 25: 453–473.

14. Mingorance C, Rodriguez-Rodriguez R, Justo ML, Alvarez de Sotomayor M, Herrera MD (2011) Critical update for the clinical use of L-carnitine analogs in cardiometabolic disorders. Vasc Health Risk Manag 7: 169–176.

15. Malaguarre M (2012) Carnitine derivatives: clinical usefulness. Curr Opin Gastroenterol 28: 166–176.

16. Li J, Head E, Kuratsune H, Cotman CW, Ames BN (2004) Comparison of the effects of L-carnitine and acetyl-L-carnitine on cardiac metabolic analogs in cardiometabolic disorders. Vasc Health Risk Manag 7: 169–176.

17. Mingorance C, Rodriguez-Rodriguez R, Justo ML, Alvarez de Sotomayor M, Herrera MD (2011) Critical update for the clinical use of L-carnitine analogs in cardiometabolic disorders. Vasc Health Risk Manag 7: 169–176.

18. Liu J, Head E, Kuratsune H, Cotman CW, Ames BN (2004) Comparison of the effects of L-carnitine and acetyl-L-carnitine on cardiac metabolic analogs in cardiometabolic disorders. Vasc Health Risk Manag 7: 169–176.

19. Schinetti ML, Rossi D, Greco R, Berrilli A (1987) Protective action of acetyl-carnitine on NADPH-induced lipid peroxidation of cardiac microsomes. Drugs Exp Clin Res 13: 509–515.
15. Tarpeinen AK, Koikka JT, Vanninen E, Yang J, Uusitupa MI (2000) Long-term effect of acetyl-L-carnitine on myocardial 123I-MIBG uptake in patients with diabetes. Clin Auton Res 10(1): 13–16.
16. Rupp H, Zarrin-Heizberg A, Mains B (2002) The use of partial fatty acid oxidation inhibitors for metabolic therapy of angina pectoris and heart failure. Herz 27: 621–636.
17. Chang KC, Tereng CD, Lu SC, Liang JT, Wu MS et al. (2010) Effects of acetyl-L-carnitine and oxefinone on aorta stiffness in diabetic rats. Eur J Clin Invest 40: 1092–1101.
18. Slatter DA, Paul RG, Murray M, Bailey AJ (1999) Reactions of lipid-derived malondialdehyde with collagen. J Biol Chem 274: 19661–19669.
19. Slatter DA, Belson CH, Bailey AJ (2000) The importance of lipid-derived malondialdehyde in diabetes mellitus. Diabetologia 43: 536–537.
20. Hunter WC, Janicki JS, Weber KT, Noordergraaf A (1983) Systolic mechanical properties of the left ventricle: effects of volume and contractile state. Circ Res 52: 519–527.
21. Shroff SG, Janicki JS, Weber KT (1983) Left ventricular systolic dynamics in terms of its chamber mechanical properties. Am J Physiol 245(Heart Circ Physiol 14): H110–H124.
22. Campbell KB, Ringo JA, Knowles GG, Kirkpatrick GD, Schmidt SL (1986) Validation of optimal elastance-resistance left ventricle pump models. Am J Physiol 251: H392–H397.
23. Shroff SG, Janicki JS, Weber KT (1992) Mechanical and energetic behavior of the intact left ventricle. In: Fozzard HA, Eds. The Heart and Cardiovascular System, Second Edition. NY: Raven, p.129–136.
24. Malone JJ, Cuthbertson DD, Malone MA, Schocken DD (2002) Cardioprotective effects of carnitine in streptozotocin-induced diabetic rats. Cardiovas Diabetol 5: 2.
25. Chang KC, Lo HM, Tseng YZ (2002) Systolic elastance and resistance in the regulation of cardiac pumping function in early streptozotocin-diabetic rats. Exp Biol Med 227: 251–259.
26. Chang KC, Kuo TS (1997) Single beat estimation of the ventricular pumping mechanics in terms of the systolic elastance and resistance. J theor Biol 189: 89–105.
27. Chang KC (1998) Theoretical maximum flow of the left ventricle is sensitive to change in ventricular afterload. J theor Biol 194: 407–417.
28. Sunagawa K, Yamada A, Sendai Y, Kikuchi Y, Nakamura M et al. (1980) Estimation of the hydromotive source pressure from ejection beats of the left ventricle. IEEE Trans Biomed Eng 27: 299–305.
29. Takeshita M, Igarashi Y, Tomimoto S, Oshike M, Hayashi T et al. (1991) Single-beat estimation of the slope of the end-systolic pressure-volume relation in the human left ventricle. Circulation 83: 202–212.
30. Dennis JE, Woods DJ (1987) New Computing Environments. In: Wouk A, Eds. Microcomputers in Large-Scale Computing. Philadelphia: SIAM, p.116–122.
31. Barnea O, Jaron D (1990) A new method for the estimation of the left ventricular pressure-volume area. IEEE Trans Biomed Eng 37, 109–111.
32. Weiss JL, Frederiksen JW, Weinfield ML (1976) Hemodynamic determinants of the time-course of fall in canine left ventricular pressure. J Clin Invest 55 (3): 731–760.
33. Wu MS, Chang CY, Chang RW, Chang KC (2012) Early return of augmented wave reflection impairs left ventricular relaxation in aged Fisher 344 rats. Exp Gerontol 47: 680–686.
34. Del Rio D, Stewart AJ, Pellegrini N (2005) A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutr Metab Cardiovasc Dis 15: 316–328.
35. Beuge JA, Aust SD (1978) Microsomal lipids peroxidation. Methods Enzymol 52: 309–310.
36. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254.
37. Nagawa K, Sagawa K, Shoukka A (1973) Load independence of the instantaneous pressure-volume ratio of the canine left ventricle and effects of epinephrine and heart rate on the ratio. Circ Res 32: 314–322.
38. Dilloo WH (1980) Diabetes mellitus induces changes in cardiac myosin in the rat. Diabetes 29: 579–582.
39. Malhotra A, Penzparkal S, Fein FS, Sonnenblick EH, Scheuer J (1981) The effects of streptozotocin-induced diabetes in rats on cardiac contractile proteins. Circ Res 49: 1243–1250.
40. Penzporkal S, Fein FS, Sonnenblick EH, Scheuer J (1981) Depressed cardiac sarcoplasmic reticular function from diabetic rats. J Mol Cell Cardiol 13: 303–309.
41. Rice JJ, Winslow RL, Hunter WC (1999) Comparison of putative cooperative mechanisms in cardiac muscle: length dependence and dynamic responses. Am J Physiol 276(Heart Circ Physiol 45): H1174–H1175.
42. Paulom DJ, Sanjak M, Shug AL (1992) Carnitine deficiency and the diabetic heart. In: Carter AL (ed). Current Concepts in Carnitine Research. CRC Press, Boca Raton, Florida, 215–230.
43. Nochy JP, Morgan HE (1974) Relationship between carbohydrate and lipid metabolism and the energy balance of the heart muscle. Anna Rev Physiol 36: 413–459.
44. Folden DV, Gupta A, Sharma AC, Li SY, Saari JT et al. (2003) Malondialdehyde inhibits cardiac contractile function in ventricular myocytes via a p38 mitogen-activated protein kinase-dependent mechanism. Brit J Pharmacol 139: 1310–1316.
45. Zarrin-Heizberg A, Rupp H (2002) Therapeutic potential of CPT I inhibitors: modulation of gene transcription as a target. Expert Opin Investig Drugs 11: 345–356.
46. Wu MS, Liang JT, Lin YD, Wu ET, TsengYZ et al. (2008) Aminoguanidine prevents the impairment of cardiac pumping mechanics in rats with streptozotocin and nicotinamide-induced type 2 diabetes. Brit J Pharmacol 154: 746–764.
47. Rodrigues B, Xiang H, McNeill JH (1988) Effect of L-Carnitine Treatment on Lipid Metabolism and Cardiac Performance in Chronically Diabetic Rats. Diabetes 37: 1338–674.
48. Burcardi M, Rupp H (1997) Etomoxir improves left ventricular performance of pressure-overloaded rat heart. Circulation 96: 3681–3686.
49. Bergman G, Atkinson L, Metcalfe J, Jackson N, Jewitt DE (1980) Beneficial effect of enhanced myocardial carbohydrate utilisation after oxefinone (L-hydroxyphenylglycine) in angina pectoris. Eur Heart J 1: 247–253.
50. Bachmann E, Weber E (1988) Biochemical mechanisms of oxefinone cardiotoxicity. Pharmacol 36(4): 238–48.
51. Stanley WC (2004) Myocardial energy metabolism during ischemia and the mechanisms of metabolic therapies. J Cardiovasc Pharmacol Therapeut 9 (Supplement 1): S31–S45.