### 4. TITLE AND SUBTITLE
Cardiac Ischemia Model for +Gz Using Miniature Swine and Baboons

### 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. **Conclusions:** In the swine and the baboon extended high levels of 1 Gz were associated with evidence of myocardial ischemia.

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

### 16. SECURITY CLASSIFICATION OF: Unclassified

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### 19a. NAME OF RESPONSIBLE PERSON
John Burns
Cardiac Ischemia Model for $+\mathbf{G}_z$ Using Miniature Swine and Baboons


Military aircrew with even nonocclusive or minimal coronary artery disease (MCAD) may be restricted from flying high-performance aircraft due to possible ischemia during high $+\mathbf{G}_z$. 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 $+\mathbf{G}_z$ exposure and scanned for myocardial perfusion. Results: Five swine died unexpectedly before $+\mathbf{G}_z$ exposure with moderate-to-severe CAD. Dysrhythmias during $+\mathbf{G}_z$ were seen equally in both the control and experimental swine and in the baboons before and after stenosis, with or without propranolol. During $+\mathbf{G}_z$, 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 $+\mathbf{G}_z$ 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.

From the Biosciences and Protection Division, Air Force Research Laboratory (J. W. Burns), the Clinical Sciences Division, USAF School of Aerospace Medicine (W. B. Kruyer, P. V. Celio, J. Deering, T. H. Loecker, L. A. Richardson); and the Veterinary Sciences Division (J. W. Fanton, H. Davis, E. J. Dick, Jr.), Brooks City-Base, TX. This manuscript was received for review in April 2007. It was accepted for publication in December 2007. Address reprint requests to: John W. Burns, Ph.D., 128 River Bluff Dr, Boerne, TX 78006; john.burns@amedd.army.mil. Dr. Burns is currently an Emeritus member of the Biosciences and Protection Division, Air Force Research Laboratory, Brooks City-Base, TX.

* John W. Fanton, DVM, is deceased. Reprint & Copyright © by the Aerospace Medical Association, Alexandria, VA.

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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 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 +Gz as soon as possible.

+Gz Preparation

During +Gz 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 +Gz 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 (+Iz) was parallel to the spine in a head-to-buttocks direction, similar to the +Gz exposure of pilots of high performance aircraft. The anti-G suit was connected to a remotely controlled inflation 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.

During +Gz the swine were connected to an 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 Gz. 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 +Gz 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” +Gz exposures. Immediately prior to the final (data) +Gz 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 +Gz exposure. Approximately 1 h after isoﬂ ate 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 +Gz exposure. The swine were anesthetized with sodium pentobarbital (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) +Gz exposure followed by a SPECT cardiac scan; and 4) +Gz 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 Gz/30 s, following preliminary warm-up exposures of +3, +5, and +7 Gz/15 s. The Cardiolite/Tc-99m was remotely infused during the last 10 s of the +7 Gz/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 radio-opaque 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) +Gz exposure followed by a SPECT cardiac scan; and 4) +Gz exposure after infusion of propranolol (0.3 mg·kg\(^{-1}\), i.v.), followed by a SPECT cardiac scan. The +Gz exposures were the same as those used before stenosis. All +Gz exposures were at an onset rate of +6 Gz·s\(^{-1}\). A 12-lead ECG (chest and limbs) was obtained during all +Gz exposures.
CARDIAC ISCHEMIA MODEL—BURNS ET AL.

+Gz 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 +Gz exposure an additional lap belt was added to secure the pelvis against the back plate to prevent submariining during +Gz. Also, for all +Gz 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 +Gz exposures. The animals were allowed to recover from the ketamine and placed on the centrifuge around 1300 for +Gz exposure. Approximately 1 h after the +Gz 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 52 wk, suggesting ischemia (Table II), and 8 of 10 SPECT scans were abnormal. There was modest agreement between abnormal ECG and abnormal SPECT (9 of 10). In this study a normal ECG may contain some 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.

Swine 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 +Gz stress and the post-stress SPECT scans were generally normal (Table II). In the study a normal ECG may contain some dysrhythmias but no ST-T changes, indicating no ischemia. Table II. Coronary histopathology showed measurable stenoses ranging from 20–95% in 9 of 10 swine. Three of the swine died unexpectedly before +Gz 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.

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 t-test was used to compare the swine weight and blood lipid data from the restraint chair for a cardiac SPECT scan.

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 +Gz stress and the post-stress SPECT scans were generally normal (Table II). In this study a normal ECG may contain some dysrhythmias but no ST-T changes, indicating no ischemia. Coronary histopathology showed measurable stenoses ranging from 20–95% in 9 of 10 swine. Three of the swine died unexpectedly before +Gz 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 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 +Gz 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 +Gz 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 atherosclerosis 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 +Gz 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.
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\textsubscript{z} at 24 wk. The +G\textsubscript{z} ECG and the SPECT scan were both normal. The animal was necropsied immediately after +G\textsubscript{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\textsubscript{z}. The dysrhythmia spontaneously converted to a normal rhythm without further episodes. One swine fibrillated and died at 1 min post +G\textsubscript{z} with only 0 to 44% stenoses throughout the coronary arteries.

### TABLE I. MINIATURE SWINE HEART RATE DURING +G\textsubscript{z} SACM.

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

Data are mean ± SD; * = P < 0.05. Three swine from Study A and two swine from Study B died unexpectedly before +G\textsubscript{z} exposure at 25, 38, 39, 18, and 24 wk, respectively.
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 +Gz exposures. However, none of the control swine had ST-T segment elevation or depression during any of the +Gz 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 +Gz 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 angigram 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 +Gz exposure before stenosis, whereas 8 of 13 SPECT scans were abnormal following +Gz 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 +Gz and at 1.5 min and 2 min post +Gz (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

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**TABLE II. MINIATURE SWINE + Gz SACM DATA.**

<table>
<thead>
<tr>
<th>Control (N = 5 Swine)</th>
<th>Study A (N = 10 Swine)</th>
<th>Study B (N = 8 Swine)</th>
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<tbody>
<tr>
<td>% Stenosis (Histopathology)</td>
<td>% Stenosis (Histopathology)</td>
<td>% Stenosis (Histopathology)</td>
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<td></td>
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<tr>
<td>LAD</td>
<td>LC</td>
<td>RC</td>
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<tr>
<td>Gz w/o Stenosis</td>
<td>Gz w/o Stenosis, with Propranolol</td>
<td>Gz with Stenosis</td>
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<td>Gz w/o Stenosis</td>
<td>Gz w/o Stenosis, with Propranolol</td>
<td>Gz with Stenosis</td>
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<td>Gz w/o Stenosis</td>
<td>Gz w/o Stenosis, with Propranolol</td>
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<td>Gz w/o Stenosis</td>
<td>Gz w/o Stenosis, with Propranolol</td>
<td>Gz with Stenosis</td>
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<td>Gz w/o Stenosis</td>
<td>Gz w/o Stenosis, with Propranolol</td>
<td>Gz with Stenosis</td>
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<td>Gz w/o Stenosis, with Propranolol</td>
<td>Gz with Stenosis</td>
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**TABLE III. BABOON CARDIAC SPECT AND ECG (N = 9 BABOONS).**

<table>
<thead>
<tr>
<th>Gz w/o Stenosis</th>
<th>Gz w/o Stenosis, with Propranolol</th>
<th>Gz with Stenosis</th>
<th>Gz with Stenosis, with Propranolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPECT</td>
<td>ECG</td>
<td>% LAD Stenosis (approx.)</td>
<td>SPECT</td>
</tr>
<tr>
<td>4 nl; 5 missing</td>
<td>1 nl; 6 abnl; 2 missing</td>
<td>3 nl; 6 missing</td>
<td>2 nl; 7 abnl</td>
</tr>
</tbody>
</table>
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 $37.6 \pm 15\%$) 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 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 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 $G \times$ time exposure (average $= 28.2 G \cdot \text{min}^{-1}$). 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
Significant stenosis effect. with propranolol effect both before and after stenosis.

Possible 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 1 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 +Gz, and a normal SPECT scan following +Gz 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 +Gz (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 +Gz-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.


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

Data are mean ± SD. All Gz exposures were +7 Gz · 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 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 +Gz (1,8,13,23,27,28) and during treadmill exercise (27), although they appear to be more severe during +Gz (27). The stresses of +Gz, including an increase in sympathetic activity during +Gz and parasympathetic activity immediately following +Gz, 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 +Gz. 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 +Gz 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.

REFERENCES


