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The evaluation and use of the pig (*Sus domesticus*) as a model for determining the effects of dietary lipids on lipid metabolism and thrombosis in human beings.

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Frazer James Allan

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Dedicated to my children,
Sophie, Fergus and Phoebe

Abstract

The domestic pig (*Sus domesticus*) has been used extensively as a model in atherothrombosis research because this species has many physiological similarities to human beings. In the series of studies presented here, the domestic pig was used and evaluated as a model for determining the effects of dietary lipids on lipid metabolism and thrombosis in human beings.

In the first study, serum specimens were collected from five 8-week-old Large White pigs in the fasting and postprandial states to determine whether cholesterol fractions can be estimated without use of the ultracentrifuge. Sequential ultracentrifugation was used to determine cholesterol in VLDL (VLDL^{fuge}), LDL (LDL^{fuge}) and HDL (HDL^{fuge}) fractions. VLDL^{fuge} was compared with VLDL cholesterol (VLDL-C) concentration estimated as serum triglyceride concentration divided by four (VLDL^{trig}). HDL^{fuge} was compared with cholesterol remaining in the supernatant after precipitation of apolipoprotein B-containing lipoproteins with Mn²⁺ and heparin (HDL^{ppt}). LDL^{fuge} was compared with the concentration determined from the Friedewald formula (total cholesterol less HDL^{ppt} less VLDL^{trig}). After correcting the centrifuged fractions for recovery of total cholesterol, the mean difference between the LDL^{friede} and LDL^{fuge} of fasting samples was <5% and the mean difference between fasting HDL^{ppt} and HDL^{fuge} was <8%. Fasting VLDL^{trig} was more than twice VLDL^{fuge}, after correction for cholesterol recovery, possibly because of very low recoveries in the ultracentrifuge or because of an incorrect divisor of total serum triglyceride. This study showed that whereas HDL cholesterol (HDL-C) and LDL cholesterol (LDL-C) can be reliably estimated in these specimens by simple methods, VLDL-C estimation requires further investigation. A secondary finding was that feeding significantly reduced the serum concentrations of total cholesterol and LDL-C but raised HDL-C.

Using the simple methods for determining serum HDL-C and LDL-C concentrations established in the first study, fasting serum cholesterol concentrations were then determined in eight-week-old pigs (6 pigs per group) fed diets containing either fish oil, milkfat, coconut oil, olive oil or cornstarch at an inclusion rate of 4% (w/w) for 3 weeks in the second study. Serum total cholesterol concentration was significantly higher in pigs fed coconut oil than in pigs fed cornstarch or fish oil ($p < 0.05$). Pigs fed coconut oil, olive oil and milkfat had a significantly higher serum HDL-C concentration than those fed cornstarch or fish oil. There were no significant differences in serum LDL-C concentrations between groups. The serum triglyceride concentrations were higher in pigs receiving coconut oil and milkfat. This study showed that dietary fats that have a hypercholesterolaemic effect in humans tended to raise serum HDL-C rather than LDL-C concentrations in pigs. These findings suggest differences in lipoprotein metabolism between human beings and growing pigs and caution is warranted when making inferences about human lipoprotein metabolism from porcine studies.

A balloon angioplasty model of arterial injury in pigs for the assessment of the thrombogenicity of dietary fats was evaluated in the third study. Eight-week-old pigs (8 pigs per group) were fed diets containing milkfat, fish oil or cornstarch at an inclusion rate of 10% w/w for 12 weeks. The group receiving the fish oil diet was withdrawn from the study after 6 weeks because the diet was unpalatable. At the end of the feeding period, angioplasty was performed on the left and right external iliac arteries simultaneously in each pig. One artery was randomly assigned to receive an angioplasty catheter with a 10-mm balloon while the contralateral artery was distended to 12 mm. One hour later, the damaged arterial segments were harvested and the size of the thrombi that formed in each artery were estimated with a technique using autologous, radiolabelled platelets and by a morphometric technique. The thrombi that formed in this study were platelet-rich, typical of those found at the site of atheroma rupture in human beings. Five of 32 arterial segments sustained deep injury, indicated by the presence of tears through the internal elastic lamina. Thrombi could also be

seen at the site of balloon injury in arteries that did not sustain deep injury, a finding that contrasts with earlier angioplasty studies in pigs, which have shown that thrombi will only form at sites of deep injury. The presence of deep injury, and the length of the tear through the internal elastic lamina, appeared to influence the size of the thrombus that formed at the angioplasty site. Given that the length of the tear was difficult to control with balloon angioplasty, and that thrombi can be identified in the absence of tears, it may be more desirable to avoid deep injury in this model. When the arteries that sustained deep injury were excluded and thrombus size was evaluated by the radiolabelled platelet method, there was some evidence ($p = 0.1$) to suggest that the pigs fed the milkfat diet may have had a greater thrombotic tendency than those fed the cornstarch diet. This model, using autologous radiolabelled platelets for estimating thrombus size, shows promise as a method of evaluating the effects of dietary lipids on thrombosis.

The activity of the coagulation cascade was assessed by determining the activated partial thromboplastin time (APTT), prothrombin time (TT), thrombin time (TT) and factor VII activity and plasma fibrinogen concentrations during the study evaluating the balloon angioplasty model of arterial injury in pigs (described above). These variables were determined at the beginning of the study and 1, 2, 4, 6, 8, 10 and 12 weeks later. There was some evidence ($p = 0.07$) to suggest that the group receiving the milkfat diet had a longer APTT than the group receiving the cornstarch diet. This suggests that the milkfat diet may induce less activity within the intrinsic clotting cascade and/or the common clotting cascade than the carbohydrate diet. This finding therefore, does not explain the tendency for thrombus to be larger at the site arterial injury induced by angioplasty in the pigs receiving milkfat than those receiving cornstarch.

In the final study, the effect of dietary lipids on blood concentrations of markers of thrombosis, platelet indices, white and red blood cell counts and platelet reactivity in pigs was evaluated. The experimental animals and diets were the same as those presented in the second study. Variables evaluated included

plasma TAT and fibrinogen concentrations, platelet numbers, mean platelet volume, plateletcrit, white blood cell (WBC) and red blood cell counts and platelet reactivity, assessed by platelet aggregation and by filragnetometry. The inclusion of fish oil in the diet of pigs has affected some variables in a way that could be interpreted as 'prothrombotic' compared to the other dietary treatments. The fish oil group had the highest concentrations of TAT and fibrinogen, the highest platelet and WBC count and the greatest plateletcrit. In contrast, platelet function, as assessed by aggregometry, was lowest in the group receiving fish oil. Olive oil appeared to be relatively 'antithrombotic' when compared to the other dietary treatments. The pigs receiving the olive oil had the lowest, or equal lowest, TAT and fibrinogen concentrations, and plateletcrit and WBC counts. Platelet function, as assessed by aggregometry, was the second lowest. This is noteworthy because the consumption of a Mediterranean-style diet, which is rich in olive oil, has been previously been shown to be protective against coronary artery disease. These findings highlight the difficulty associated with using indicators of thrombosis to predict final thrombus size. The establishment of a thrombus end-point in an appropriate animal model is required in order to evaluate the overall thrombogenic potential of dietary fats. Nevertheless, evaluating the effect of dietary lipids on markers of thrombosis, such as those investigated in this study, may provide important insights into mechanisms that lead to thrombosis.

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Table of contents

Abstract.....	iii
Acknowledgements	vii
Table of contents.....	viii
List of figures.....	xi
List of tables.....	xvi
Chapter 1. Literature review	1
1.1. Atherosclerosis	2
1.2. Thrombosis	6
1.3. Lipoprotein metabolism in pigs and human beings.....	9
1.4. Tests of platelet function and thrombotic potential.....	17
1.5. The effect of dietary lipids on thrombosis.	45
1.6. References	56
Chapter 2. Determination of fasting and postprandial lipoprotein cholesterol concentrations in pigs: a comparison of methods.....	80
2.1. Introduction	80
2.2. Materials and methods.....	81
2.3. Results.....	85
2.4. Discussion.	88
2.5. References	91
Chapter 3. Serum lipoprotein cholesterol and triglyceride concentrations in pigs fed diets containing fish oil, milkfat, olive oil and coconut oil.....	94
3.1. Introduction.....	94
3.2. Material and methods.....	95
3.3. Results.....	98
3.4. Discussion	100

3.5. References	105
Chapter 4. Evaluation of a balloon angioplasty model of arterial injury in pigs for the assessment of the thrombogenicity of dietary fats	111
4.1. Introduction	111
4.2. Materials and methods.....	112
4.3. Results.....	127
4.4. Discussion	136
4.5. References	144
Chapter 5. The effect of a milkfat diet and a low-fat diet on coagulation screening tests, fibrinogen concentrations and factor VII activity in pigs.....	151
5.1. Introduction	151
5.2. Materials and methods.....	154
5.3. Results.....	158
5.4. Discussion	161
5.5. References	163
Chapter 6. The effect of dietary lipids on blood concentrations of markers of thrombosis, platelet indices, white and red blood cell counts and platelet reactivity in pigs	166
6.1. Introduction	166
6.2. Materials and methods.....	169
6.3. Results.....	186
6.4. Discussion	189
6.5. References	199
Chapter 7. General discussion.....	211
7.1. Discussion	211
7.2. References	218

Appendix.....	224
Glossary.....	227

List of figures

Figure 1.1 A schematic representation of the optical method of aggregometry	21
Figure 1.2 A schematic representation of the impedance method of aggregometry	22
Figure 1.3 A typical clot signal generated by the Sonoclot® Coagulation and Platelet Function Analyzer	24
Figure 1.4 Simplified diagram of the pathways of eicosanoid production from arachidonic acid and eicosapentaenoic acid and their biological effects	45
Figure 1.5 Putative mechanism by which dietary fatty acids can affect platelet and endothelial function	47
Figure 2.1 The Friedewald formula	80
Figure 2.2 The modified Friedewald formula used in this study	84
Figure 2.3 Formula used to calculate the percent difference between the LDL cholesterol concentration as determined by sequential ultracentrifugation and derived from the Friedewald formula.....	85
Figure 4.1 The effect of the milkfat and carbohydrate diets on the quantities of docosahexaenoic acid (22:6 n-3) and eicosapentaenoic acid (20:5 n-3) within platelet phospholipids, expressed as a percentage of the total fatty acid content within platelet phospholipids	128

-
- Figure 4.2** The effect of the milkfat and carbohydrate diets on the quantity of arachidonic acid within platelet phospholipids, expressed as a percentage of the total fatty acid content within platelet phospholipids..... 128
- Figure 4.3** The effect of the milkfat and carbohydrate diets on the 5, 8, 11-eicosatrienoic acid within platelet phospholipids, expressed as a percentage of the total fatty acid content within platelet phospholipids..... 129
- Figure 4.4** Photomicrograph of a thrombus that has formed within the external iliac artery at a site of damage created by a balloon angioplasty catheter. In this section, there is no evidence of deep injury..... 130
- Figure 4.5** Higher magnification of Figure 4.4 130
- Figure 4.6** Photomicrograph of a thrombus that has formed within the external iliac artery at a site of damage created by a balloon angioplasty catheter. Unlike the artery shown in Figures 4.4 and 4.5, a tear can be seen extending into the media, indicated by the arrow 131
- Figure 4.7** Higher magnification of Figure 4.6 131
- Figure 4.8** Thrombus size, evaluated morphometrically, versus arterial tear length 132
- Figure 4.9** Number of platelets incorporated into arterial thrombus versus arterial tear length..... 132

-
- Figure 4.10** The raw data, excluding the arteries that tore, are shown for each litter..... 133
- Figure 4.11** Number of platelets within the thrombus versus the thrombus size as evaluated morphometrically. All data are shown 135
- Figure 4.12** Number of platelets within the thrombus versus the thrombus size as evaluated morphometrically. Only arteries that did not sustain tears during balloon angioplasty are shown..... 135
- Figure 5.1** The effect of feeding a carbohydrate diet (blue line, open squares) and a milkfat diet (red line, filled circles) on the activated partial thromboplastin time in growing pigs over a 12-week period 159
- Figure 5.2** The effect of feeding a carbohydrate diet (blue line, open squares) and a milkfat diet (red line, filled circles) on the prothrombin time in growing pigs over a 12-week period 159
- Figure 5.3** The effect of feeding a carbohydrate diet (blue line, open squares) and a milkfat diet (red line, filled circles) on the thrombin time in growing pigs over a 12-week period 160
- Figure 5.4** The effect of feeding a carbohydrate diet (blue line, open squares) and a milkfat diet (red line, filled circles) on plasma fibrinogen concentrations in growing pigs over a 12-week period 160
- Figure 5.5** The effect of feeding a carbohydrate diet (blue line, open squares) and a milkfat diet (red line, filled circles) on factor VII activities in growing pigs over a 12-week period 161

-
- Figure 6.1** Changes in plasma TAT concentrations in two pigs in response to intravenous infusion of thromboplastin172
- Figure 6.2** Scanning electron micrograph of the microfilament polyester filter with 20- μ m pores evaluated for suitability for filtragometry.....179
- Figure 6.3** Scanning electron micrograph of a nickel filter with 20- μ m pores following filtragometry179
- Figure 6.4** Scanning electron micrograph of a nickel filter following filtragometry using heparin at a concentration of 10 IU per mL of whole blood181
- Figure 6.5** Scanning electron micrograph of a nickel filter following filtragometry using trisodium citrate at a final concentration in blood of 0.0034 mg per mL of whole blood181
- Figure 6.6** Diagram of the filtragometry system used in this study.....184
- Figure 6.7** The change in pressure (in mmHg) that develops two minutes after drawing whole blood across a nickel filter is shown for each group of pigs who have been fed diets containing either a 4% cornstarch (carbohydrate), fish oil, milkfat, olive oil or coconut oil.....188
- Figure A-1** The effect of feeding a diet containing 10% w/w concentrated marine fish oil to pigs for 6 weeks225

Figure A-2 Calculated platelet count of platelet-rich plasma, using the formula described in Chapter 6, versus an automated platelet count of platelet-rich plasma, using a haematology analyser (Cobas Minos Vet, ABX Hematologie, Montpellier, France) in a group of growing pigs (n=16)	226
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List of tables

Table 2.1 Mean total cholesterol concentration ($\text{Chol}^{\text{total}}$) determined enzymatically and the mean cholesterol concentration determined by summation of the cholesterol content of the VLDL, LDL and HDL fractions following sequential ultracentrifugation (Chol^{sum}).....	86
Table 2.2 Mean serum LDL cholesterol concentrations prepared from sequential ultracentrifugation (LDL^{fuge}) and the mean serum LDL cholesterol concentrations derived from the modified Friedewald formula ($\text{LDL}^{\text{friede}}$).....	87
Table 2.3 Mean serum HDL cholesterol concentrations prepared from sequential ultracentrifugation (HDL^{fuge}) and the mean serum HDL cholesterol concentrations derived following the precipitation of apolipoprotein B-containing lipoproteins (HDL^{ppt}).....	87
Table 2.4 Mean serum VLDL cholesterol concentrations prepared from sequential ultracentrifugation ($\text{VLDL}^{\text{fuge}}$) and the mean serum VLDL cholesterol concentrations calculated by dividing the serum triglyceride concentration by four ($\text{VLDL}^{\text{trig}}$).....	88
Table 3.1 Ingredient composition of the experimental diets.....	96
Table 3.2 Mean weight gain (in kilograms) of each group of pigs during the 21-day study.....	99
Table 3.3 Mean serum concentrations of lipids at day 21.....	99
Table 4.1 Ingredient composition of the experimental diets.....	114

Table 4.2	Fatty acid composition of the carbohydrate and milkfat diets.....	115
Table 4.3	The effect of feeding milkfat or a carbohydrate diet for 12 weeks in growing pigs on predicted mean thrombus size in the external iliac artery following balloon angioplasty.....	134
Table 4.4	The effect of the diameter of the angioplasty balloon on the predicted mean thrombus size in the external iliac artery of growing pigs fed a milkfat or carbohydrate diet for 12 weeks.....	134
Table 6.1	A comparison between the flow rate across the filter in previous studies (Hornstra & ten Hoor, 1975; Larsson <i>et al</i> , 1990; Soderback <i>et al</i> , 1991; Broijersen <i>et al</i> , 1993) with the flow rate across the filter in the current study	177
Table 6.2	Mean concentrations of markers of thrombosis and haematological variables in growing pigs fed diets containing fish oil, milkfat, olive oil, coconut oil or cornstarch at 4% w/w	187