Lipemic Index of Pork
3B-107

Report prepared for the Co-operative Research Centre for High Integrity Australian Pork

By

Prof Manohar L Garg
Dr Melinda Phang
A/Prof Lisa G Wood
Prof Peter RC Howe

Nutraceuticals Research Group
University of Newcastle
Callaghan, NSW 2308
AUSTRALIA

March 2014

An Australian Government Initiative

Established and supported under the Australian Government's Cooperative Research Centres Program

Executive Summary

Following consumption of a meal, the metabolic response to dietary fat during the postprandial period is an important determinant of cardiovascular health, whereby an elevated and prolonged lipemic response is associated with an induced pro-atherogenic state (Poppitt 2005). Given that, in a typical Western lifestyle, a large proportion of time is spent in the postprandial state, it is important to understand the potential impact of these post meal changes with respect to the type of foods we consume. Of particular concern are fatty meals which can induce significant postprandial lipemia. Moreover, the fat content of meat is highly saturated; saturated fat can contribute to raise plasma LDL cholesterol in consumers, further increasing the risk of atherosclerosis.

Pork is the most widely consumed meat in the world and serves as an excellent source of dietary protein as well as containing other vitamins and minerals. However, pork has been perceived as a fatty meat and therefore less healthy compared to other meats; this may have contributed to the lower rates of consumption in Australia. The amount of saturated fat in a serving of pork varies according to the leanness of the cut. Regular consumption of lean pork has been shown to produce improvements in plasma lipid profiles similar to consumption of lean red meat (veal) (Rubio, Rubio et al. 2006). In fact, there is growing evidence that regular consumption of lean pork as a protein source in conjunction with regular exercise can improve cardio-metabolic health outcomes in diabetic and overweight patients (Wycherley, Noakes et al. 2010; Murphy, Thomson et al. 2012) .

Therefore the aim of this trial was to demonstrate that the impact on plasma lipids (the acute lipemic response) of consuming a pork meal is comparable to that of consuming a meal of lean red meat (lamb) with equivalent fat content.

This was an acute crossover intervention study, where healthy adults were randomly assigned to consume a single serve of pork or lamb mince each containing approximately 24g fat per serve. At baseline, blood lipid levels, weight, body mass index, and measures of body composition including % body fat and lean mass using bioelectrical impedance were assessed. Blood lipid levels were measured again at 2, 4 and 6 hours following consumption of the pork or lamb meal. Participants returned at least one week later to repeat the assessment with the alternate meal. On each occasion, prior dietary intakes and physical activity were assessed.

Results show that there were no differences in physical activity, energy or macronutrient intakes during the study period. Moreover, baseline blood lipids prior to consumption of pork and lamb were similar. Statistical analyses using paired, two-tailed t-tests showed no differences between the pork and lamb meals in the incremental changes of blood cholesterol (total, LDL and HDL) and triglycerides at 2, 4 or 6 hours. Although statistically insignificant, the Lipemic Index (i.e. the integrated change in plasma triglyceride levels calculated as the area within a trapezoid) tended to be lower for pork than lamb; the effect size suggests the possibility of a significant difference with a larger sample size.

Given that postprandial disturbances play an important role in the development and progression of cardiovascular and associated diseases, the current findings provide a proof of concept that pork is as good as lamb and possibly better with respect to postprandial lipemia and support the growing evidence that consumption of fresh pork is equally healthy to consumption of alternative meats.

Table of Contents

1. Introduction

Elevated cholesterol levels are an established risk factor for cardiovascular disease and for several years, health authorities have been advocating decreased dietary intake of saturated fat and cholesterol as an initial step in the management of hypercholesterolemia. Given that dietary fat intake plays a significant role in the development of cardiovascular disease through its effect on lipoprotein metabolism (Neaton, Blackburn et al. 1992), the immediate metabolic response to dietary fat during the postprandial period serves as an important indicator of cardiovascular health.

Postprandial lipemia is characterised by a transient accumulation of circulating blood lipids in the post meal state, mainly comprising of triglyceride-rich lipoproteins (Paglialunga and Cianflone 2007). An elevated and prolonged lipemic response is associated with an induced proatherogenic state via adverse effects on lipoprotein remodeling, inflammatory pathways, hemostatic variables, and endothelial integrity (Poppitt 2005; Delgado-Lista, Lopez-Miranda et al. 2008). For this reason the effect of dietary fat on the extent and duration of the lipemic response has stimulated significant interest.

Pork is a rich source of protein, vitamins and minerals, and studies have reported that regular consumption of lean pork may improve cardiovascular risk factors including glycemic control and blood lipid levels (Wycherley, Noakes et al. 2010; Murphy, Thomson et al. 2012 (under review)). Wycherley and colleagues (Wycherley, Noakes et al. 2010) demonstrated that an energy restricted high protein pork diet compared to an isocaloric diet over 16 weeks reduced blood pressure, lipids and glucose levels in diabetic patients. An overall reduction was reported in total cholesterol (-0.67 mmol/L), triglycerides (-0.47mmol/L) and LDL cholesterol (mmol/L) in diet and control groups, with no differences between groups.

Rubio and colleagues also demonstrated using a cross-over design that lean pork consumption produced similar effects on the lipid profile compared to that of veal consumption. Total cholesterol and LDL levels were reduced following a 6 week pork diet (-5.2% and -6.5% respectively), while total cholesterol and triglyceride levels decreased with the veal diet (-4.3% and -9.8% respectively). There were no differences in the lipid profile between the pork or the veal diets (Rubio, Rubio et al. 2006). A study by Flynn et al also reported similar effects without changes in serum lipids in healthy men and women whether strictly consuming a three month beef, poultry, fish or pork diet (Flynn, Naumann et al. 1982).

To date, no study has examined the acute, immediate effects of red meat vs. white meat on post-prandial lipemia. This project examined the clearance rate of blood lipids from consuming dietary fat provided by pork, compared to lamb, meat. The aim of the current study was to conduct a randomized cross-over study to compare the effect of consuming pork meat, with that of a lean lamb meat, on post prandial lipemia. Lipaemic index of pork versus lamb meat was derived by calculating area under the curve (AUC) by plotting postprandial triglyceride changes over time following meal consumption.

2. Methodology

2.1. *Subjects, design and dietary groups*

Free-living healthy, non-smoking men and women were recruited through local media advertisements and the Hunter Medical Research Institute volunteer register to participate in a randomized, cross-over trial. Subjects were excluded if

they reported one of the following: diagnosed diabetes or CV disease; dyslipidemia, hypercholesterolemia; gastrointestinal disease, liver disease or other conditions relation to malabsorption or metabolism of lipids; use of anti-inflammatory or hypocholesterolaemic drugs; BMI >30; current smokers; pregnant or breast feeding women; unable or unwilling to consume pork or lamb as required. Eligible volunteers were randomized using a computer software program (http://randomization.com) and allocated to one of two starting diet meals. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Human Research Ethics Committee at the University of Newcastle, Callaghan, Australia. Written informed consent was obtained from all subjects. The trial was registered on the Australia New Zealand Clinical Trials Register (ACTRN12613000922774).

2.2. *Study design*

Of a total of 28 volunteers who were screened for eligibility, 26 were randomized to commence the acute dietary intervention with either the pork or lamb meal, with lipid assessments over the 6 hour period post meal consumption (Figure 1). At the end of this period, volunteers crossed over to consume the alternate meat meal with a minimum of 1 week washout period in between; thus each volunteer acted as their own control. Volunteers attended the research unit on each occasion for an approximate 6.5 hour duration where they consumed the cooked meat meal and the following assessments were made at each study visit; weight and height (to calculate BMI (kg/m2), body composition (assessed using the InBody bioelectrical impedance analysis), medical history questionnaire, 24 hour dietary recall and blood lipid measurements at baseline, 2, 4 and 6 hours post-meal consumption.

2.3. *Dietary intervention*

All volunteers were provided with a freshly cooked minced pork or lamb meal and asked to consume and finish the meal within a 10 minute time frame. As the meats were matched on fat content per serve, the portion sizes varied slightly (pork 140g/serve, lamb 200g/serve) to match ~24g of fat per serve. All volunteers were given 2 slices of white bread and 250mL of bottled water with the respective meats and 250 mL bottled water to drink every 2 hours. Participants were asked to refrain from consuming any other foods or fluids and limit physical activity during the 6 hour study assessment.

2.4. *Outcomes measures*

The primary outcome measure was the change in plasma triglyceride levels (mmol/L) following consumption of a single meal containing pork or lamb mince. Lipemic index was calculated by integrating the change in plasma triglyceride levels (area under curve) within a trapezoid, as indicated in figure 2. Secondary outcome measures included baseline levels of plasma cholesterol, LDL cholesterol and HDL cholesterol as well as the change in cholesterol levels following consumption of meals incorporating pork or lamb mince.

2.5. *Clinical assessments*

2.5.1. *Dietary intake*

Volunteers were asked to complete a 24 hour dietary recall to report all food and beverage intake prior to each study day. Nutrient intakes were calculated using a computerized database (Foodworks 7, 2012, Xyris Software, Kenmore Hills, Australia) to ensure background diet was not a confounding factor to influence the test results. The statistical package was SPSS Statistics 21 (IBM, Chicago, USA) and significance was set at $P<0.05$ unless otherwise stated.

2.5.2. *Blood lipid profile*

Volunteers were required to have their blood collected via venipuncture from the antecubital vein using a vacutainer system. Blood was collected into a 4mL lithium heparin tubes (Becton Dickinson Bioscience, Ltd NSW, AUS) at baseline and 2, 4 and 6 hours post-meal consumption for analysis of blood lipid levels. The samples were centrifuged at 3000 x g for 10 min (Heraeus Biofuge Stratos, Radiometer Pacific, AUS) to obtain plasma. The samples were analysed for plasma cholesterol, LDL-cholesterol, HDL-cholesterol and triglyceride levels by Hunter New England Area Health Pathology Services (NSW, Australia) using standard automated analytical techniques

2.6. *Statistical analysis*

Data of subjects who completed the trial were analysed using paired 2-tailed t-test to identify differences between means in the same cohort with different treatment. The statistical package was SPSS 21 (IBM Software, Chicago, USA) and significance was set at $P<0.05$ unless otherwise stated. The area under the curve (AUC) was calculated using the trapezoidal rule as indicated in figure 2.

2.7. *Compliance*

Subjects were asked to limit physical activity and refrain from consuming any other food or beverages during the study period. During the washout period of the cross over design, subjects were asked to maintain their normal physical activity and follow a similar diet which was assessed a 24 hour food recall upon the second visit of the opposite meat meal.

3. Outcomes

3.1. *Subject characteristics*

Of the 28 subjects who were enrolled in the intervention, 4 withdrew prior to commencement (due to increased work/study commitments) and 4 withdrew after commencement (Figure 1). Among these, 2 withdrew as they could no longer commit to the study, 1 withdrew due to a personal illness and 1 due to non-compliance. Thus 18 subjects completed the cross-over study. Characteristics of volunteers are presented in Table 1. This population was on average aged 31±2 years, healthy weight (BMI 24.1±0.6kg/m^2) and normolipidaemic (plasma total cholesterol 4.88±0.2mmol/L; triglyceride 1.08±0.1mmol/L) adults.

3.2. *Body composition*

Two subjects that had greater than a one week wash-out period had their body composition reanalysed on their second visit. There was no difference in any index of adiposity between these subjects over time.

3.3. *Dietary intakes*

There was no difference in energy intake (kJ) (Table 2) or macronutrients (total fat, protein or carbohydrate) in either group 24 hours prior to each study day.

3.4. *Blood lipids*

There was no difference in the baseline blood cholesterol levels (Table 2) prior to intervention with pork or lamb meals. Changes in plasma triglyceride levels over time were also similar (Figure 2) with an exception at 4 hour post-intervention when the triglyceride levels were significantly lower following consumption of the pork compared to the lamb meal ($p=0.02$) (Table 3 and Figure 2). Plasma cholesterol levels over 6 hours were not significantly different whether consuming the pork or the lamb meat (Table 3). A similar pattern was also observed for LDL-

cholesterol and HDL-cholesterol levels indicating a similar cholesterolaemic response with both meals (Table 3).

However area under the curve (AUC) for the change in plasma triglycerides levels (Lipemic Index) tended to be lower when participants were consuming pork compared to lamb (p=0.116 with an effect size of 0.39 mmol/L) (Figure 3)

Figure 1 - Consort diagram.

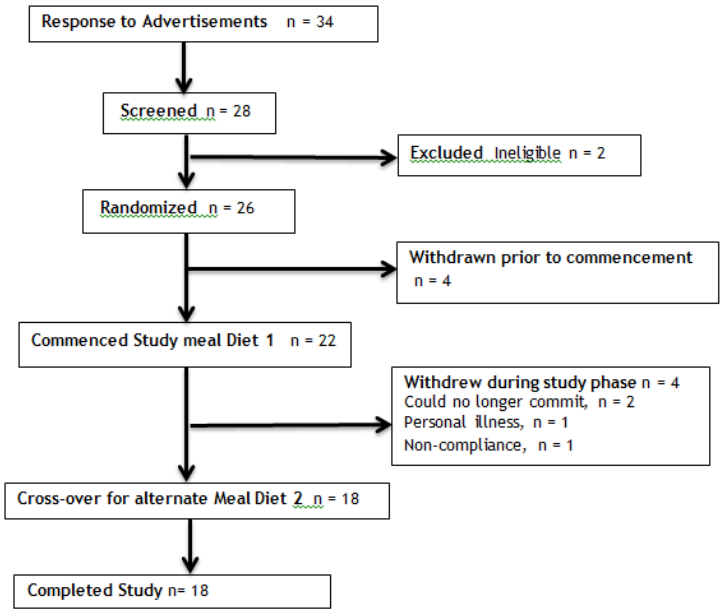


Table 1 - Baseline characteristics of study participants.

	Mean±SD
Gender *n*	10 M / 8 W
Age (yrs)	31±2
Weight (kg)	78±4
BMI (kg/m^2)	24.1±0.6
Fat Mass (kg)	14.6±0.9
% Body Fat	21±1.1
Dietary Intake	
Energy (kJ/day)	7635±359
Protein (g/d)	93.1±6.1
CHO (g/d)	76.9±12.8
Fat (g/d)	70.5±4.5
SFA (g/d)	26.3±1.8
Total cholesterol (mmol/L)	4.88±0.2
Triglycerides (mmol/L)	1.08±0.1
LDL-cholesterol (mmol/L)	2.9±0.1
HDL-cholesterol (mmol/L)	1.4±0.04

*Dietary intake was analyzed using FoodWorks 7 (Xyris Software), mean ± standard error. Abbreviations: yrs, years; kg, kilograms; BMI, body mass index; m, metres; LDL, low-density lipoprotein; HDL, high-density lipoprotein; mmol, millimoles; L, litre; kJ, kilojoule; g, grams; SFA, saturated fatty acid; CHO, carbohydrates

Table 2 - Baseline blood lipid profile and macronutrient intakes.

	Pork	Lamb	P value
Total cholesterol (mmol/L)	4.71±0.3	5.05±0.3	0.309
Triglycerides (mmol/L)	0.97±0.1	1.21±0.2	0.204
LDL –cholesterol (mmol/L)	2.83±0.2	2.96±0.4	0.692
HDL-cholesterol (mmol/L)	1.38±0.1	1.45±0.1	0.450
Energy (kJ)	7669±483	7224±522	0.536
Protein (g)	89.8±6.9	94.7±11.1	0.715
CHO (g)	193±20	178±14.7	0.555
Fat (g)	72.1±6.5	65.3±6.5	0.469
SFA (g)	26.5±2.5	23.9±2.3	0.449

Data presented as mean ± standard error.
Abbreviations: mmol, millimoles; L, litre; kJ, kilojoule; SFA, saturated fatty acid; g, grams; CHO, carbohydrate

Table 3 - Blood lipid levels over 6 hours following dietary intervention.

		Baseline	2 Hours	4 Hours	6 Hours
Total Cholesterol (mmol/L)	Pork	4.71±0.3	4.67±0.2	4.76±0.2	4.95±0.2
	Lamb	5.05±0.3	4.80±0.3	5.02±0.2	5.13±0.2
		P = 0.14	*P = 0.61*	*P = 0.13*	*P = 0.27*
LDL-Cholesterol (mmol/L)	Pork	2.83±0.2	2.69±0.2	2.84±0.2	3.03±0.2
	Lamb	2.98±0.2	2.86±0.2	2.97±0.2	3.16±0.2
		P = 0.24	*P = 0.19*	*P = 0.31*	*P = 0.38*
HDL-Cholesterol (mmol/L)	Pork	1.38±0.1	1.36±0.1	1.38±0.1	1.41±0.1
	Lamb	1.45±0.1	1.44±0.1	1.45±0.1	1.46±0.1
		P = 0.09	***P =0.047***	*P = 0.11*	*P = 0.24*
Triglycerides (mmol/L)	Pork	0.98±0.1	1.23±0.1	1.11±0.1	0.98±0.1
	Lamb	1.19±0.2	1.47±0.1	1.36±0.1	1.09±0.1
		P = 0.07	*P = 0.11*	***P = 0.02***	*P = 0.13*

Data presented as Mean ± standard error
Abbreviations: LDL, low-density lipoprotein; HDL, high-density lipoprotein; mmol/L, millimoles/litre.

Figure 2 - Plasma triglyceride levels over time following consumption of the pork or lamb meal. Values are mean±SEM

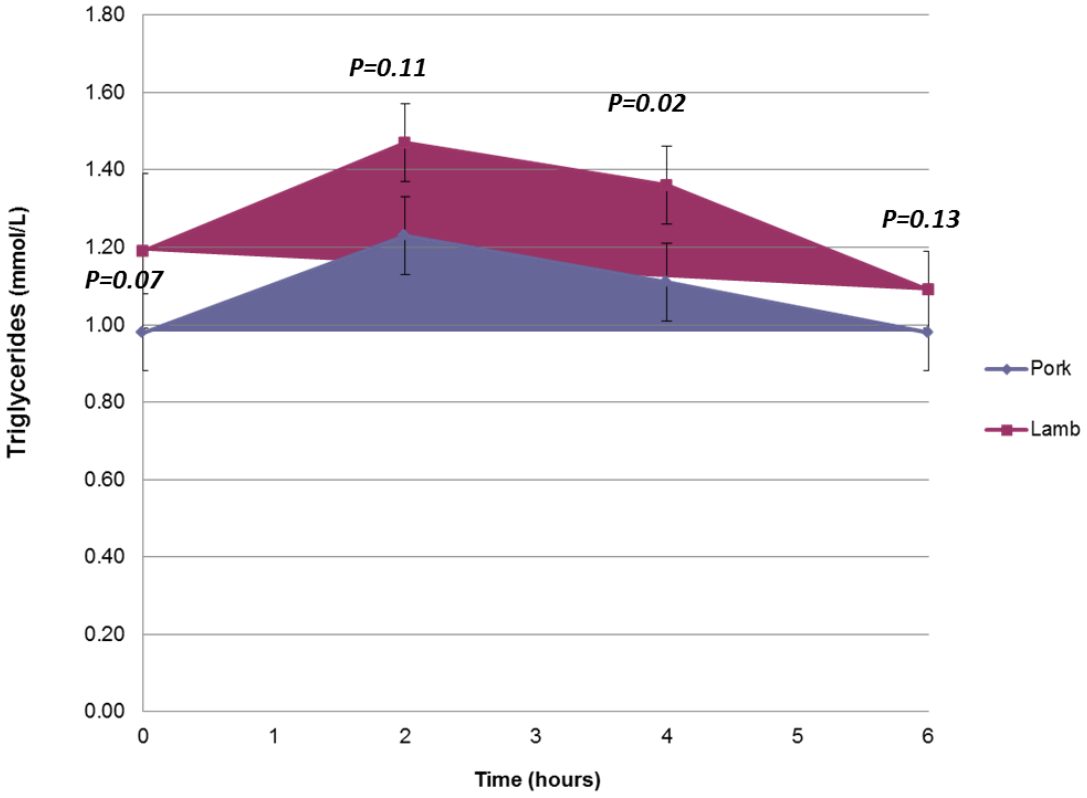
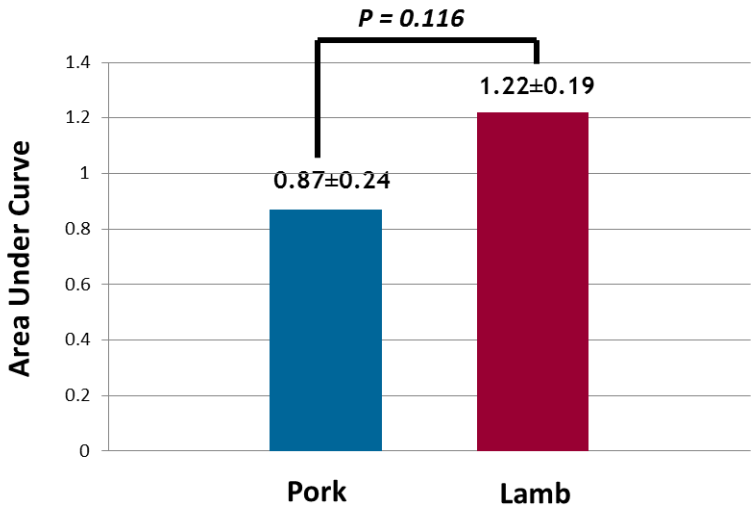


Figure 3 - The area under the curve (AUC) was calculated using the integrated change in plasma triglyceride levels calculated as the area within a trapezoid as indicated in figure 2. Values are mean±SEM.



4. Conclusion

This is the first study to investigate effects of a single meal containing fresh pork or lamb on the triglyceride kinetics in healthy human subjects. The shape and area of the change in postprandial plasma triglyceride curve (Lipemic Index) indicates that lipemia caused by pork consumption was similar to that of lamb. Although statistically insignificant, AUC (change in plasma triglyceride levels or relative lipemic index) for pork tended to be lower than that of lamb with an effect size suggestive of a significant difference with a larger sample size.

This report adds to the growing body of evidence for the healthiness of pork. Howe et al (Pork CRC 3A-111) recently demonstrated that regular consumption of lean pork was equally healthy as beef or chicken showing no difference between the pork, beef or chicken diet for body weight, body mass index (BMI) or any other index of adiposity. However, the project did not include collection and analysis of blood samples for biomarkers of cardio-metabolic health.

Caution must be exercised when interpreting lipemic index results. We purchased lamb and pork mince from Coles Supermarket in Newcastle. The label on the two products stated that the fat content is 17%, weight/weight (Coles Lamb Regular Mince, 17g fat/100 grams and Coles Pork Regular Mince, 17g fat/100 grams). Analysis in our laboratory found that the fat content of pork mince was close (16.8%) to that reported on the label while the fat content of lamb mince was much lower (11.9%) than stated on the label.

For proper comparison, the meats had to be matched on fat content per serve, therefore, the portion sizes varied (pork 140g/ serve, lamb 200g/ serve) to match ~24g of fat per serve. Consequently, although unlikely, differences in the protein and micronutrient content of the two meals may not have been accounted for in calculation of the lipemic index. Other factors such as the level of physical activity, amount of fluid intake, time to consume the meal were controlled in our clinical facilities.

A number of other factors have been shown to influence postprandial lipemia including genetic makeup, age, gender, menopausal status, smoking, alcohol consumption, blood pressure, diabetes, cardiovascular disease, obesity, hyperlipidemias, lipid-lowering drugs and food matrix (review see Garg et al 2014, unpublished). However, the cross-over design involving the same subjects in both arms of the study rules out any influence of these factors. Also since all the study participants had blood lipid levels in the normal range at baseline, hyperlipidemia was not a confounding factor in this study.

In conclusion, we provide proof of concept that the lipemic index of pork is at least comparable, possibly lower, than that of lamb. These encouraging observations require confirmation in a larger study population where the pork and lamb meat cuts or mince are matched not only for fat content but also for the amount of meat consumed. Further studies are also warranted to compare the lipemic index of pork with other comparator meats including chicken and beef as well as with processed meats.

5. References

Delgado-Lista, J., J. Lopez-Miranda, et al. (2008). "Chronic dietary fat intake modifies the postprandial response of hemostatic markers to a single fatty test meal." Am J Clin Nutr 87(2): 317-322.

Flynn, M. A., H. D. Naumann, et al. (1982). "Dietary "meats" and serum lipids." Am J Clin Nutr 35(5): 935-942.

Murphy, K. J., R. L. Thomson, et al. (2012). "Effects of eating fresh lean pork on cardiometabolic health parameters." Nutrients 4(7): 711-723.

Murphy, K. J., R. L. Thomson, et al. (2012 (under review)). "Effects of eating fresh lean pork on cardiometabolic health parameters." Journal of the American College of Nutrition.

Neaton, J. D., H. Blackburn, et al. (1992). "Serum cholesterol level and mortality findings for men screened in the Multiple Risk Factor Intervention Trial. Multiple Risk Factor Intervention Trial Research Group." Arch Intern Med 152(7): 1490-1500.

Paglialunga, S. and K. Cianflone (2007). "Regulation of postprandial lipemia: an update on current trends." Appl Physiol Nutr Metab 32(1): 61-75.

Poppitt, S. D. (2005). "Postprandial Lipaemia, Haemostasis, inflammatory response and other emerging risk factors for cardiovascular disease: the influence of fatty meals." Curr Nutr Food Sci 1: 23-34.

Rubio, J. A., M. A. Rubio, et al. (2006). "Effects of pork vs veal consumption on serum lipids in healthy subjects." Nutr Hosp 21(1): 75-83.

Wycherley, T. P., M. Noakes, et al. (2010). "A high-protein diet with resistance exercise training improves weight loss and body composition in overweight and obese patients with type 2 diabetes." Diabetes Care 33(5): 969-976.