C1

Sex differences in ventricular repolarization in Langendorff-perfused guinea-pig hearts

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It is well established that women have longer rate-corrected QT (QTc) intervals and a higher incidence of drug-induced *torsades de pointes* than do men. The underlying mechanisms remain unclear but are likely to involve the gonadal steroids. The female guinea-pig has a conventional oestrus cycle and has been suggested to be a suitable model for the investigation of sex differences in ventricular repolarization (1, 2).

Age-matched male and female guinea-pigs (700-900g) were humanely killed, the hearts excised and mounted on a Langendorff perfusion-apparatus. Following stabilization, ventricular monophasic action potentials (MAP) were recorded under normal sinus rhythm using a closed bipolar suction electrode placed on the epicardial surface of the left ventricle, close to a branch of the left anterior descending coronary artery. The electrocardiogram (ECG) was also recorded. The right atrium and sinus atrial node were then removed and the hearts paced at cycle lengths (CL) of 150 ms — 375 ms. Data are presented as mean ± standard error of the mean, and statistical analysis was carried out using unpaired Student's *t* tests or ANOVA, as appropriate. $P<0.05$ was considered significant.

Under sinus rhythm, hearts from female guinea-pigs had longer QTc intervals (293.6 ± 2.1 ms, n=30) than those from males (276 ± 2.9 ms, n=13; $P<0.01$), while there were no significant differences in PR interval, RR interval and QRS duration. In paced hearts, the MAP duration at 90% repolarization ($MAPD_{90}$) was found to increase in a CL-dependent manner in both male (CL = 150 ms: 77.1 ± 1.7 ms; CL = 375 ms: 123.2 ± 1.5 ms; n=13, $P<0.0001$) and female (CL = 150 ms: 79.5 ± 1.3 ms; CL = 375 ms: 132.8 ± 1.7 ms; n=17, $P<0.0001$) hearts such that the $MAPD_{90}$ was significantly longer in female hearts than in male hearts at CL of 250 ms and greater (two-way ANOVA, $P<0.0001$).

In conclusion, the present results demonstrate that guinea-pig intact hearts show sex differences in ventricular repolarization.

James AF & Hancox JC (2003). *Cardiovasc Res* **57(1)**, 1-4.

James AF, Arberry LA & Hancox JC (2004). *Basic Res Cardiol* **99(3)**, 183-192.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C2

Resistance to metabolic stress and K_{ATP} channel activity in cardiac myocytes isolated from male and female rats

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In guinea-pigs ventricular myocytes from female animals have been reported to have higher K_{ATP} current density than those from males, and also to show higher levels of mRNA for SUR2A estimated by RT-PCR[1]. Further, female cells showed less Ca^{2+} loading in response to simulated ischaemia and reperfusion[1]. We have investigated whether similar gender differences in tolerance to metabolic stress and cardiac K_{ATP} current density exist in the rat.

Ventricular myocytes were isolated enzymatically from humanely killed adult rats. Cellular responses to metabolic stress and reperfusion were assessed by exposing cells to 7 min of metabolic inhibition (MI) with 2 mM CN^- and 1mM iodoacetate followed by 10 min reperfusion with normal Tyrode. Cells were field stimulated at 1 Hz throughout, fura-2 was used to measure $[Ca^{2+}]_i$ and contractile recovery was measured with a video-imaging system. $[Ca^{2+}]_i$ after 10 min reperfusion was higher in male than female myocytes (232 ± 14 nM vs 170 ± 8 nM, mean ± S.E.M. n=109 and 100 cells respectively, $p<0.01$, Students t test). Further, the percentage of female cells that had recovered the ability to contract in response to field stimulation 10 min after reperfusion was higher than that of male cells (74 ± 7% vs 42 ± 9%, n = 18,16 experiments respectively, $p<0.05$). Whole-cell K_{ATP} currents were measured using patch clamp in isolated male and female rat ventricular myocytes in response to MI, and normalised to cell capacitance. There was no significant difference between the maximal K_{ATP}current density induced in male and female cells by MI (29.82 ± 2.91 pA/pF and 26.83 ± 1.79 pA/pF respectively, n = 27 and 24 cells respectively, 3 animals in each case). Single channel recordings from patches on cells exposed to MI indicated that neither the single channel conductance nor the time to K_{ATP} channel activation during MI differed between male and female myocytes. Transcript levels of genes encoding cardiac K_{ATP} channel subunits were estimated using quantitative PCR. These experiments failed to show any difference between male and female rats in the transcript level of genes encoding Kir6.2 or SUR2A.

Thus although we found increased resistance to metabolic stress in cardiac myocytes from female rats, we did not find differences in K_{ATP} channel activity during MI, nor in transcript levels for Kir6.2 or SUR2A. This suggests that gender differences in stress resistance may be unrelated to K_{ATP} channel activity, or may result from differences in the pattern of channel activation that were not revealed by our experiments.

Ranki HJ et al (2001). J Am Coll Cardiol 38, 906-915.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C3

Sex differences in cardiac morphology of adult sheep following moderate early gestation undernutrition with or without undernutrition in early postnatal life

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Undernutrition is implicated in linking *in utero* and postnatal growth to the development of cardiovascular disease in later life (Eriksson *et al.*, 1999; Roseboom *et al.*, 2000). We therefore investigated the effects of early gestation and postnatal undernutrition on cardiac morphology and left ventricular function in adulthood.

Welsh Mountain ewes received 100% (group C, n=25) or 50% of global nutrient requirements (group U, n=26) from conception to day 30 of gestation, and 100% thereafter. Offspring were then fed either *ad libitum* (CC, n=15 and UC, n=13) or at a level that reduced body weight to 85% of individual target weight (predicted from 0-12 wk growth trajectory) from 12 to 25 weeks postnatal age and *ad libitum* thereafter (CU, n=10 and UU, n=13). Each group contained approximately equal numbers of males (n=27) and females (n=24). At 2.5 years cardiac morphology and left ventricular function was determined by transthoracic echocardiography under general anaesthesia (2% halothane in oxygen). Data (mean ± S.E.M.) were analysed by ANOVA and a Bonferroni post-hoc test.

In male but not female offspring an increase was seen in the interventricular septal wall thickness (UC, 11.2 ± 0.3mm vs. CC, 8.5 ± 0.5mm, $p<0.01$) in the early gestation undernutrition group (UC) compared to the control group (CC), and also in the mean left ventricular wall thickness (UC, 10.4 ± 0.5mm vs. CC, 8.5 ± 0.4mm, $p<0.05$). This effect was not seen with exposure to both periods of undernutrition (UU). These results were independent of blood pressure. No changes seen in left ventricular fractional shortening.

This study suggests that the increase in left ventricular wall thickness following early gestation undernutrition is sex specific. Mismatches between the *in utero* and postnatal environment may have important consequences for adult cardiac morphology. The absence of this phenomenon in the female adult offspring may reflect different growth rates and susceptibility between the sexes in early gestation.

Eriksson, JG *et al.* (1999). *BMJ* **318**, 427-31.

Roseboom, TJ *et al.* (2000). *Heart* **84.6**, 595-98.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C4

Moderate postconceptional and post-weaning undernutrition causes sex specific impairment of coronary artery function in adult sheep

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The early gestation and postnatal environments are implicated in the development of coronary heart disease in adult life [1,2]. Previous studies have shown that undernutrition in utero leads to vascular dysfunction in fetal and adult offspring [3]. However to date it has not been determined whether these phenomenona are sex specific. In sheep we investigated the effect of moderate post-conceptional and post-weaning nutrient restriction on the vascular reactivity of the left anterior interventicular artery in male and female adult offspring.

Welsh mountain sheep offspring were exposed to either 100% nutrient requirements throughout gestation and postnatal life (group CC, n=17), or to 50% nutrient requirements from conception to day 30 of gestation (term = 147 days) and a post-weaning (12-25 weeks nutrient restriction to reduced body weight to 85% of individual target weight (predicted from 0–12 week growth trajectory) and ad libitum thereafter (group UU, n=15). At 2.5 years, male (n=17) and female offspring (n=15) were killed humanely; the distal anterior interventricular artery was dissected and mounted on a wire myograph. Vascular relaxation was determined in thromboxane (10^{-6} M) preconstricted vessels using bradykinin (10^{-12}-10^{-6} M) and adenosine (10^{-9}-10^{-4} M). Data are expressed as mean ± S.E.M. and were analysed by ANOVA and a Bonferroni post-hoc test.

In terms of bradykinin, there was a significant decrease in the maximal response (84.0 ± 3.3 vs 66.3 ± 5.4*, $p<0.05$) in UU compared to CC males. This was not seen in the females.

The EC_{50} values of the CC and UC animals did not differ.

There were no inter-group differences in the EC_{50} or maximal responses to adenosine in either sex.

Our results suggest a sex-specific impairment of adult coronary artery dilatation in adult life by moderate early gestation and post-weaning undernutrition which could have consequences for coronary function in adult life.

	Males		Females	
PEC_{50} (-logM)	Group CC	Group UU	Group CC	Group UU
	(n=10)	(n=7)	(n=7)	(n=8)
Bradykinin	8.49 ± 0.33	7.77 ± 0.22	7.86 ± 0.38	8.56 ± 0.24
Adenosine	5.59 ± 0.20	5.26 ± 0.19	5.77 ± 0.15	5.98 ± 0.11
Maximal relaxation				
Bradykinin	84.0 ± 3.3	66.3 ± 5.4*	80.3 ± 4.7	75.7 ± 4.9
Adenosine	50.9 ± 9.5	53.2 ± 10.1	78.1 ± 7.9	53.3 ± 10.3

Table 1. *P<0.05, UU significantly different from CC.

Eriksson JG et al. (1999). BMJ 318, 427-431.

Roseboom TJ et al. (2000). Heart 84, 595-598.

Brawley L et al. (2003). Pediatr Res 54, 83-90.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

SA1

Gender differences in cardiac electrophysiology and arrhythmias

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There are important male-female differences in human cardiac rhythm and arrhythmias. For example, women have faster basal heart rates and longer QT intervals than men. These differences relate not only to gender but to gonadal steroids. For example, drugs that prolong repolarization induce torsades de pointes more frequently in women than men; female gender is an independent risk factor for syncope and sudden death in the congenital long QT syndrome; and the higher propensity toward arrhythmia in normal females is associated with fundamental male-female differences in repolarization. Many of these male-female differences appear related to the molecular determinants of the ion channels that control cardiac impulse initiation and repolarization. Studies performed in human subjects and, especially, in animal models have demonstrated a number of differences among ion channel properties, most importantly, in repolarizing potassium currents that appear to be responsible for many of the basal repolarization differences. Beyond this, and impacting particularly on drug-induced arrhythmias, both the presence and the metabolism of gonadal steroids can modify the metabolism of a variety of drugs, resulting in elevated plasma levels and accentuated actions. Another important variable is age, with male-female differences being most prominent when gonadal steroid activity is greatest, and being of lesser importance in earlier and later years. The variety of male-female differences in cardiac rhythm and the mechanisms determining the differences in repolarization and arrhythmias in males and females will be discussed.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

SA2

Gender differences in diseases with a genetic background

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Males and females differ in their basic cardiac electrophysiology, although little is known of the underlying mechanisms. Data from animal models suggest the following general scheme: female ventricular myocytes have a higher L-type Ca^{2+} current density, but a lower rapid delayed rectifier current and transient outward current density than do male myocytes. These differences do not necessarily apply to all cell layers. At present, it seems far-fetched to extrapolate these differences to the human. However, a different composition of membrane currents is of interest, because it may explain the existence of gender differences in the incidence of cardiac arrhythmias in diseases with a genetic background or component (e.g. the long QT syndrome or the Brugada syndrome). It is well known that the incidence of cardiac arrhythmias is higher in males in the Brugada syndrome, but lower in males in the long QT (LQT) syndrome. Bazett, famous for the much-debated QT interval rate correction (QTc), also pointed to the fact that the QTc interval is 370 ms in males and 400 ms in females as early as 1920. In the general population the QTc interval is similar between boys and girls. After puberty, the female QTc interval remains unchanged, but the male QTc interval shortens.

We have assessed these gender differences in patient groups with either LQT1 or LQT2, in whom the density of the slow or the rapid components of the delayed rectifier current, respectively, is impaired. We measured 12-lead ECGs before and during β-adrenergic blockade in 87 patients (48 women, 14 men, 12 girls and 13 boys). Lead V4 was used for analysis. QT dispersion was the difference between the longest and shortest QT interval in any lead. We assessed (1) differences in QTc intervals, (2) differences in QT dispersion and (3) differences in responsiveness to β-adrenergic blockade.

(1) Although the number of patients in our study became small when we subdivided our patients according to gender or LQT subgroups, which imposes a serious limitation, we were quite surprised to find that relevant differences in QTc intervals seemed to be present in LQT1 patients, but not in LQT2 patients. Thus, differences in QTc intervals between boys and girls remained insignificant, both in LQT1 and LQT2 patients, irrespective of β-adrenergic blockade. In adult patients the differences in QTc intervals as known from the general population, were completely absent in LQT2 patients (491 ± 10.5 ms [average ± SEM] in females and 494 ± 12.5 ms in males) and were insignificant in LQT1 patients (502 ± 19.3 ms in females and 472 ± 9.9 ms in males), possibly by the large inhomogeneity within the female LQT1 patient group. During treatment with β-adrenergic blockade this picture did not change, although the difference between female and male LQT1 patients became significant (463 ± 8.5 ms in female LQT1 patients and 422 ± 9.1 ms in male LQT1 patients, $p<0.01$).

(2) In addition we observed that female LQT2 patients had a 50% higher QT dispersion than female LQT1 patients both before and during treatment (about 40 ms in LQT1 patients and circa 62 ms in LQT2 patients). Remarkably, these differences were completely absent in adult male patients, but not in girls.

(3) Finally, we observed that adult male LQT1 patients were the only patients that responded with a marked, highly significant, decrease in QTc intervals and with a moderate (but not significant) decrease in dispersion of QT intervals. Decreases in QTc intervals in response to β-adrenergic blockade must, however, be appreciated against the background that they result from a simple mathematical procedure. The QT intervals in fact increase after β-adrenergic blockade, but the RR intervals increase even more, leading to decreased QTc intervals. The male adult LQT1 patients were the only ones with unchanged QT intervals during treatment. Thereby their decrease in QTc intervals was more prominent.

We conclude that, in addition to underlying differences in repolarization between males and females, responses to β-adrenergic modulation appear to be modulated by gender-related factors. Although our observations are at a descriptive level and do not permit mechanistic conclusions, differences in QTc intervals between male and female LQT patients seem to require the presence of male sex hormones, whereas the differences in dispersion between female LQT1 and LQT2 patients are as prominent in girls as in adult females, but are absent in adult males. Male LQT patients, therefore, seem to be relatively protected against cardiac arrhythmias compared to female LQT patients by a (i) constitutively lower dispersion in QT intervals in combination with (ii) a shorter QTc interval due to the effect of male sex hormones and by (iii) a stronger shortening of their QTc interval in response to β-adrenergic blockade.

Conrath CE, Wilde AAM, Jongbloed RJE, Alders M, Van Langen IM, Van Tintelen JP, Doevendans PA, Opthof T. (2002). Gender differences in the long QT syndrome. Effects of β-adrenoceptor blockade. *Cardiovasc Res* **53,** 770-776

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

SA3

Gender differences in subcellular Ca^{2+} regulation in the heart

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Intrinsic gender differences in cardiac function have been known to exist for some time, although the precise mechanisms responsible remain poorly understood. Recent evidence suggests that sex hormones can alter the expression and function of key proteins involved in intracellular Ca^{2+} regulation. We tested the hypothesis that gender differences in cardiac function can be related to differences in subcellular Ca^{2+} regulation, particularly at the level of the sarcoplasmic reticulum (SR) and Na^+/Ca^{2+} exchanger (NCX). Electrically-induced and caffeine-induced Ca^{2+} transients were recorded in left ventricular myocytes isolated from humanely killed male and female rats and guinea-pigs using the Ca^{2+}-sensitive fluorescent indicators indo-1 and fluo-4. Electrophysiological parameters were measured using discontinuous single electrode voltage-clamp. There was no significant gender difference in Ca^{2+} transient amplitude. Peak L-type Ca^{2+} current was smaller in females of the same age but the relative sizes of the current varied with maturation of the male. We measured SR Ca^{2+} contents by integrating the NCX current induced by rapid caffeine application. We found that SR Ca^{2+} content was greater in myocytes from females compared with those from males. This could be related to lower activity of the NCX in females, assessed by measuring repolarizing tail currents and Ca^{2+} transient decay kinetics following thapsigargin treatment. Further, females consistently display greater fractional release of Ca^{2+}. The results allow us to begin to piece together the subcellular mechanisms responsible for gender differences in cardiac function.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

SA4

Oestrogen receptor alpha signalling contributes to gender based differences in cardiac phenotype

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Male and female individuals display differences in the cardiovascular phenotype, e.g. the QT interval in the electrocardiogram (ECG) is shorter in men than in women. Sex hormones like androgens and oestrogens may be responsible for these gender specific cardiovascular characteristics. A cardioprotective action of oestrogens has been reported in various studies, especially since premenopausal women suffer less often from heart diseases than men (Review: Babiker et al., 2002). Oestrogens operate via oestrogen receptor alpha (ERα) and beta (ERβ) which both act as transcription factors. ERα and ERβ deficient mice were investigated here to characterize the influence of ER dependent signalling on the cardiovascular system.

In ERα deficient male and female mice and their homozygous (+/+) littermates (WT) heart weight (HW), peripheral and left ventricular blood pressure and ECGs were measured. ECGs and blood pressure were recorded under anaesthesia as approved by the local committee for animal research (3% isoflurane for handling, 1% for recording). After blood pressure recording hearts were excised from anaesthetised ERα and ERβ deficient mice and their WT littermates. Single cardiac myocytes were isolated from hearts retrogradely perfused by trypsin and collagenase. In externally stimulated cells sarcomere shortening was monitored optically at frequencies between 0.5 and 10 Hz. Action potentials (APs) as well as L-type calcium current ($I_{Ca,L}$) were recorded by patch clamp electrodes in whole cell mode. All single cell experiments were performed at 36°C. APs were elicited at the same stimulation frequencies as shortenings. Western blot analyses were used to examine ERα dependent expression of sarcoplasmic reticulum Ca-ATPase 2a (SERCA) and phospholamban (PL). Body weight (BW) and HW were significantly smaller in female WT compared to male WT. ERα deficiency increased BW and HW in females but decreased HW in males resulting in equilibration of HW and HW/tibia length differences. In the ECG the QTc interval was shorter in male than in female WT hearts. ERα deficiency did not affect QTc in males, but in ERα deficient females the QTc interval was shortened to the level of males. Mean arterial blood pressure (MABP) as well as left ventricular systolic pressure (LVSP) were higher in male than in female WT. ERα deficiency lowered MABP and LVSP in both genders and pressures of ERα deficient males approached those of female WT. In WT isolated cells sarcomere shortening exhibited a biphasic shortening frequency relation with minimum around 2 Hz. Gender differences in shortening were not detected among WT cells. ERα deficiency reduced sarcomere shortening in both genders, but more pronounced in females. ERβ deficiency, however, did not affect shortening frequency relation neither in male nor in female cells, therefore ERβ deficient hearts were not analysed further. In murine cardiac cells increasing stimulation frequency is accompanied by a prolongation of AP duration (APD), e.g. APD_{90} in female WT was 34 ± 10 ms (± SD; n=32) at 0.5 Hz and 49 ± 15 ms (± SD; n=18) at 6 Hz. AP duration in WT cardiac myocytes was not different among genders. In contrast to QT interval APD was not affected by ERα deficiency. In WT cells of both genders $I_{Ca,L}$ exhibited the typical bell shaped voltage dependency with a maximum of 10 ± 3 pA/pF (± SD; n=12) at 0 mV clamp potential. In male cardiomyocytes ERα deficiency lead to an increase in $I_{Ca,L}$ current amplitude, whereas $I_{Ca,L}$ in female cells remained unchanged. Expression of SERCA and PL did not differ between female WT and ERα deficient hearts, but both proteins were reduced in male ERα deficient hearts compared to male WT hearts. The increase of $I_{Ca,L}$ in male ERα deficient cells seems to be counterbalanced by a down regulation of SERCA and PL.

Gender differences in heart weight, peripheral and LV blood pressure as well as in QT interval were abolished by ERα deficiency.

Decreased sarcomere shortening of isolated ERα deficient cardiomyocytes seems to be in agreement with reduced LV pressure. However, neither APD nor changes $I_{Ca,L}$ are able to explain the *in vivo* recorded differences nor the reduced sarcomere shortening. Interestingly ERα deficiency causes significant changes in cardiac phenotype of both female and male mice. Taken together, ERα dependent signalling plays an important role in the development of gender based differences in cardiac phenotype.

Babiker *et al.*(2002). *Cardiovasc Res* **53**, 709-719.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.