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14. ABSTRACT Military aircrew with minimal coronary artery disease (MCAD) may be restricted from flying high-performance aircraft due to possible ischemia during high 1 Gz. An animal model is presented to provide ischemia data for a more informed decision. Methods: 18 swine were placed on a high cholesterol/high fat diet for up to 57 wk. Five control swine were maintained on a standard swine diet. Also, nine male baboons had a constrictor placed around the left anterior descending coronary artery. Two baboons were sham-operated controls. The unanesthetized swine and baboons were infused with Tc-99m at the end of 1 Gz exposure and scanned for myocardial perfusion. Results: Five swine died unexpectedly before 1 Gz exposure with moderate-to-severe CAD. Dysrhythmias during 1 Gz were seen equally in both the control and experimental swine and in the baboons before and after stenosis, with or without propranolol. During 1 Gz, ECG ST-T wave changes suggesting ischemia were observed in the cholesterol swine but not the control swine, and in the baboons before and after stenosis, with or without propranolol. There was a positive relationship between a normal/abnormal ECG and a normal/abnormal myocardial perfusion scan in the swine and a weak relationship in the baboon before stenosis, but somewhat better after stenosis. Coronary histopathology showed normal vessels from the control swine and stenoses ranging from 0 – 95% from the cholesterol swine. Baboon stenosis averaged 37.6 ± 15.0%. Conclusions: In the swine and the baboon extended high levels of 1 Gz were associated with evidence of myocardial ischemia					
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Cardiac Ischemia Model for +G_z Using Miniature Swine and Baboons

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Background: Military aircrew with minimal coronary artery disease (MCAD) may be restricted from flying high-performance aircraft due to possible ischemia during high +G_z. An animal model is presented to provide ischemia data for a more informed decision. **Methods:** There were 18 swine that were placed on a high cholesterol/high fat diet for up to 57 wk. Five control swine were maintained on a standard swine diet. Also, nine male baboons had a constrictor placed around the left anterior descending coronary artery. Two baboons were sham-operated controls. The unanesthetized swine and baboons were infused with Tc-99m at the end of +G_z exposure and scanned for myocardial perfusion. **Results:** Five swine died unexpectedly before +G_z exposure with moderate-to-severe CAD. Dysrhythmias during +G_z were seen equally in both the control and experimental swine and in the baboons before and after stenosis, with or without propranolol. During +G_z, ECG ST-T wave changes suggesting ischemia were observed in the cholesterol swine but not the control swine, and in the baboons before and after stenosis, with or without propranolol. There was a positive relationship between a normal/abnormal ECG and a normal/abnormal myocardial perfusion scan in the swine and a weak relationship in the baboon before stenosis, but somewhat better after stenosis. Coronary histopathology showed normal vessels from the control swine and stenoses ranging from 0–95% from the cholesterol swine. Baboon stenosis averaged 37.6 ± 15.0%. **Conclusions:** In the swine and the baboon extended high levels of +G_z were associated with evidence of myocardial ischemia.

Keywords: ECG, atherogenic diet, serum lipids, mechanical stenosis, coronary artery disease, histopathology, Cardiolite/Tc-99m SPECT scan, vascular access port, acceleration, inertial force, propranolol.

MILITARY AIRCREW with even nonocclusive or minimal coronary artery disease (MCAD) may be restricted from flying high-performance aircraft. The primary concern is that even nonocclusive lesions might become physiologically significant, capable of causing myocardial ischemia during high +G_z (18,22). Moreover, many high-performance aircraft are single pilot. Using coronary angiography, MCAD is defined as maximum stenosis < 50% diameter reduction of the lumen. Currently, U.S. Air Force aircrew with maximum coronary stenosis < 50% and aggregate (arithmetic sum) of stenoses < 120% are waived to fly in low-performance aircraft if they exhibit no evidence of ischemia and have normal left ventricular function. Generally, flying status is not recommended for aircrew with lesions > 50% or

aggregate ≥120% (16). Supportive data are needed to define the +G_z-related myocardial ischemia threshold at various levels of coronary artery disease (CAD), especially MCAD, to help validate or refute this concern. However, acquiring the necessary data from human subjects or pilots would be unethical. Therefore, the objective of this study was to develop an animal cardiac ischemia model for +G_z exposure to explore critical ischemia thresholds for safety of flight in high performance aircraft.

Current thinking regarding CAD and the genesis of atherosclerotic plaque (atheromas) is that inflammation plays a major role throughout the process of plaque development. Plaque formation follows a cascade of cellular responses to inflammation, including the release of cytokines, chemokines, C-reactive protein, and the up-regulation of many other cellular mechanisms. Hyperlipidemia is considered to be one of a number of vascular inflammatory agents. Irritated or injured endothelium allows monocyte migration into the intima. Monocytes are converted to macrophages which become foam cells as they accumulate lipids (cholesterol and oxidized LDL). Also, smooth muscle cells migrate from the vascular media into the intima, accumulate lipids, and produce collagen, which, with the smooth muscle cells, forms the fibromuscular cap over the lesion (17). Coronary plaque has been classified as hard, intermediate, or soft. The hard plaque is more stable with a thicker cap

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and likely to contain calcium, whereas the soft plaque has a thin cellular covering. Nonocclusive soft plaques are currently of greatest concern and interest clinically because of the possibility of erosion and rupture of the cap followed by thrombotic emboli and distal occlusion ischemia, resulting in an acute coronary event (20). In this study, the occlusive, stable plaque is of greatest importance since restricted coronary flow plays an important role in possible ischemia during +G_z exposure.

Over the past three decades the unanesthetized miniature swine has been shown to be an excellent animal model for +G_z research (2,6). The swine does a spontaneous (no training) cyclical straining maneuver while wearing an anti-G suit (2,4), very similar to the anti-G straining maneuver (AGSM) that centrifuge subjects and aircrew are trained to perform. The AGSM raises blood pressure by 100–150 mmHg, and is a supplement to the anti-G suit for high +G_z exposure. Moreover, the swine is responsive to diet-induced CAD (14). Thus, the miniature swine was chosen as the primary animal model for this study. In addition, the baboon was chosen as a second animal model since it is more closely related to man phylogenetically. However, the baboon does not consistently develop CAD when provided a high lipid diet (9,10,26). Therefore, a mechanical coronary stenosis was devised for the baboon.

METHODS

The animals used in these studies were procured, maintained, and used in accordance with the Animal Welfare Act and the “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Laboratory Animal Resources, National Research Council. These studies were approved by the Animal Care and Use Committee at Brooks Air Force Base.

There were 23 healthy, mature, female miniature swine with an average weight of 33.4 ± 4.0 kg (SD) and a weight range of 28–44.5 kg obtained from Sinclair Research Center, Inc., Columbia, MO (Hormel strain), used in this study. In addition, 11 healthy, mature, male baboons (*papio cynocephalus anubis*) weighing an average of 26.7 ± 2.9 kg (range = 22.7–31.0 kg) and ranging in age from 7–22 yr were also used in this study.

Swine Experimental Protocol

The swine were divided into three experimental groups: Study A (10 swine); Study B (8 swine); and a Control group of 5 swine. A preliminary report (technical paper) has been published previously containing a brief description and discussion of the methods and results from Study A and Control groups (5). Using isoflurane anesthesia and sterile surgical technique, a vascular access port (VAP; Model GPV-7S, Access Technologies, Norfolk Medical, Inc., Skokie, IL) for blood sampling and isotope injection was placed in each swine. The access port was placed subcutaneously at the dorsum of the neck behind the ear. The catheter end of the VAP was tunneled subcutaneously to an incision in the ventral neck and placed in the superior vena cava just cranial to

the right atrium through the external jugular vein, using fluoroscopy. For blood sampling or injection, the skin over the VAP was anesthetized with a freezing anesthetic spray and the silicone center of the VAP was penetrated through the skin using a noncoring Huber needle. The VAP was flushed weekly and filled with a heparin/betadine solution to maintain patency and sterility. Some of the VAPs remained patent for the length of the study. If a VAP failed (clotted) it was replaced using the opposite external jugular vein.

After collection of baseline data, Study A and B swine were placed on a high cholesterol/high fat diet (standard laboratory swine diet blended with 1.5% cholesterol and 15% beef tallow) for 18–57 wks. The five control swine were maintained on a standard laboratory swine diet (no cholesterol) for 44–58 wks. The standard diet contained 15% crude protein, 3% fat, 8% fiber, 8% ash, 0.8–1.5% calcium, 0.7% phosphorous, 0.65% lysine, 0.25–0.75% salt, 150 ppm zinc, selenium, and multiple other minerals and vitamins. The animals were sampled monthly for serum lipids (total cholesterol, LDL, HDL, total cholesterol/HDL ratio, and triglycerides) and weighed. The following procedures were followed for each group.

Control swine: 1) Baseline coronary angiogram to define any preexisting CAD or coronary abnormalities; 2) monthly serum lipid profile; 3) one or two +G_z exposures with 12-lead ECG at 39 to 49 wk after start of study using a simulated aerial combat maneuver (SACM) centrifuge exposure, with a Cardiolite (Du Pont Radiopharmaceuticals, No. Billerica, MA)/Tc-99m VAP infusion during the last 10 s of the SACM; 4) a single photon emission computed tomography (SPECT) myocardial perfusion scan 1 h later; and 5) necropsy at 44 to 58 wks after start of study. The SACM was an alternating +4 to +7 G_z or +4 to +8 G_z exposure with 15 s at each level. These swine were fed a standard pig chow (no cholesterol) at 1–2% of their pre-study bodyweight until approximately 28 wk when the volume of food had to be reduced because the swine were becoming too large for the centrifuge restraint system (“couch”).

Study A swine: 1) Baseline coronary angiogram; 2) atherogenic diet started; 3) monthly serum lipid profile; 4) one or two +G_z exposures with 12-lead ECG after 42 to 52 wks on atherogenic diet using a +4 to +8 G_z SACM centrifuge exposure, with a Cardiolite/Tc-99m VAP infusion during the last 10 s of the SACM; 5) a SPECT cardiac scan 1 h later; and 6) necropsy at 25 to 55 wks after beginning of atherogenic diet. These swine were fed the special diet at 1–2% of their pre-study bodyweight until approximately 28 wks when the special diet had to be diluted with standard pig chow because the swine were becoming too large.

Study B swine: 1) Baseline SPECT cardiac scan; 2) baseline cardiac fluoroscopy for identification of existing coronary calcium deposits; 3) start atherogenic diet; 4) monthly serum lipid profile; 5) monthly cardiac fluoroscopy; 6) coronary angiogram after 21–22 wk and 44–45 wk on atherogenic diet; 7) +G_z exposure with 12-lead ECG at 24–57 wk using a +5 to +9 G_z SACM with

Cardiolite/Tc-99m infusion during the last 10 s of the SACM; 8) a SPECT cardiac scan 1 h later; and 9) necropsy at 18–57 wk. These swine were fed a blended combination of the special diet and standard pig chow in an attempt to control weight gain and serum total cholesterol levels between 400–500 mg percent.

Three of the Study A swine died unexpectedly early. In an attempt to identify early onset of CAD, monthly fluoroscopy and angiography at 21–22 wk and 44–45 wk were added to Study B. If fluoroscopy or angiography demonstrated CAD, the swine would be placed on the control diet (no cholesterol) and exposed to +G_z as soon as possible.

+G_z Preparation

During +G_z the swine were protected with either an abdominal pneumatic bladder anti-G suit (Control and Study A) or a custom-made extended coverage anti-G suit with pneumatic bladder coverage from below the diaphragm to the ankles of the rear legs (Study B). The swine were sedated with ketamine (20 mg · kg⁻¹, i.m.) at approximately 0800 on the morning of the +G_z exposure. The 12-lead (chest and limbs) ECG leads were positioned, followed by fitting of the anti-G suit. The swine were then placed in a form-fitted aluminum and fiberglass restraint system (“couch”) and held in place with a strap behind the head, at mid-chest, and across the hips. The restraint system positioned the swine in a normal standing position with the weight of the animal supported along the ventral surface. The four legs passed through holes in the couch and did not support any weight. The animal and restraint system were lifted onto the centrifuge using a chain hoist and secured on the centrifuge. The head of the swine was facing the center of centrifuge rotation; therefore, during rotation the inertial load (+G_z) was parallel to the spine in a head-to-buttocks direction, similar to the +G_z exposure of pilots of high performance aircraft. The anti-G suit was connected to a compressed air source through an anti-G valve which supplied air pressure to the anti-G suit at 1.5 psi · G⁻¹ starting at +2 G_z. The VAP was connected to a remotely controlled infusion pump for injection of the Tc-99m labeled Cardiolite during the last 10 s of the +G_z profile. The swine were allowed the remainder of the morning to recover from the ketamine sedation. At approximately 1400, the unanesthetized swine received several preliminary “warm up” +G_z exposures. Immediately prior to the final (data) +G_z exposure (SACM), a qualified isotope technician loaded the Cardiolite/Tc-99m (approximately 10.0–15.0 mCi) into the VAP tubing for injection during the final +G_z exposure. Approximately 1 h after isotope infusion the swine were sedated with rompun/telezol (1 mg · kg⁻¹/3 mg · kg⁻¹) or rompun/ketamine (1 mg · kg⁻¹/20 mg · kg⁻¹), i.v., and the heart was scanned for myocardial perfusion using SPECT.

Necropsy

Each animal was necropsied after their last +G_z exposure. The swine were anesthetized with sodium pen-

tobarbital (30 mg · kg⁻¹, i.v.) and respired manually with an Ambu bag. The chest and abdomen were opened, and the heart was retrograde perfused by gravity flow at approximately 100 mmHg through a cannula secured in the abdominal aorta just cranial to the kidneys. The right atrium was opened and the perfusion was initiated with a 5% dextrose solution, followed by buffered glutaraldehyde or formaldehyde when the effluent from the right atrium became clear. After fixation, the heart was removed and soaked in formaldehyde for several days. The coronary arteries were sampled by taking approximately 5–7 mm sections, with surrounding tissue, at four to six locations along each of the left circumflex, left anterior descending (LAD), right, and the posterior descending coronary arteries. The coronary artery tissues were investigated histologically for evidence of CAD and percent stenosis (percent cross-sectional area reduction).

Baboon Experimental Protocol

The following baseline studies were performed on each animal before coronary stenosis: 1) coronary angiogram to determine any preexisting disease or abnormalities; 2) resting SPECT cardiac scan; 3) +G_z exposure followed by a SPECT cardiac scan; and 4) +G_z exposure after infusion of propranolol (0.3 mg · kg⁻¹, i.v.), followed by a SPECT cardiac scan. Unanesthetized acceleration data collection occurred during an exposure of +7 G_z/30 s, following preliminary warm-up exposures of +3, +5, and +7 G_z/15 s. The Cardiolite/Tc-99m was remotely infused during the last 10 s of the +7 G_z/30 s profile.

Following baseline data collection, nine of the animals were anesthetized with nitrous oxide/isoflurane, the chest was opened in the left third intercostal space using sterile technique, the pericardium was opened, and the LAD coronary artery was isolated as close to its origin as possible. A piece of radiolucent nylon tubing, 0.5 cm in length and opened longitudinally, was placed around the LAD coronary artery and held closed by two radiopaque tantalum clips. The nylon tubing was bored to various diameters to achieve an approximate 30–40% constriction of the artery using the baseline coronary angiogram diameters as reference. The pericardium and chest were closed and the animals were allowed to recover with standard antibiotic therapy. Two animals were sham operated using the identical anesthetic and surgical procedures without placement of the nylon tubing. Following surgical recovery [avg. 6.0 ± 1.5 wk (range = 5–10 wk)], each animal again underwent the following procedures: 1) coronary angiography for determination of degree of stenosis; 2) resting SPECT cardiac scan; 3) +G_z exposure followed by a SPECT cardiac scan; and 4) +G_z exposure after infusion of propranolol (0.3 mg · kg⁻¹, i.v.), followed by a SPECT cardiac scan. The +G_z exposures were the same as those used before stenosis. All +G_z exposures were at an onset rate of +6 G_z · s⁻¹. A 12-lead ECG (chest and limbs) was obtained during all +G_z exposures.

+G_z Preparation

For all procedures the animals were restrained in the seated position in a customized restraint chair made of tubular aluminum and Plexiglas. The animals wore a custom-made jacket which covered the entire thorax down to the waist. The jacket had sewn-in shoulder and waist straps which were passed through slots in a Plexiglas back plate and bolted in place to bring the back of the animal snugly against the back plate. For +G_z exposures an additional lap belt was added to secure the pelvis against the back plate to prevent submarining during +G_z. Also, for all +G_z exposures the animals wore an abdominal bladder pneumatic anti-G suit. The anti-G suit was pressurized as described for the swine. The arms and legs were secured to the restraint chair at the wrists and ankles with 1-in wide nylon straps.

For placement in the restraint chair the animals were anesthetized with ketamine (avg. of 7.6 mg · kg⁻¹, i.m.); at approximately 0800. ECG leads were attached and a sterile 24-in catheter was passed transcutaneously to the superior vena cava from the arm or to the inferior vena cava from the lower leg, using fluoroscopy. The catheter was used for the infusion of propranolol (when used) and for the remotely controlled infusion of Cardiolite/Tc-99m during the last 10 s of the +G_z exposures. The animals were allowed to recover from the ketamine and placed on the centrifuge around 1300 for +G_z exposure. Approximately 1 h after the +G_z exposure and the Tc-99m infusion the animals were anesthetized with ketamine (7.6 mg · kg⁻¹, i.m.) and valium (10 mg, i.v.) and removed from the restraint chair for a cardiac SPECT scan.

Statistical Analysis

Swine weights and blood lipids: A one-way ANOVA was used to compare the swine weight and blood lipid data (Fig. 1) between the three groups (Control, Study A, and Study B) at baseline, 4 wk, 12 wk, 24 wk, 36 wk, and 48 wk. If significance was observed an unpaired, two-tailed independent samples *t*-test was used to compare the three studies. Significance was accepted as *P* ≤ 0.05.

Swine heart rate: In the Control study the swine were exposed to a +4 to +7 G_z SACM for their first exposure and a +4 to +8 G_z SACM for their second exposure. Whereas in Study A, the swine were exposed to a +4 to +8 G_z SACM for their first and second exposures, and in Study B, the swine were exposed to only one +5 to +9 G_z SACM. In an effort to normalize +G_z level and time at +G_z as well as time of exposure after the beginning of the study, a G × time at peak G and number of weeks after beginning of the study were used as covariants in a one-way ANOVA comparing heart rate from the three studies at control (baseline), peak heart rate during +G_z, 1 min post-exposure, and 2 min post-exposure (Table I).

Baboon heart rate: A two-tailed paired *t*-test was used to compare heart rate before stenosis, with or without propranolol, and after stenosis, with or without propranolol, at pre +G_z exposure, during +G_z, immediately post +G_z, and at 1 min, 1.5 min, and 2 min post +G_z exposure.

Baboon cardiac SPECT scans and ECG: Cardiac SPECT data could not be analyzed because of missing or poor quality scans. ECG was of no value as an ischemia indicator since it was abnormal before stenosis.

RESULTS

Swine

Swine weights and blood lipids: Study A swine weights were consistently and significantly higher than Control and Study B throughout the investigation (Fig. 1). Moreover, total cholesterol, HDL, LDL, and the total cholesterol/HDL ratio for studies A and B were significantly higher than Control through most of the study. Overall mean triglycerides from Study B were significantly lower than both Control and Study A.

Heart rate: When comparing Control versus Study A versus Study B heart rate the only significant difference was between Study A and Study B at 1 min post-exposure.

Control group: Baseline coronary angiography was normal. Although dysrhythmias were common, and similar to Studies A and B there were no ST segment or T wave (ST-T) changes in the ECG during +G_z stress and the post-stress SPECT scans were generally normal (Table II). In this study a normal ECG may contain many dysrhythmias but no ST-T changes, indicating no ischemia, whereas an abnormal ECG containing ST-T changes or an abnormal SPECT scan are suggestive of ischemia. Coronary histopathology after necropsy showed normal vessels with no stenosis.

Study A: Baseline coronary angiography was normal. After start of the special diet there was good agreement between abnormal ECG and abnormal SPECT (9 of 10). Of 11 ECGs during +G_z stress, 8 were abnormal at 42 to 52 wk, suggesting ischemia (Table II), and 8 of 10 SPECT scans were abnormal. There was modest agreement between abnormal ECG/SPECT and abnormal histopathology. Coronary histopathology showed measurable stenoses ranging from 20–95% in 9 of 10 swine. Three of the swine died unexpectedly before +G_z exposure; one died at 25 wk and the other two died at 38 and 39 wk. Histopathology showed moderate to severe CAD in these three animals.

Study B: Baseline SPECT cardiac scans and cardiac fluoroscopies were normal on all animals. Cardiac fluoroscopy for identification of calcification within the coronary artery walls during atherogenesis was negative throughout the study. Coronary angiography at 21–22 wk following the start of the special diet was normal in six of seven swine and was still normal at 44–45 wk in two of the swine. Two swine died unexpectedly before +G_z exposure; one died from anesthesia complications during surgical repair of the vascular access port, and the other was found dead during a morning health check. Both swine had significant coronary stenoses. Coronary histopathology after necropsy of all swine showed stenoses ranging from 0 to 91% and in most cases correlated well with the angiography data.

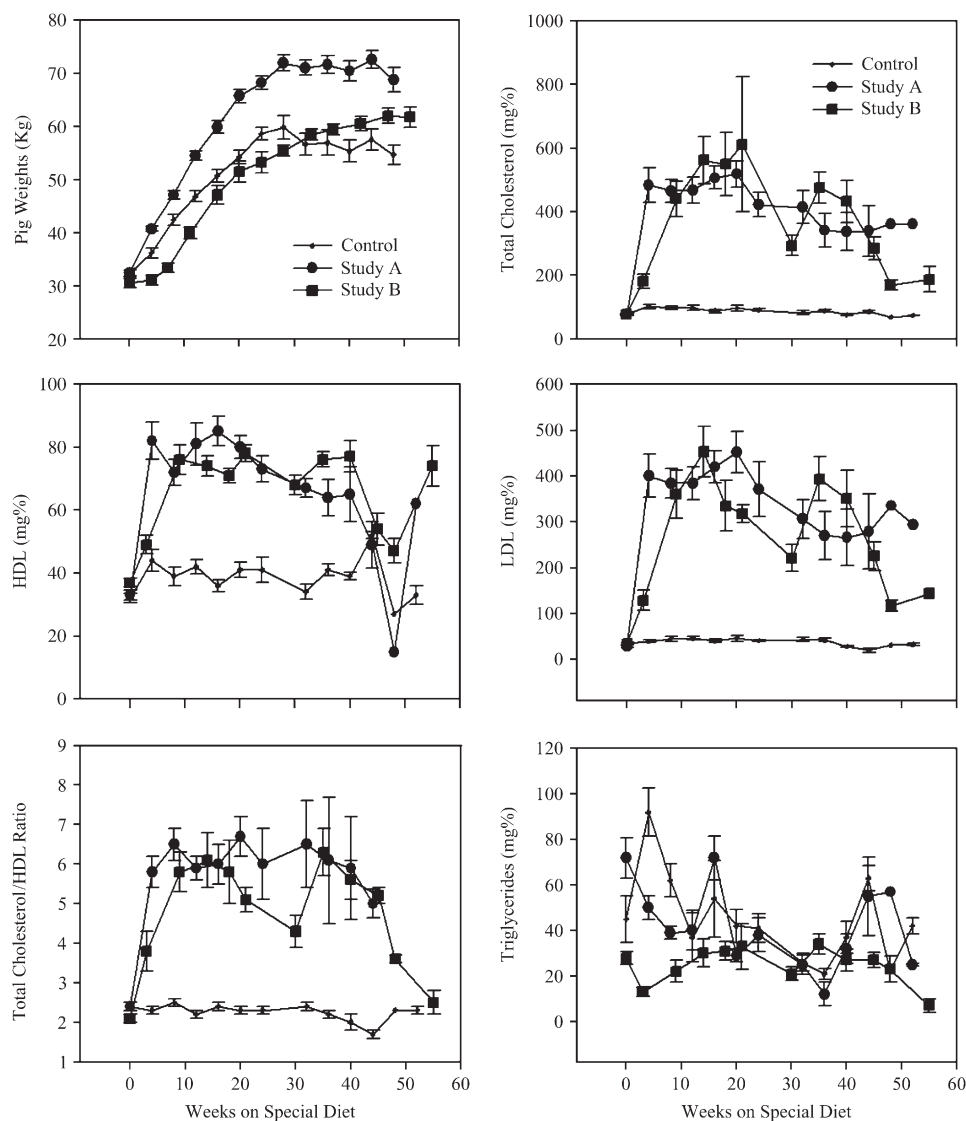


Fig. 1. Swine weights and plasma lipid data from Control, Study A, and Study B miniature swine at zero through 58 wk on high cholesterol/high fat diet. Data are mean \pm SE.

The one swine which showed an abnormal angiogram at 21 wk was placed on a standard pig chow diet (no cholesterol) and exposed to +G_z at 24 wk. The +G_z ECG and the SPECT scan were both normal. The animal was necropsied immediately after +G_z and histopathology demonstrated stenoses of 16–91% throughout the coronary arteries.

Two of the remaining five swine were generally normal throughout the study with minimal to mild histopathology. One of the two swine had a severe ventricular tachycardia at 2 min post +G_z. The dysrhythmia spontaneously converted to a normal rhythm without further episodes. One swine fibrillated and died at 1 min post +G_z with only 0 to 44% stenoses throughout the coro-

TABLE I. MINIATURE SWINE HEART RATE DURING +G_z SACM.

	<i>n</i>	G x Time (G Minutes)	Control	Peak	1 Minute Post	2 Minute Post
Control (N = 5 Swine)						
18.8 \pm 5.7 +7 G _z peaks at 40 \pm 0.82 wk	4	32.9 \pm 9.9	91 \pm 15.5	220 \pm 4.5	211 \pm 9.1	186 \pm 39.8
12.2 \pm 3.5 +8 G _z peaks at 47 \pm 2.8 wk	5	24.4 \pm 7.0	95 \pm 15.4	220 \pm 6.2	162 \pm 53.7	166 \pm 21.3
Study A (N = 10 Swine)						
12.1 \pm 3.4 +8 G _z peaks at 44.3 \pm 1.6 wk	7	24.3 \pm 6.9	101 \pm 13.7	215 \pm 7.2	196 \pm 40	199 \pm 22.9
14 \pm 0.0 +8 G _z peaks at 50 \pm 1.4 wk	4	28 \pm 0.0	107 \pm 17.2	217 \pm 6	207 \pm 9.1*	204 \pm 8.2
Study B (N = 8 Swine)						
8.8 \pm 1.5 +9 G _z peaks at 47.5 \pm 12.1 wk	6	13.3 \pm 2.2	119 \pm 37.2	223 \pm 13.2	153 \pm 17.1*	184 \pm 87.5

Data are mean \pm SD; * = *P* < 0.05. Three swine from Study A and two swine from Study B died unexpectedly before +G_z exposure at 25, 38, 39, 18, and 24 wk, respectively.

TABLE II. MINIATURE SWINE +G_z SACM DATA.

	n	G x Time (G Minutes)	Centrifuge ECG	SPECT	% Stenosis (Histopathology)				
					LAD	LC	RC	PD	
Control (N = 5 Swine)									
18.8 ± 5.7 +7 G _z peaks at 40 ± 0.82 wk	4	32.9 ± 9.9	4 nl	4 nl					
12.2 ± 3.5 +8 G _z peaks at 47 ± 2.8 wk	5	24.4 ± 7.0	5 nl	3 nl; 2 abnl	wnl	wnl	wnl	wnl	
Study A (N = 10 Swine)									
12.1 ± 3.4 +8 G _z peaks at 44.3 ± 1.6 wk	7	24.3 ± 6.9	1 nl; 6 abnl	6 abnl					
14 ± 0.0 +8 G _z peaks at 50 ± 1.4 wk	4	28 ± 0.0	2 nl; 2 abnl	2 nl; 2 abnl	min-90; p-d	min-75; p-d	min-95; p-d	min-95; p-d	
Study B (N = 8 Swine)									
8.8 ± 1.5 +9 G _z peaks at 47.5 ± 12.1 wk	6	13.3 ± 2.2	4 nl; 2 abnl	2 nl; 3 abnl	0-73; p-d	0-90; p-d	1-91; p-d	0-84; p-d	

Data are mean ± SD; three swine from Study A and two swine from Study B died unexpectedly before +G_z exposure at 25, 38, 39, 18, and 24 wk, respectively. SACM = simulated aerial combat maneuver; SPECT = single photon emission computed tomography; LAD = left anterior descending coronary artery; LC = left circumflex coronary artery; RC = right coronary artery; PD = posterior descending coronary artery; nl = normal; abnl = abnormal (suggesting ischemia); wnl = within normal limits; min = minimal; p = proximal; d = distal.

naries. Of the two remaining animals, both had mild to moderate histopathology and abnormal SPECT scans, whereas one had a normal ECG and the other had an abnormal ECG. SPECT scans demonstrated septal, lateral, and posterior left ventricular wall ischemia. Apical ischemia was masked by apical thinning, seen in both control and diseased swine and, therefore, could not be diagnosed.

ECG: Dysrhythmias, such as sinus tachycardia (ST), premature ventricular contractions, sinus bradycardia, and ventricular tachycardia, as well as p-wave and dramatic T-wave changes and left and right axis shift were observed equally in both control and cholesterol swine during all +G_z exposures. However, none of the control swine had ST-T segment elevation or depression during any of the +G_z exposures. ST segment elevation of up to 4 mm was seen in leads II, III, aVF, V5, or V6 of the cholesterol animals. Of 17 +G_z exposures of the cholesterol swine, 10 exhibited abnormal ECGs, usually ST-T segment changes, indicative of ischemia.

Single-site stenosis in the cholesterol swine ranged from 0–95% in the proximal, mid, and distal regions of the left circumflex, LAD, right, and posterior descending coronary arteries, with aggregate stenosis over the whole heart ranging from 0–800%. The morphology of the coronary lesions resulting in stenosis of varying degrees was consistent among the study A and B swine. The lesions consisted of a fibromuscular cap containing foam cells, macrophages, scattered lymphocytes, extracellular vacuoles, and mineralization. Other affected vessels included the ascending, thoracic, and abdominal aorta, and mesenteric, iliac, and renal arteries.

Baboons

Coronary stenosis using a fixed-diameter nylon clip around the LAD coronary artery averaged 37.6 ± 15.0% with a range of 18–58% (Table III). Fig. 2 is an angiogram of a constrictor in place on the LAD coronary artery. Fig. 3 illustrates the LAD after removal of the retaining clips and the nylon constrictor. Note the severe atrophy of the vessel media and thickening of the intima (observed). There were no abnormal SPECT scans following +G_z exposure before stenosis, whereas 8 of 13 SPECT scans were abnormal following +G_z exposure after stenosis, with or without propranolol.

ECG: The 12 lead ECG data was determined to be either normal or abnormal and was compared with the SPECT data. There were 17 missing or poor quality SPECT scans and 2 missing ECGs. Of the available SPECT and ECG data, 59% of the data agreed and 41% disagreed. The baboon ECG demonstrated the same dysrhythmias observed in the swine. An abnormal ECG indicated possible/probable ischemia.

Heart rate: As anticipated, heart rate was consistently significantly reduced by propranolol both before and after stenosis (Table IV). There was also a significant stenosis effect with propranolol at pre +G_z and at 1.5 min and 2 min post +G_z (heart rate was higher with stenosis and propranolol than before stenosis with propranolol at each of the three times).

DISCUSSION

The data demonstrate that across the spectrum of mild-moderate-severe CAD, noninvasive evidence of

TABLE III. BABOON CARDIAC SPECT AND ECG (N = 9 BABOONS).

G _z w/o Stenosis		G _z w/o Stenosis, with Propranolol		% LAD Stenosis (approx.)	G _z with Stenosis		G _z with Stenosis, with Propranolol	
SPECT	ECG	SPECT	ECG		SPECT	ECG	SPECT	ECG
4 nl; 5 missing	1 nl; 6 abnl; 2 missing	3 nl; 6 missing	2 nl; 7 abnl	37.6 ± 15	3 nl; 4 abnl; 2 missing	9 abnl	2 nl; 4 abnl; 3 missing	4 nl; 5 abnl

All +G_z data exposures were +7 G_z · 30 s⁻¹; symbols are the same as in Table II. Sham operated baboons are not included.

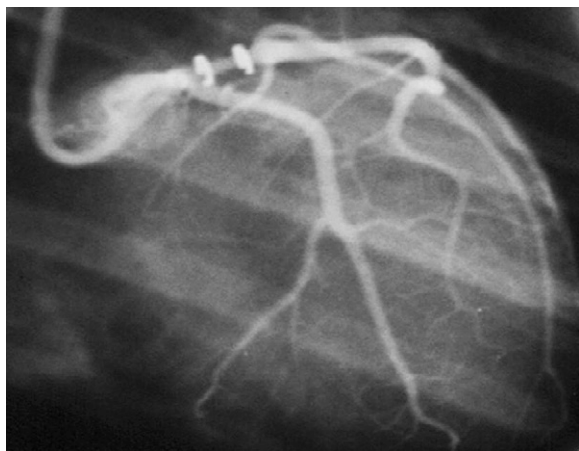


Fig. 2. Baboon coronary angiogram showing stenotic implant on the proximal left anterior descending coronary artery.

myocardial ischemia was frequently present during high +G_z. There was only one fatality related to high +G_z exposure. One swine fibrillated and died during the recovery phase immediately after +G_z with only mild CAD. Similarly, in a previous coronary blood flow study (18), an unanesthetized, asymptomatic female miniature swine fibrillated at 49 s of +7 G_z. Necropsy demonstrated an approximate 90% occlusion of the LAD coronary artery and an infarct distal to the stenosis. The baboons in this study with coronary stenoses ranging from 18–58% (average = 37.6 ± 15%) tolerated +G_z without a fatality.

The stress that these unanesthetized animals were exposed to is considered to be as great, or greater than, that of aircrew in combat. Not only were the animals exposed to a physical stress (+G_z), but also a very significant emotional stress due to restraint and loss of situational control. It has been previously demonstrated that unanesthetized, restrained swine generate a tremendous increase in plasma catecholamines upon initial exposure

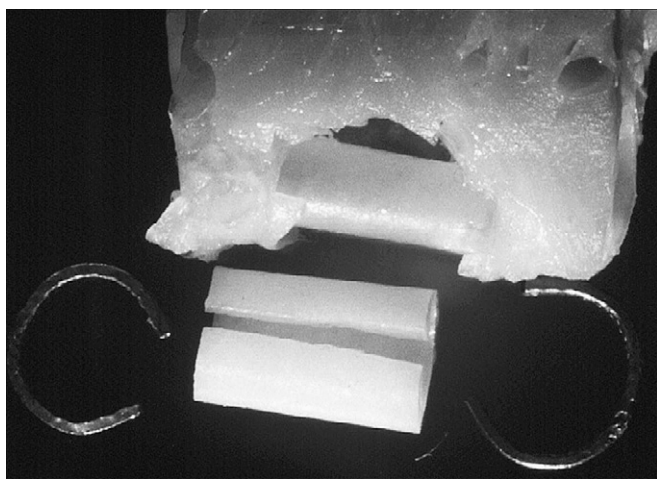


Fig. 3. Top: Left anterior descending coronary artery after removal of the nylon tubing at necropsy. Note the severe atrophy of the vessel media and thickening of the intima. Bottom: Nylon tubing (0.5 cm long) and tantalum clips used on the left anterior descending coronary artery of the baboon to produce stenosis.

to +G_z (3). Although propranolol significantly reduced heart rate in the baboon it did not significantly influence the severity of the SPECT or ECG response to +G_z before or after stenosis.

The miniature swine has again proven to be an excellent animal model for +G_z research (2,4,5) and this study demonstrated that the Sinclair/Hormel strain of miniature swine is also an excellent model for diet-induced CAD. Surprisingly, CAD can develop very quickly. The five swine that died unexpectedly early (18 to 39 wk on the special diet) were found to have moderate to severe CAD and significantly higher mean total cholesterol and mean LDL values than the other swine. Four of the swine that died early were found dead in their cages at morning health check. Except for the moderate to severe CAD determined from necropsy, there was no obvious cause of death. However, they could have experienced rupture of an unobserved, nonocclusive coronary soft plaque, resulting in thrombotic emboli and severe occlusion ischemia and fibrillation.

A previous study (14) using Gottingen miniature swine fed a diet which included 11.2% egg yolk and 0.5% cholesterol demonstrated coronary atherosclerosis after 18 mo, but had no reported unexpected early deaths. The growth rate of the Gottingen swine was approximately 0.5 kg · wk⁻¹, whereas in the current study the growth rate for the first 28 wk (before reduced food intake) was approximately 2.6 kg · wk⁻¹ for the Study A swine and 2.0 kg · wk⁻¹ for the Study B swine. The higher growth rate and the early onset of significant coronary lesions in the present study is probably a result of the higher lipid content of the feed (15% beef tallow and 1.5% cholesterol), although the difference in swine strain could also be a factor.

As in humans, not all of the swine developed significant CAD. Of the 18 swine in Study A and B, 7 developed minimal CAD, 4 developed minimal to moderate or moderate CAD, and 7 had severe CAD (80% or greater stenosis). Substantial coronary calcification was observed in the swine hearts from Study A, both histologically and visually from isolated heart X-rays. However, failure to observe calcification during the monthly cardiac fluoroscopic exams in Study B suggests that the amount of calcification may not have been great enough for fluoroscopic observation in the intact beating heart, even though calcification was observed histologically. Calcification is usually seen in established “hard” stenotic plaque.

Except for two abnormal SPECT scans the control swine showed a good correlation between a normal ECG during +G_z, a normal SPECT scan following +G_z, and normal histopathology at necropsy, indicating no ischemia during +G_z, even though the control swine had the greatest G × time exposure (average = 28.2 G · min⁻¹). Using ECG and SPECT data from Control, Study A and Study B (23 swine) compared to the histopathologic data from the same animals resulted in sensitivity for both ECG and SPECT of 0.8 with four true positives and one false negative for each test. In addition, the specificity for ECG was 0.71 (15 true negatives and 6

TABLE IV. BABOON +G_z HEART RATE (N = 9 BABOONS).

	Before Stenosis	n	Before Stenosis with Propranolol	n	After Stenosis	n	After Stenosis with Propranolol	n
Pre G	143 ± 21.2	8	91 ± 5.6*	9	144 ± 23.6	9	95.6 ± 6*	8
During G	205 ± 16.1	7	128 ± 23.5	9	214 ± 12.7	9	131 ± 11.1	8
Immediately Post G	201 ± 12.1	8	116 ± 21.9	9	196 ± 34	9	118 ± 22.9	8
1 min Post G	178 ± 21.5	8	94 ± 7.9	9	177 ± 11.3	9	109 ± 12.5	8
1.5 min Post G	191 ± 15.6	8	93 ± 6.5*	9	169 ± 11.8	9	102 ± 10*	8
2 min Post G	171 ± 21.2	2	90 ± 7.3*	5	170 ± 9.6	8	103 ± 8.4*	8

Data are mean ± SD. All G_z exposures were +7 G_z · 30 s⁻¹. Sham operated baboons are not included. Significant (*P* < 0.05) decrease in heart rate with propranolol effect both before and after stenosis.

*Significant stenosis effect.

false positives) and 0.52 for SPECT (10 true negatives and 9 false positives).

Several unexplained discrepancies occurred in Study B. One animal demonstrated a normal angiogram at 22 wk after start of the special diet but died during surgery for VAP repair at 24 wk with mild to severe CAD throughout the coronary bed. Moreover, another swine had a severely abnormal angiogram at 21 wk but a normal ECG during +G_z and a normal SPECT scan following +G_z with severe stenoses at necropsy.

Those aspects of the ECG that normally suggest myocardial ischemia (ST segment and T-wave changes) occur infrequently in normal human subjects during +G_z (8). If they do occur it is usually immediately upon acceleration, too soon for ischemia to develop, and they disappear immediately upon deceleration, too soon for ischemia to subside, suggesting an erroneous +G_z-related aberration in the ECG (13). In this study, the observation of ST-T changes (abnormal ECG) supported by an abnormal SPECT scan and histologic findings of mild to severe stenoses would suggest that the interpretation of an abnormal ECG was accurate.

Limitations of the Study

As described, the number of study animals was small, especially with the unexpected deaths before data collection in some. The swine developed significant CAD too rapidly and the CAD spectrum was very broad (minimal, moderate, and severe), limiting further the numbers with the desired mild to moderate CAD. Thus, drawing meaningful conclusions from the data regarding MCAD was not possible. No reliable method was available to detect nonsignificant lesions other than frequent serial angiography, which was not feasible. Constrictors used in the baboon created specific single-site CAD more reliably in the desired range of severity, but such lesions are not true atherosclerotic disease. And the baboon proved to be a less desirable animal model than the swine for cardiac noninvasive testing. Moreover, the angiograms were recorded on videotape and filmed using portable fluoroscopy, providing less than ideal images for interpretation.

Every aspect of the normal ECG is altered during +G_z. Typical dysrhythmias seen in this study in both the swine and baboon were: sinus tachycardia, prema-

ture ventricular contractions, left and right axis shift, major inconsistent T-wave changes (flattening, biphasic, inverted, decreased, and dramatically increased), ST elevation or depression, R > S transition, premature atrial contractions (PACs), right atrial enlargement (RAE), left atrial enlargement (LAE), and ectopic tachycardias. Many of these same dysrhythmias are seen in normal human subjects during +G_z (1,8,13,23,27,28) and during treadmill exercise (27), although they appear to be more severe during +G_z (27). The stresses of +G_z, including an increase in sympathetic activity during +G_z and parasympathetic activity immediately following +G_z, distortion and movement of the heart in the thorax, including flattening of the heart on the G-suit-induced elevated diaphragm, changes in cardiac volume, and electrical interference from the AGSM make interpretation of the ECG difficult during and immediately following +G_z. The lingering effects of ketamine, although minimal, could be a contributing factor for dysrhythmias (29). Full recovery of mature swine from a ketamine injection of 15 mg · kg⁻¹, i.m., is reported to occur within 4.1 h (21). The swine and baboons in this study were not exposed to +G_z until approximately 6 h following i.m. ketamine injection.

The diagnostic quality of the SPECT scans was disappointingly inconsistent for several possible reasons. SPECT software was developed for humans. The thorax of the swine is larger than man in the anteroposterior plane. Moreover, the base-to-apex plane of the swine heart is parallel to the sternum and centered under the sternum with the apex pointing ventrally. These anatomical differences could lead to SPECT imaging and interpretation errors. Also, the apex of the swine heart is thin, leading to possible false positive interpretations of apical ischemia. The thorax of the baboon is smaller than the human or the swine thorax. Thus, the gall bladder, which collects a measurable amount of the Tc-99m, is considerably closer to the heart in the baboon than it is in either man or the swine. Shielding was required to separate the images of the gall bladder and the heart. However, shielding may not have been adequate in some baboons, possibly resulting in an erroneous cardiac scan.

Although invasive coronary angiography is the "gold standard" for detection of coronary stenosis, and SPECT

perfusion scan for global ischemia was the accepted technique at the time of this study new noninvasive techniques are being developed and evaluated. These include 64-slice computed tomography (11,12,15,19) and MRI (7) for identification of plaque and determination of percent stenosis, and rubidium-82 positron emission tomography perfusion imaging for determination of ischemia (24,25). If available for animal research, some of these newer modalities, especially coronary angiography by computed tomography, might be more helpful for future research.

Helpful Findings

Regardless of the severity of CAD (minimal, moderate, or severe) clearly the majority of the special diet swine studies suggested ischemia by ECG ST-T changes and/or SPECT imaging. Control animals, on the other hand, reliably had normal studies with normal angiography and normal histopathology. Thus, ECG changes and SPECT scans were fairly reliable. The swine is a good animal model for investigation of CAD as it reliably develops CAD with a high cholesterol/high fat diet yet does not develop CAD on standard chow. The swine coronary anatomy is similar to humans and the swine does a spontaneous straining maneuver similar to trained aircrew.

Summary and Conclusions

The frequency and type of dysrhythmias were seen equally in control and cholesterol swine and in the baboon before and after stenosis. ST-T changes in the swine during +G_z, usually indicative of myocardial ischemia, were seen only in the cholesterol swine and were positively related with histopathologic coronary stenosis findings, and to a lesser degree with SPECT perfusion scans. In the baboon, ST-T changes during +G_z were observed in most animals before and after stenosis and with or without propranolol. The swine has proved to be an excellent model for diet-induced CAD and an excellent model for +G_z-induced myocardial ischemia in the diseased animal. On the other hand, the baboon was not a good animal model because of SPECT problems, ECG interpretation, and a lower +G_z tolerance. The rapid development of moderate to severe CAD in this swine study and the unexpected early animal deaths did not provide adequate data to evaluate the ischemic response to MCAD. The use of frequent noninvasive CT coronary angiography to identify MCAD as early as possible would be beneficial in future studies. In spite of the study limitations, the majority of studies showed ischemia by ECG changes and/or SPECT scan. Control animals, on the other hand, reliably had normal studies with normal angiography and normal histopathology. Recommendations regarding MCAD cannot be formulated from these data. However, these data do highlight the importance of additional studies to investigate the potential clinical ramifications of MCAD during +G_z exposures.

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