Enhanced contractility in coronary arteries of diabetic pigs is prevented by exercise

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Abstract
We hypothesized that hyperlipidaemia, diabetes and diabetic dyslipidaemia increase the contractility of coronary arteries in swine, and that exercise would prevent this enhanced contractility. We further hypothesized that this enhanced contractility is associated with elevated potassium (K⁺) channel activity, consistent with the idea that certain disease states, as in hypertension, result in a compensatory upregulation in K⁺ channels. Swine were assigned to one of the following groups: control, standard chow (C; n = 6); hyperlipidaemic, high-fat chow (H; n = 5); diabetic, standard chow (D; n = 7); diabetic, high-fat chow (‘diabetic dyslipidaemic’, DD; n = 12); or exercise-trained DD (DDX; n = 9). High-fat chow consisted of standard pig chow with added cholesterol (2%) and coconut oil. Endothelium-denuded segments from D, DD and DDX animals showed enhanced contractility to prostaglandin F₂α (PGF₂α) compared with C, while segments from H, D and DD showed enhanced contractility to endothelin-1 (ET-1) compared with C and DDX (P < 0.05). The enhanced contractility was not accompanied by differences in K⁺ channel contribution to force reduction. There was no effect of the treatments on expression of the endothelin receptor A or endothelin receptor B. A possible mechanism for the enhanced vasoreactivity of coronary arteries of H, D and DD swine is an alteration in the signalling pathways of ET-1- and PGF₂α-induced contraction. Exercise prevented the increase in contractility to ET-1, but not to PGF₂α, reinforcing the concept of vasocostriclor specificity.

Keywords: diabetic dyslipidaemia; endothelin-1; prostaglandin; smooth muscle; potassium channels

Introduction
Diabetes is a major independent cardiovascular risk factor and causes acceleration in the progression of atherosclerosis¹,². The mechanisms by which diabetes contributes to these cardiovascular complications are not well understood; however, there is strong evidence that diabetes induces alterations in calcium (Ca²⁺) handling, both in isolated arteries and also in vascular smooth muscle cells³–⁶. Additionally, it has been shown that the potassium (K⁺) channel current is altered in diabetic dyslipidaemia⁷. Altered Ca²⁺ homeostasis and K⁺ channel activity could cause significant changes in vascular tone since both are tightly coupled to the contractility of the vessel.

The major role of K⁺ channels in vascular smooth muscle is to provide a mechanism for vasorelaxation via K⁺ efflux and membrane hyperpolarization⁸. In addition to basal activity of K⁺ channels in intact arteries, Ca²⁺ can stimulate K⁺ channel activity in smooth muscle cells. The channels that contribute significantly to vascular tone are the voltage-dependent K⁺ channels (Kᵥ) and Ca²⁺-activated K⁺ channels (KCa). Thus, it is the balance of processes that modulate K⁺ channel activity and intracellular Ca²⁺ concentration which largely determines the vessel lumen diameter. Because changes in lumen radius alter blood flow to the fourth power, coronary perfusion could be decreased by relatively small decreases
Both hyperlipidaemia and diabetes have been shown to alter the properties of vascular smooth muscle cells, which can result in changes in the contractility of blood vessels. While the majority of studies report increases in agonist-induced isometric force in a variety of vascular tissues obtained from animals with diabetes, there are several reports of decreases in arterial vasoreactivity. The reasons for the discrepancies are not clear; however, the conflicting data could be a result of the use of different species, vessels and vasoconstrictors, or differences in the duration of experimental conditions. These findings, however, are consistent with the idea that diabetes alters Ca\(^{2+}\) channel activity, or both. We have previously measured free Ca\(^{2+}\) concentrations and K\(^{+}\) channel activity in single coronary smooth muscle (CSM) cells.

The purpose of this study was to test the hypothesis that chronic hyperlipidaemia, diabetes and diabetic dyslipidaemia would result in enhanced contractility of coronary arteries, and that this enhanced contractility would be associated with an increase in the K\(^{+}\) channel activity measured in intact tissues. This is consistent with the idea that in hyperlipidaemia, diabetes and diabetic dyslipidaemia, as in hypertension, there is a compensatory upregulation in K\(^{+}\) channels. We studied the effects of the widely used vasoconstrictors prostaglandin F\(_2\alpha\) (PGF\(_2\alpha\)) and endothelin-1 (ET-1). Furthermore, based on our previous report that exercise training attenuated the coronary contractile response to ET-1 in non-diabetic animals, we hypothesized that endurance exercise training would prevent this increase in vascular reactivity in diabetic dyslipidaemia. The porcine model was used because it shares many important similarities with the human, such as cardiac anatomy, resting and exercise heart rates, and metabolism of dietary carbohydrates and lipids. Indeed, the response of the swine to exercise is also similar to that of equine models.

**Methods**

**Porcine model of diabetes and diabetic dyslipidaemia**

All procedures were approved by the University of Missouri Animal Care and Use Committee in accordance with the ‘Principles for the utilization and care of vertebrate animals used in testing, research and training’. The procurement of animals, the husbandry and the experiments reported in this paper conform to the ‘European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes’ (Council of Europe No. 123, Strasbourg, 1985). Male miniature Yucatan swine (Sinclair Research Center, Columbia, MO) weighing 40–50 kg were randomly placed into five groups similar to those described previously. The groups were: (1) control, standard pig chow, sedentary (C; n = 6); (2) hyperlipidaemic, standard pig chow supplemented with cholesterol (2%) and coconut oil, sedentary (H; n = 5); (3) diabetic, standard pig chow, sedentary (D; n = 7); (4) diabetic, high-fat diet, sedentary (‘diabetic dyslipidaemic’, DD; n = 12); and (5) diabetic, high-fat diet, endurance exercise-trained, (DDX; n = 9). The high-fat/cholesterol diet elevated the percentage of energy provided by fat from 8% (standard chow) to 46%. Individual body weights and fasting blood glucose levels were taken weekly to ensure a weight gain of 1% of initial body weight per week to match control pigs. This was accomplished by adjusting the amount of feed, and daily insulin dosages. Animals were fed once, at the same time of day, and water was made freely available.

Diabetes was induced in the D, DD and DDX animals by injecting allophan (125 mg kg\(^{-1}\)); Sigma Chemical Co., St. Louis, MO) into the superior vena cava via a surgically implanted vascular access port. Allophan specifically destroys the insulin-producing β cells of the pancreas. To accelerate atherosclerosis and to ensure that the effects of diabetes would be marked in the 20-week period, the animals were maintained at fasting blood glucose levels of 300–400 mg dl\(^{-1}\). Additionally, the DD and DDX animals were fed a high-fat diet to mimic the dyslipidaemia that is common in the human diabetic population (see above). Blood glucose, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol were measured as previously described.

**Treadmill exercise protocol**

DDX animals were acclimated to a motorized treadmill (Good Horsekeeping Inc., Ash Grove, MO) over a two-week period during which the grade and speed were increased in small increments as described in detail elsewhere. The goal was to reach a workload that elicited a heart rate between 65 and 75% of maximal predicted heart rate. The exercise protocol consisted of a 10 min warm-up period, followed by 30 min of walking at the target heart rate, followed by a 5 min cooling-down period. To maintain this target heart rate, the grade was elevated as necessary during the remaining 16-week exercise-training programme. This protocol was followed for four days per week.

**Right coronary artery ring preparation**

The heart was removed from all animals while under deep isoflurane anaesthesia. The right coronary artery (RCA) was immediately dissected away and placed in ice-cold sterile storage medium. Within 24 h, four 3 mm serial segments were denuded.
of endothelium by rubbing a thin dowel along the luminal surface. Efficacy of removing the endothelial layer was confirmed in experiments by the lack of a response to the endothelium-dependent vasodilator bradykinin, after preconstricting with PGF$_2$-$\alpha$. Arterial rings were hung on force transducers (Grass-Telefactor, West Warwick, RI) and placed in a modified Krebs-Henseleit bicarbonate-based buffer that contained, in mM: 2 CaCl$_2$, 118 NaCl, 1 MgCl$_2$, 5 KCl, 24.8 NaHCO$_3$ and 10 glucose. The pH was adjusted to 7.4, and the buffer continuously gassed with a mixture of 5% CO$_2$/21% O$_2$/74% N$_2$. Force data were collected at a sampling frequency of 0.2 Hz using AxoBASIC software (Axon Instruments, Union City, CA) and stored on a personal computer for off-line analysis.

The rings were allowed to equilibrate for 1 h before beginning a length versus force protocol. The purpose of the length versus force protocol was to ensure that all subsequent protocols were performed after all rings had been set to their optimal lengths. A predetermined amount of stock KCl solution was added to the tissue bath to reach a final depolarizing concentration of 60 mM KCl. After 5 min, the solution was washed out and replaced with the Krebs-Henseleit.

The length of the ring was increased by 0.5 mm. This sequence was repeated as many times as necessary until the force developed in response to one 60 mM KCl challenge did not differ from that of the previous 60 mM KCl challenge by more than 10%. Once optimal length was reached, the rings were allowed to equilibrate for an additional 45 min.

Several experimental protocols were run in the four tissue baths. PGF$_2$-$\alpha$ (30 $\mu$M final concentration) was added to evaluate vessel reactivity. This concentration was selected because of previously published data showing enhanced contraction in porcine coronary arteries$^{12}$. After allowing the rings to equilibrate, the fraction of force inhibited by K$^+$ channel activation during PGF$_2$-$\alpha$-induced tone was determined. The K$^+$ channel blocker 4-aminopyridine (4AP; final concentration 1 mM) or tetraethylammonium (TEA; final concentration 1 mM) was added and peak responses to these inhibitors were assessed. After washing out with fresh Krebs-Henseleit solution several times, and the rings had reached basal force, these protocols were repeated using ET-1 ($1 \times 10^{-8}$ M final concentration) as the agonist. This concentration was selected because this approximates the EC$_{50}$ (concentration

![Fig. 1](attachment:image1.png)

**Fig. 1** Typical responses of porcine right coronary artery ring preparations to the application of 60 mM KCl, prostaglandin F$_2$ (PGF$_2$-$\alpha$) or endothelin-1 (ET-1) and K$^+$ channel antagonists (4AP – 4-aminopyridine; TEA – tetraethylammonium); horizontal bars indicate the duration of agonist/antagonist application. (a) Responses to agonists were determined by taking the difference of peak developed force and baseline force, which are indicated by the double-headed arrows. (b) After application of PGF$_2$-$\alpha$ or ET-1, K$^+$ channel inhibitors were added which resulted in additional developed force. This portion of developed force shown by the double-headed arrow is the result of the inhibition of agonist-activated K$^+$ channels.
giving half-maximal response) for ET-1 with porcine coronary arteries (data not shown). Figure 1 contains representative force recordings collected during the application of PGF$_2\alpha$ and ET-1 as the agonists (Fig. 1a), and the response to application of specific K$^+\,$ channel inhibitors (Fig. 1b).

Developed force to PGF$_2\alpha$ and ET-1 was calculated by determining peak force and subtracting the initial baseline force. The developed force was then normalized to the response to the 60 mM KCl application. The fraction of force inhibited by agonist-induced K$^+$ channel activation was determined by obtaining the developed force for the 4AP or TEA and normalizing to the 60 mM KCl response.

**Right coronary artery immunohistochemistry**

Samples of RCA were fixed by immersion in neutral buffered 10% formalin for a minimum of 24 h, processed routinely to paraffin, sectioned at 5 μm thickness, and floated onto positively charged slides (Fisher Scientific, St. Louis, MO). Sections were deparaffinized and steamed in citrate buffer at pH 6.0 (target retrieval solution S1699; Dako, Carpintera, CA) for 20 min to achieve antigen retrieval, and then cooled for 20 min. Sequential Tris buffer and water wash steps were performed after each step in the staining protocol. Sections were incubated with avidin biotin two-step blocking solution (Dako X590) to inhibit background staining and in 3% hydrogen peroxide to inhibit endogenous peroxidase. Non-serum protein block (Dako X909) was applied to inhibit non-specific protein binding, and serial sections were incubated overnight at 4°C in 1:400 dilutions of primary rabbit antibody in phosphate-buffered saline containing 15 mM sodium azide and peroxidase-labelled streptavidin (Dako). Diaminobenzidine (Dako) was applied for 1 min, dehydrated, and covered with a cover slip.

Images of the tunica media were captured at a magnification of 400 X using an Olympus BX40 microscope (Hitschfel Instruments, St. Louis, MO) and a Spot Insight digital camera (Diagnostic Instruments, Sterling Heights, MI). The area of positive immunohistochemical staining was analysed using Image Pro Plus (Media Cybernetics, Silver Springs, MD).

**Statistics**

One-way analysis of variance (ANOVA) was performed using standard statistical packages (SPSS 10.0 and SigmaStat; SPSS Inc., Chicago, IL). Where data were not normally distributed or had unequal variances, a one-way ANOVA on ranks was performed. The Dunn’s and Games–Howell multiple comparison tests were used for post hoc analysis. Differences were considered significant if the P-value was < 0.05.

**Results**

**Characteristics of animal model**

Table 1 contains mean body weight (BW) and selected blood chemistry profiles of the experimental animals. All groups had similar BW with the exception of the D group, which was significantly lower than for the C and DD groups. Blood glucose values in D, DD and DDX were approximately five-fold greater ($P < 0.05$) compared with C and H. Plasma lipids have been characterized in more detail in other reports.$^7,12,14,21$, which provided the data from which the LDL:HDL ratio was calculated. Exercise training had no effect on lipids, similar to the lack of effect on blood glucose. Blood urea nitrogen, creatinine and liver function tests all remained within normal limits in all animals (data not shown).

**Exercise training status**

As with the bulk of the blood lipid data, specific adaptations of the DDX animals that demonstrate the efficacy of the exercise regimen, such as decreased resting heart rate, decreased heart rate during submaximal exercise were counter-stained with Mayer’s haematoxylin stain for 1 min, dehydrated, and covered with a cover slip.

Images of the tunica media were captured at a magnification of 400 X using an Olympus BX40 microscope (Hitschfel Instruments, St. Louis, MO) and a Spot Insight digital camera (Diagnostic Instruments, Sterling Heights, MI). The area of positive immunohistochemical staining was analysed using Image Pro Plus (Media Cybernetics, Silver Springs, MD).

**Table 1** Summary of animal body weight, blood glucose and LDL:HDL ratio. By design, D, DD and DDX animals were chronically hyperglycaemic, while H, DD and DDX animals were chronically hyperlipidaemic.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>C</th>
<th>H</th>
<th>D</th>
<th>DD</th>
<th>DDX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>57.5 ± 1.8$^a$</td>
<td>53.5 ± 2.7</td>
<td>45.0 ± 3.6</td>
<td>56.8 ± 3.0$^a$</td>
<td>53.2 ± 1.0</td>
</tr>
<tr>
<td>Blood glucose (mg dl$^{-1}$)</td>
<td>56 ± 5.1</td>
<td>57 ± 7.0</td>
<td>340 ± 26.4$^b$</td>
<td>341 ± 11.1$^b$</td>
<td>329 ± 16.7$^b$</td>
</tr>
<tr>
<td>LDL:HDL ratio</td>
<td>0.78 ± 0.1</td>
<td>2.0 ± 0.5$^c$</td>
<td>2.8 ± 0.2</td>
<td>2.4 ± 0.5$^c$</td>
<td>2.7 ± 0.5$^d$</td>
</tr>
</tbody>
</table>

$^a$Greater than D.

$^b$Greater than C and D.

$^c$Greater than C.

$^d$Greater than C and D.

LDL = low-density lipoprotein cholesterol; HDL = high-density lipoprotein cholesterol. Experimental group: C – control; H – hyperglycaemic; D – diabetic; DD – diabetic dyslipidaemic; DDX – exercise-trained DD.
and citrate synthase activity, have been published previously.\textsuperscript{7,22}

**Response to 60 mM KCl**

The developed force response to 60 mM KCl is contained in Table 2, which indicates that there is no difference in the developed force between the treatment groups. Because the responses to the 60 mM KCl application were not different, the normalization of subsequent contractile responses as a percentage of the response to 60 mM KCl was justified.

**Response to PGF\textsubscript{2}\textalpha{} and K\textsuperscript{+} channel inhibition with PGF\textsubscript{2}\textalpha{}-contracted rings**

Figure 2 represents summary data of the response to PGF\textsubscript{2}\textalpha{}. When PGF\textsubscript{2}\textalpha{} was used as the agonist, the resulting developed force in the D, DD and DDX groups was significantly greater when compared with the C group ($P < 0.05$). These results reflect an increased reactivity to PGF\textsubscript{2}\textalpha{} of 36, 36 and 24%, respectively, over the C group. Table 2 contains peak developed force values from the 4AP and TEA applications. The addition of the K\textsubscript{v} channel inhibitor 4AP caused an increase in the developed force in all rings as the hyperpolarizing function of the K\textsubscript{v} channel was inhibited, but there were no between-group differences in these 4AP-induced responses. Similarly, the increases in developed force after the addition of the KCa channel inhibitor TEA were not significantly different across groups. These data suggest that the vessels of hyperlipidaemic and diabetic animals are more reactive to PGF\textsubscript{2}\textalpha{}, and that the differences in the contribution of both types of K\textsuperscript{+} channel did not reach statistical significance.

**Response to ET-1 and K\textsuperscript{+} channel inhibitors during ET-1 protocol**

Figure 3 represents summary data for the responses to the agonist ET-1. The ET-1-induced developed force

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2}
\caption{Response of porcine right coronary artery rings preparations to prostaglandin F\textsubscript{2}\textalpha{} (PGF\textsubscript{2}\textalpha{}), with developed force expressed as a percentage of the response to application of 60 mM KCl (% 60 KCl). Experimental groups: C – control; H – hyperlipidaemic; D – diabetic; DD – diabetic dyslipidaemic; DDX – exercise-trained DD. Rings from D and DD animals exhibited enhanced vasoreactivity to PGF\textsubscript{2}\textalpha{}, which was not prevented by endurance exercise. *Developed force significantly greater than C; †developed force significantly greater than H, $P < 0.05$}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3}
\caption{Response of porcine right coronary artery rings preparations to endothelin-1 (ET-1), with developed force expressed as a percentage of the response to application of 60 mM KCl (% 60 KCl). Experimental groups: C – control; H – hyperlipidaemic; D – diabetic; DD – diabetic dyslipidaemic; DDX – exercise-trained DD. Rings from D and DD animals exhibited enhanced vasoreactivity to ET-1, which was completely prevented by endurance exercise. *Developed force significantly greater than C; †developed force significantly greater than DDX, $P < 0.05$}
\end{figure}
was significantly greater in the H, D and DD groups compared with both the C and the DDX group. The developed force in the H, D and DD groups represents an increase in developed force of 80, 94 and 89% respectively compared with the C group, and a 32, 40 and 36% increase respectively compared with the DDX group. The responses to the addition of $K_v^+$ channel inhibitors are shown in Table 2. The addition of the $K_v$ channel inhibitor 4AP caused an additional increase in developed force in all groups as expected, indicating that there was a large contribution of the $K_v$ channels to ET-1-induced tone. However, this contribution of the $K_v$ channels was not different between experimental groups. Similarly, there was an increase in the developed force after the addition of the $K_{Ca}$ channel inhibitor TEA, reflecting a marked contribution of the $K_{Ca}$ channels to ET-1-induced tone. There were no statistically significant differences in the changes in developed force after TEA addition did not reach statistical significance.

**Endothelin A and B receptor immunohistochemistry**

To determine whether the increase in the constriction to ET-1 could be explained by an increase in the endothelin receptor density, we performed quantitative immunohistochemistry for endothelin receptor A ($ET_A$) and B ($ET_B$) subtypes. There was no statistically significant difference in the percentage staining for $ET_A$ or $ET_B$ across experimental groups. Summary data are shown in Fig. 4a for $ET_A$ and in Fig. 4b for $ET_B$.

**Discussion**

One of the main findings of this study was that a 20-week porcine model of diabetic dyslipidaemia was sufficient to cause enhanced vasoreactivity of coronary arteries in response to the naturally occurring agonists PGF$_2$ and ET-1. We demonstrate here for the first time in an animal model, with high relevance to humans, that a programme of moderate-endurance exercise training prevents this elevated vasoreactivity to ET-1.

**Characteristics of animal model**

By design, all animals were maintained on a growth rate of 1% of body weight gain per week to match control, non-diabetic pigs, and blood glucose levels were maintained in the range of 300–400 mg dl$^{-1}$ in an attempt to accelerate diabetic complications, in particular coronary atherosclerosis. All animals were monitored weekly to ensure the linear growth rate, while maintaining appropriate blood glucose levels by manipulations of feed and insulin therapy. Despite these experimental constraints, we succeeded in maintaining elevated blood glucose levels as well as positive energy balance in all groups. However, there was a significant difference in body weight between the D, C and DD groups. It is important to note, however, that the animals in the D group did not suffer from negative energy balance, as the group weight in the final week of the experiment was approximately 10 kg greater than their weight during the initial week of the experiment.

**Efficacy of the training protocol**

A regular programme of treadmill running has been shown to elicit cardiovascular changes associated with improved cardiac function in miniature Yucatan swine both by us and by others. The intensity in the current study was moderate compared with that of the previous studies. The purpose for selecting a more moderate intensity was to ensure that the diabetic animals would be able to perform the full protocol without complications for the entire study period. Despite the more moderate exercise intensity, the efficacy of this protocol was shown by the elevation in citrate synthase activity in the biceps muscle (data not shown). Additionally, the DDX group exhibited exercise-induced resting bradycardia, which has long been known to be another marker for the trained state.
Vascular contractility in diabetic swine

Response to 60 mM KCl
The steady-state force of a vessel is achieved by the balance of vasoconstricting agents and vasorelaxing agents in coronary segments. We selected several different agents to cause vasoconstriction via different pathways to give a better definition of the pathways affected in our pathophysiological model. Our finding that the response to 60 mM KCl was not different across groups is similar to the results reported by Weber et al. and Abebe and MacLeod. The mechanism of KCl-induced contraction is by membrane depolarization and Ca influx through activated voltage-gated Ca channels (VGCC). The contractile data in the present study are not consistent with a hyperlipidaemia and diabetes-induced decrease in whole-cell Ca current (I$_{Ca}$) shown by Wamhoff et al. using the patch clamp technique on CSM from the same animal model. However, Wamhoff et al. did report that endurance exercise training resulted in the recovery of I$_{Ca}$ to control values. One possible explanation for this incongruence is the fact that the patch clamp results were from freshly dispersed CSM, whereas the isometric force data were from endothelium-denuded rings. The force developed in intact rings in response to KCl depolarization (VGCC activation) is the result of various factors which are intact rings in response to KCl depolarization (VGCC). The contractile data in the present study are not consistent with a hyperlipidaemia and diabetes-induced decrease in whole-cell Ca current (I$_{Ca}$) shown by Wamhoff et al. using the patch clamp technique on CSM from the same animal model. However, Wamhoff et al. did report that endurance exercise training resulted in the recovery of I$_{Ca}$ to control values. One possible explanation for this incongruence is the fact that the patch clamp results were from freshly dispersed CSM, whereas the isometric force data were from endothelium-denuded rings. The force developed in intact rings in response to KCl depolarization (VGCC activation) is the result of various factors which are absent when measuring activation) is the result of various factors which are intact rings in response to KCl depolarization (VGCC). The contractile data in the present study are not consistent with a hyperlipidaemia and diabetes-induced decrease in whole-cell Ca current (I$_{Ca}$) shown by Wamhoff et al. using the patch clamp technique on CSM from the same animal model. However, Wamhoff et al. did report that endurance exercise training resulted in the recovery of I$_{Ca}$ to control values. One possible explanation for this incongruence is the fact that the patch clamp results were from freshly dispersed CSM, whereas the isometric force data were from endothelium-denuded rings. The force developed in intact rings in response to KCl depolarization (VGCC activation) is the result of various factors which are absent when measuring activation) is the result of various factors which are intact rings in response to KCl depolarization (VGCC).

Response to agonists and K$^+$ channel inhibition
The contractile response to PGF$_2$ or ET-1 can be summarized as an increase in vasoreactivity in hyperlipidaemic, diabetic and diabetic dyslipidaemic animals. However, the group mean values for the C versus H group, with PGF$_2$ as the agonist, were not significantly different. The responses to ET-1 and PGF$_2$ are consistent with other findings which report that chronic hyperlipidaemia and diabetes alone, or in combination, caused increases in contractile force. These investigators used a variety of agonists such as noradrenaline and methoxamine, α$_2$-adrenoceptor agonists, PGF$_2$, serotonin and ET-1. This body of work is in line with the idea that in the early stages of coronary artery disease, coronary arteries exhibit enhanced reactivity before the formation of overt atherosclerotic lesions. In fact, Dixon et al. reported similar increases in steady-state force to PGF$_2$ application in porcine left circumflex artery ring preparations in hyperlipidaemia and diabetic dyslipidaemia. The porcine model used by Dixon et al. was very similar to the model used in the current study and, importantly, the diabetic dyslipidaemic animals showed accelerated deposition of lipids in vascular tissue, evidenced by an increased Sudan IV staining compared with control animals.

The possible mechanisms for enhanced contractility to ET-1 include: (1) an increase in ET-1 receptors; (2) an alteration in the signalling pathway of ET-1-induced contraction; (3) an alteration in the K$^+$ channel regulation; or (4) a combination of these factors. Our results indicate that the experimental conditions of hyperlipidaemia, diabetes and diabetic dyslipidaemia did not cause an increase in either ET$_A$ or ET$_B$ receptor density. It is possible that in our model, despite a lack of change in the ET-1 receptor density, the experimental treatments increased the ET-1-dependent force via pathways downstream from the ET-1 receptor, including elevated tyrosine kinase activity. This suggestion stems from previous work from Liu and Sturek who reported that ET-1 stimulates trans-sarcolemmal Ca$^{2+}$ influx via tyrosine kinase activity, which could be inhibited by the addition of genistein, a tyrosine kinase antagonist. Lee et al. have shown that basal and ET-1-stimulated tyrosine phosphorylation was elevated in CSM from a similar model of porcine diabetic dyslipidaemia. The current data do not conclusively rule out any of these possibilities; therefore further study is needed to determine the exact mechanism for enhanced ET-1-induced contractility.

We tested the hypothesis that chronic hyperlipidaemia, diabetes and diabetic dyslipidaemia would result in enhanced contractility of coronary arteries associated with an increase in the K$^+$ channel contribution to attenuation of agonist-induced force. Although there was no statistically significant difference in the developed force when 4AP or TEA was added to ET-1-preconstricted rings, there was a clear trend for greater force production in the H, D and DD groups when TEA was added to the bath. This suggests that there was an increase in the K$_{Ca}$ activity in the rings from these groups during ET-1 activation, thus when these channels were inhibited, the resulting developed force was potentiated compared with the rings from the C and DDX animals. We conclude that KV and K$_{Ca}$ channels contribute to steady-state developed force in response to ET-1 stimulation, and we speculate that the contribution was greater in the hyperlipidaemic, diabetic and diabetic dyslipidaemic groups compared with control and exercise-trained groups, although this did not reach statistical significance. This is consistent with our hypothesis, and has been reported by us previously. Furthermore, this has been reported by others in a hypertensive model, where it has been suggested that an increase in K$_{Ca}$ channel expression is a compensatory response to the elevated [Ca$^{2+}$].
The prostanoid PGF₂α is a vasoconstrictor whose action depends on activation of the G-protein-coupled PGF₂α receptor activating phospholipase C, which in turn stimulates the production of inositol-1,4,5-triphosphate (IP₃) and diacylglycerol (DAG). The production of IP₃ and DAG causes an increase in intracellular Ca²⁺, which in turn activates the contractile elements. Therefore, possible mechanisms for enhanced vasoreactivity to PGF₂α include an increase in PGF₂α receptor number, an alteration in the signaling pathway of PGF₂α-induced contraction, an increase in the PGF₂α-sensitive Ca²⁺ stores, or a combination of these factors. There are very little data on the effect of hyperlipidaemia or diabetes on PGF₂α receptor density, and we did not quantify PGF₂α receptor number in the current study. A potential explanation for the PGF₂α response is an increase in the IP₃ production secondary to chronic diabetes, which has been shown to occur in CSM. Additionally, the VGCC is not the sole Ca²⁺ channel through which Ca²⁺ influx occurs when stimulated by PGF₂α. Usune et al. have reported that PGF₂α application may enhance Ca²⁺ influx through a receptor-operated Ca²⁺ channel. Clearly, an increase in influx via the ROC in the tissue from the hyperlipidaemic or diabetic animals could result in enhanced reactivity to the agonist. This study does not intend to clarify which mechanism is the most likely, and emphasizes the fact that these potential mechanisms need further study.

With ET-1 as the agonist, the increased contractility that was observed in the DD group was completely and remarkably prevented by the endurance exercise programme. Exercise alone has previously been shown to reduce ET-1-induced contraction. One possible explanation is that the endurance exercise regimen attenuated the ET-1-stimulated elevation in intracellular Ca²⁺ by either reducing Ca²⁺ influx or membrane-bound Ca²⁺ release. A reduction in ET-1-sensitive Ca²⁺ influx via the VGCC is not the likely mechanism, since Wamhoff et al. have shown that VGCC current is actually normalized (returns to values in control animal) with exercise, whereas whole-cell Ca²⁺ current density is depressed in hyperlipidaemia, diabetes and diabetic dyslipidaemia. On the other hand, if there is involvement of the VGCC, such that intracellular Ca²⁺ is depressed despite the normalization of VGCC current density, it may occur via a potentiated ability to buffer the Ca²⁺ influx before activation of the myofilaments occurs. The endurance exercise-induced reduction in vasoreactivity may be due to the improved Ca²⁺ buffering of CSM such that, regardless of the increase in VGCC current density, the inherent Ca²⁺ buffers are capable of preventing influx from enhanced activation of the contractile elements.

While it is remarkable that exercise did prevent the increase in the contractile response to ET-1 in the DD animals, it is not altogether surprising that the effect of exercise did not result in a statistically significant reduction in the contraction in response to PGF₂α. The most straightforward reason is that the high variability of the data in the D group prevented statistical significance from being reached. Another plausible explanation for the lack of effect of endurance exercise in the PGF₂α condition is that PGF₂α and ET-1 stimulate contractions using different pathways. Endurance exercise may have elicited changes in the signal transduction pathway that are not common to both. Because PGF₂α stimulates a G-protein-mediated signaling pathway, it is possible that diabetes and diabetic dyslipidaemia cause an increase in G-protein-mediated Ca²⁺ influx that is unaffected by endurance exercise training. On the other hand, if the dose of PGF₂α was not near the EC₅₀, then the possibility exists that there was a shift in the sensitivity of the tissue to PGF₂α, which was not detected in this series of experiments.

**Limitations of the study**

Although there was a statistically significant increase in contractility in coronary arteries from hyperlipidaemic, diabetic and diabetic dyslipidaemic animals resulting from application of one dose of both PGF₂α and ET-1, a full dose–response relationship would have provided important information regarding sensitivity of the preparation to each agonist. Without this information we cannot address any shift in the EC₅₀ of PGF₂α or ET-1 in this preparation. There was no difference across groups in the receptor density shown by the percentage staining for ETA or ET B; however, this does not preclude the possibility that the sensitivity of the receptors to activation by ET-1 was altered by the experimental treatments.

**Conclusion**

Using a porcine model, we have shown that coronary arteries are hyper-reactive to certain agonists in hyperlipidaemia, diabetes and diabetic dyslipidaemia. The enhanced reactivity does not appear to be as a result of changes in the contribution of K⁺ channels to the development of tone. A more likely mechanism is an alteration in the specific signalling pathway of ET-1- and PGF₂α-induced contraction. Finally, endurance exercise prevented the increase in contractility to ET-1, but not to PGF₂α, reinforcing the concept of vasoconstrictor specificity.

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