Fatty acids in animals: thrombosis and hemostasis

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ABSTRACT  Long-chain fatty acids, including stearic acid, can cause thrombosis when they are injected into the systemic circulation of animals. The toxicity is decreased if the fatty acids are bound to albumin. To date, the effect of individual fatty acid feeding is not known. In general, feeding animals diets high in saturated fat followed by the injection of a thrombogenic stimulus is associated with a greater incidence of thrombosis than when a normal diet or a diet high in unsaturated or polyunsaturated fat is fed. A high dietary intake of long-chain polyunsaturated fatty acids of the n–3 family may prevent thrombosis in animals. In assessing the effects of dietary fatty acids, the ability of the organism, be it animal or human, to convert fatty acids to less thrombogenic fatty acids (stearic to oleic) or create an antithrombotic fatty acid (linolenic to eicosapentaenoic) is a major attribute. Further studies should consider the storage of fatty acids in tissues and their release into the blood and the potential impairment of albumin binding and fatty acid transport mechanisms.  Am J Clin Nutr 1997;65(suppl):1683S–6S.

KEY WORDS  Fatty acids, thrombosis, blood platelets, blood coagulation, animal study, n–3 fatty acid

ANIMAL STUDIES

Background
To assess the potential thrombogenic effects of individual long-chain fatty acids, it seems logical to approach the problem by injecting the individual fatty acids into the vascular system of experimental animals to determine whether thrombosis occurs and whether there is variation in the effects of the different fatty acids. Support for this approach is provided by the results of in vitro studies showing that certain fatty acids accelerate blood coagulation by activating factor XII (Hageman factor) and also cause the blood platelets to aggregate, a prelude to thrombus formation. Although this approach provides a measure of the potential thrombogenic effect, its relevance to the effect of individual fatty acids in the diet is more remote because of other influences related to the manner and mechanisms by which the animals and humans absorb, transport, convert, and metabolize the fatty acids once they are ingested. Another issue, always a consideration with studies in animals, is whether the species under study is a suitable model for humans and whether the results can be considered relevant to what would occur in humans.

Injections of individual fatty acids
Injections of unbound, long-chain, saturated fatty acids into the systemic circulation of dogs was followed by massive generalized thrombosis and sudden death (1). In contrast, the infusion of either long-chain unsaturated or short-chain saturated fatty acids did not kill any animal nor were there overt signs of toxicity (Table 1). The lethal effect was seen with fatty acids 14:0 or longer, including myristic (14:0) through behenic (22:0). Infusions of five different unsaturated fatty acids were given to 16 dogs. These included oleic acid (18:1) and erucic acid (22:1) and the polyunsaturated fatty acids linoleic acid (18:2n–6), linolenic acid (18:3n–3), and arachidonic acid (20:4n–6). None of these fatty acids was lethal.

Thrombus formation in the isolated jugular vein segment occurred in most dogs receiving long-chain fatty acids, whether saturated or unsaturated. Long-chain saturated fatty acids caused jugular vein thrombosis in 9 of 11 dogs. Short-chain fatty acids (12:0 or less) produced thrombi in only 5 of 13 dogs. The infusion of unsaturated fatty acids, in contrast with their lack of effect in causing systemic thrombosis, caused jugular thrombi in 14 of 16 dogs.

The infusion of stearic acid (18:0) or 22:0 caused a shortening of the whole-blood clotting time in silicone-coated tubes from 32 ± 11 to 17 ± 11 min (P < 0.001), but no significant changes occurred in the one-stage prothrombin time, two-stage prothrombin assay, assay for factors V and VII, or plasma fibrinogen concentration.

Injection of fatty acid–albumin into rabbits
When fatty acid–albumin solutions were infused intravenously into rabbits, none died but 8 of 15 given 18:0-albumin (1400–2100 μmol fatty acid/L) and 4 of 10 given 18:1-albumin (1960–2100 μmol fatty acid/L) developed thrombi in the lungs (Table 1). The whole-blood silicone clotting time decreased from 37.8 ± 3 to 23.2 ± 3 min (P < 0.01) in rabbits given 18:0 and from 38.0 ± 2.6 to 23.9 ± 2 min (P < 0.01) in rabbits given 18:1. None of 10 rabbits given a 5% albumin solution (200–400 μmol/L) developed lung thrombi and whole-blood silicone clotting times were not shortened (2).

Mobilization of fatty acids in rabbits
Endogenous fatty acids are released from tissue lipids by the action of lipases in response to various stimuli. The effect is to increase the fatty acid concentration in the blood, sometimes to very high concentrations. In humans, pigs, and dogs, this lipid mobilization results from the action of catecholamines whereas in rabbits it is seen in response to pituitary hormones such as

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corticotropin and in birds it occurs after the injection of glucagon.

In rabbits given corticotropin to mobilize endogenous lipid, the mean plasma fatty acid concentration increased to 1770 μmol/L at 2 h and remained elevated at 1269 μmol/L after 5 h. Toxic signs developed within 1 h and included an increase in respiratory rate followed by weakness, labored respirations, and decreased alertness. Four of 10 rabbits given corticotropin died during the experiment. The hearts and lungs from these rabbits contained thrombi and pulmonary edema and congestion were common findings at necropsy. Controls did not show any significant increase in plasma fatty acid concentration, toxic signs, or pathologic findings. Increases in the plasma fatty acid concentrations in the corticotropin group were associated with shortening of the whole-blood silicone clotting time from 41.4 ± 6.2 to 21.9 ± 6.1 min (P < 0.001). Controls did not have a decrease in the clotting time (11).

In additional studies, Connor and Hoak (unpublished observations, 1964) gave corticotropin to rabbits that had been fed diets supplemented with cocoa butter to increase the content of 18:0 in their adipose tissue. Contrary to expectation, these rabbits did not show any increased thrombogenic tendency when given corticotropin. Analysis of the adipose tissue suggested that the rabbits fed the diet supplemented with cocoa butter converted much of the 18:0 to 18:1.

### DIETARY STUDIES IN ANIMALS

#### Background

Results in the literature provide little information about studies of the effects of individual fatty acids in the diet on thrombosis. Several studies suggest that diets high in saturated fats increased the thrombogenic effect of agents known to produce thrombosis. In contrast, unsaturated fats either caused little or no effect and in some instances appeared to diminish the thrombotic tendency. It is difficult to separate the effect of decreasing a thrombogenic influence by removing long-chain saturated fatty acids from the diet, if they do exert such an effect in vivo, from a possible antithrombotic effect of certain long-chain unsaturated acids that are added to the diet. In animals and humans 18:0 can be converted to 18:1. Consequently, 18:0 feeding might not exhibit thrombogenic effects if its conversion to 18:1 is sufficient to maintain 18:0 concentrations below thrombogenic concentrations, if there is such a concentration in vivo.

Numerous reports indicate that dietary n−3 fatty acids interrupt vascular thrombus formation and the development of vascular lesions. The fatty acids considered responsible for this action are eicosapentaenoic (20:5n−3) and docosahexaenoic (22:6n−3), both of which are found in fish oil. Animals and humans can convert 18:3n−3 to 20:5n−3. Therefore, the thrombosis-reducing effect of a diet rich in 18:3n−3 might represent the influence of the formation of 20:5n−3 in larger amounts.

Obviously, the effects of dietary fatty acids are complex. The studies in animals to date do not allow a delineation that separates individual fatty acids according to a thrombogenic index nor do they define dietary composition that would either cause or prevent thrombosis.

#### Results

In 1959, Thomas and Hartoft (3) produced thrombosis and myocardial infarction without atherosclerosis in rats by feeding a diet rich in butter and cholesterol and giving cholic acid and thiouracil. Gresham and Howard (4) repeated these studies with similar results and produced atherosclerosis without thrombosis or myocardial infarction by replacing the butter in the diet with arachis oil. Arachis oil contained 40−60% 18:1, 20−35% 18:2n−6, 5% 18:0, and 15% 16:0 (palmitic acid). The butter diet contained 20% 16:0, 10% 18:0, 30% 18:1, 5% 18:2n−6, and 30% short-chain fatty acids.

Numerous studies in rats fed diets rich in butter or 18:0 showed hepatic vein thrombi when the rats were given Salmonella typhosa toxin. Rats given corn oil−enriched diets did not develop these lesions. Thrombin-induced platelet aggregation...
was enhanced in samples from the rats fed the 18:0-rich diets (5, 6).

Feeding rats for 20 wk a diet high in 16:0 and 18:0 (provided by hydrogenated palm oil) caused thrombosis, a shortening of the plasma clotting time, and an increased platelet aggregation to thrombin. This was not seen in rats fed a diet rich in unsaturated fatty acids (7). Nordoy (8), using a rat model in which formalin was applied to the jugular vein to induce thrombosis, found that rats fed a saturated fat diet (hydrogenated coconut oil) had a 66% incidence of thrombosis, controls had a 44% incidence, and rats given linseed oil in addition to the saturated fat diet had only a 27% incidence. When thrombosis was induced in rats given intravenous injections of ADP, 90% of those fed a 40%–saturated fat diet for 6 wk, 50% of those fed a 10%–saturated fat diet, and 47% of those fed a 32%–saturated fat diet supplemented with linseed oil developed thrombi (9).

Hornstra (10), using an aortic loop in rats, measured the obstruction time as a function of the type of fatty acids in the diet. Dietary saturated fatty acids produced the shortest obstruction times, polyunsaturated fatty acids (both n–3 and n–6) produced the longest, and monounsaturated fatty acids appeared to have no effect. The individual contributions of the major dietary saturated fatty acids (14:0, 16:0, and 18:0) to thrombogenesis was difficult to determine from the statistical correlations because the diets contained complex mixtures of these fatty acids (10). In 1963 Mustard et al (12) studied the deposit of thrombus material in extracorporeal shunts in pigs fed diets supplemented with lard and egg yolk. The highest deposit was in the egg yolk group. There were no changes in silicone clotting times or in plasma fatty acid concentrations.

n–3 FATTY ACIDS AND THROMBOSIS

Background

Epidemiologic, clinical, experimental, and laboratory studies have suggested that a high dietary intake of long-chain polyunsaturated fatty acids of the n–3 family from seafood may prevent thrombosis and myocardial infarction. The effects of these fatty acids, 20:5n–3 and 22:6n–3, have been associated with both atherosclerosis and thrombosis. In nonhuman primates a high intake of these fatty acids can eliminate vascular thrombus formation and vascular lesion formation after mechanical vascular injury. Suggested mechanisms for this effect include impaired platelet function by interference with thromboxane formation, reduction in serum triacylglycerols, altered cell membrane composition that influences cellular responses, and decreased blood viscosity.

Results

In a study with nonhuman primates, a diet containing 40% of energy from fat was given over a period of 30 wk (13). The control diet contained olive oil and the intervention diet contained a similar amount of 85% ethyl ester concentrate of n–3 fatty acids, mainly 20:5n–3 and 22:6n–3. The bleeding time in the n–3 group was increased from 4.3 to 7.6 min, and platelet deposition onto thrombogenic synthetic polyester grafts in femoral arteriovenous shunts was reduced, a process that was resistant to the effects of both aspirin and heparin. A similar protective effect was observed at sites of surgical carotid endarterectomy. In crossover experiments, endarterectomy of aortic segments from n–3 donors showed little tendency to induce thrombus formation when tested in the arteriovenous shunts of control recipient animals. In contrast, aortic segments from control animals accumulated platelets when placed in the arteriovenous shunts of n–3-treated animals.

Lam et al (14) fed normolipemic pigs regular feed or feed supplemented with cod liver oil (1 mL · kg⁻¹ · d⁻¹) for 4 wk. Deep carotid injury was caused by balloon angioplasty. Platelet deposition and injury-related vasoconstriction were both significantly reduced in the cod liver oil group. In perfusion studies, arterial blood from pigs treated with cod liver oil resulted in less platelet deposition to normal aortic media than did blood from control pigs. Kim et al (15) studied the effect of fish-oil additives to a butter-cholesterol hyperlipidemic diet in pigs. Average plasma cholesterol concentrations were similar in the two groups. The fish-oil diets included 30 mL cod liver oil or menhaden oil/d for 4 mo. The studies included counts of adherent monocytes and microthrombi over lesions directly attached to the endothelium. Both the numbers of microthrombi and attached monocytes were greatly reduced in the fish-oil group, but there was no retardation of the growth of the lesions. n–3 Fatty acids have also been shown in animal models to increase the endothelium-dependent relaxation of preconstricted coronary arteries in response to several agonists (16).

COMMENTS

It is apparent that long-chain fatty acids, including 18:0, can cause thrombosis when they are unbound. An important question relates to whether fatty acid transport might become deregulated either as a result of excessively high plasma fatty acid concentrations from lipid mobilization as seen with stress or when the plasma albumin is extremely low as a result of proteinuria or impaired protein synthesis. Under normal conditions, most plasma fatty acids are bound to albumin. However, as the fatty acid–albumin molar ratio exceeds 2, an increasing amount of fatty acid will become associated with other plasma proteins and cells. Coagulation proteins, platelets, and proteins of the vessel wall become candidates for these associations.

In feeding studies in animals, dietary fatty acids can have a demonstrable effect on platelet function and saturated fatty acids can cause a predisposition to a prothrombotic state. It has not been possible to separate the influence of individual saturated fatty acids in assessing this effect. It is difficult to separate the influence of removal of saturated fatty acids from the diet apart from the effect of increasing the amount of polyunsaturated fatty acids, particularly n–3 and n–6, in the diet.

The feeding studies in animals that have been reported have made important suggestions that long-chain saturated fatty acids may predispose to thrombosis, particularly in the presence of a known thrombogenic stimulus. In many instances the concentration of saturated fats was extremely high and displaced other normal constituents of the diet. Unfortunately for the purposes of this paper, none of the studies used individual fatty acids.

In assessing the effects of dietary fatty acids, the ability of the organism, be it animal or human, to convert fatty acids to...
less thrombogenic fatty acids (18:0 to 18:1) or create an antithrombotic fatty acid (18:3n−3 to 20:5n−3) is a major attribute. Worthy of consideration for further study are questions related to the storage of fatty acids in the tissues, mechanisms for release into the blood, and potential impairment of binding by albumin. Is there a differential or prioritized release of fatty acid from adipose tissue in response to stress? Is it related to chain length or degree of unsaturation? Is it influenced by diet? Can monounsaturated or polyunsaturated fatty acids displace saturated fatty acids from albumin and cause them to be more available to platelets and coagulation factors? These questions have implications that go much beyond the concern about what is good or bad to eat. They relate to fundamental concepts of lipid metabolism and transport and may have particular relevance in disease states, especially in individuals with genetic or acquired abnormalities in which there is a defect that could culminate in the development of thrombosis.

REFERENCES