



Atherosclerosis

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Diet- Induced Atherosclerosis/Hypercholesterolemia in Rodent Models

Brief Scientific Literature Review- October 2008

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Atherosclerosis is a complex chronic disease characterized by the accumulation of lipids within arterial walls that eventually go on to form plaques, which can cause narrowing, hardening, and/or complete blockage of arteries. One well known risk factor in humans is hypercholesterolemia (i.e. elevated total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C)) (1), and other important contributors to this disease include inflammation, oxidative stress, and insulin resistance (2, 3). Foods high in dietary saturated fat (SF) and cholesterol (i.e. “Western-type diets”) have been linked to elevations in circulating cholesterol levels (in particular, LDL-C) (4), prompting the recommendation that humans limit the intake of these dietary constituents (1). Like humans, Western-type diets can induce elevated LDL-C and atherosclerosis in certain rodent models (i.e. mice, hamsters, guinea pigs). Therefore, the use of such diets for promoting atherosclerosis in these models has been a valuable tool for both gaining more understanding of this disease and testing therapies that can potentially reverse it.

In the past, Western-type diets have been made by adding high levels of fat and cholesterol to grain-based chow diets. The addition of ingredients to chows can dilute other nutrients (i.e. vitamins, minerals, protein, etc) and non-nutrients (i.e. phytoestrogens), so their use in atherosclerosis research has been criticized (5). In order to avoid this issue, purified ingredient diet formulas which are open to the public (i.e. AIN-76A) can be easily revised to intentionally alter the atherosclerosis phenotype by adding in fat calories (i.e. dairy butter, hydrogenated coconut oil) in place of purified carbohydrate calories only (i.e. sucrose, corn starch). This method of modification maintains nutrient to calorie ratios, which is important as animals typically eat for calories, not weight of food. Furthermore, casein-based purified diets contain no phytoestrogens (or other phytochemicals) unlike typical grain-based chows which can have highly variable levels (6). Since the presence of phytoestrogen containing sources (i.e. soy protein and isolated isoflavones) has been found to influence atherosclerosis and lipoprotein metabolism in various rodent models (7-13), the use of purified Western-type diets provides a clean ‘reagent’ for inducing this disease. That being said, not all rodent models respond the same to a given Western-type purified diet due to genetic differences. This review will highlight commonly used dietary factors able to influence LDL-C and atherosclerosis in various rodent models, as well as some of the potential benefits and drawbacks associated with using these models for disease induction.

Wild Type Mice and Rats

Wild type mice, such as the C57BL/6 mouse, are relatively resistant to atherosclerosis, but have the capacity to develop mild atherosclerosis under certain conditions. Unlike humans, they typically have a very low proportion of TC as LDL-C, and most cholesterol in circulation

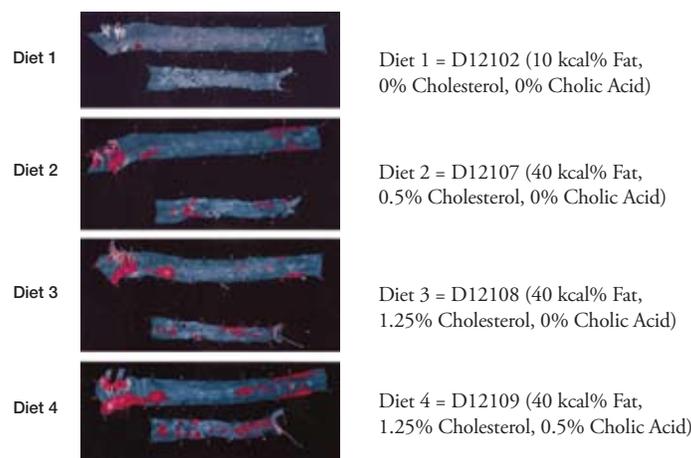


FIGURE 1. Oil red O-stained atherosclerotic lesions in aortas of LDLR2/2 mice fed diets varying in fat, cholesterol, and cholate content. Mice were killed after being fed defined diets for 12 weeks. Aortas were prepared and stained with oil red O as described in Methods. One representative aorta from a total of 6 in each of the 4 dietary groups is shown. Graphic representation - for details see reference (38).

is found in high density lipoproteins (HDL-C), which contributes to their reduced susceptibility to atherosclerosis. Western-type diets containing moderately high levels of SF (~35 kcal% fat,), as (cocoa butter, palm oil, or dairy butter), cholesterol (~0.5 to 1% w/w) and cholic acid (~0.1% to 0.5% w/w), are capable of inducing elevations in TC and LDL-C and mild atherosclerosis (i.e. cholesterol laden foam cells, fatty deposits or streaks) in some mouse strains after 12 weeks (14-16). The presence of cholic acid aids cholesterol and fat absorption, and also suppresses conversion of cholesterol to bile acids (17). This latter effect reduces removal of cholesterol and increases cholesterol levels (in particular, non-HDL-C) which allows atherosclerosis induction to occur in susceptible mouse strains. While it is possible to induce atherosclerosis in some mice, it is important to realize that the presence of cholic acid can influence transcription factors controlling genes involved with regulating lipoprotein metabolism and inflammation, both of which are important in the development of atherosclerosis (16, 17), and can also promote gallstones in certain mouse strains (18, 19).

Like mice, commonly used outbred rat strains (i.e. Sprague-Dawley, Wistar) typically have high levels of HDL-C and low levels of LDL-C. Western-type diets (~45 kcal% fat as hydrogenated coconut oil) or even low-fat diets (~12 kcal% fat as corn oil) with high levels of cholesterol (~1% w/w) and cholic acid (0.25% - 0.5% w/w) are capable of promoting elevations in TC and LDL-C in Sprague-Dawley or Wistar rats (20-22) likely by reducing bile acid production (23). Despite such elevations, this alone will not promote atherosclerosis in rats unless a thyroid hormone inhibitor (2-thiouracil, ~0.5% w/w) is added to the diet (24). However, rats that have been selectively bred with spontaneous gene mutations such as the JCR:LA Corpulent rat can develop hypercholesterolemia and are sensitive to dietary cholesterol without the need for cholic acid or thyroid hormone inhibitors (25).

Hamsters

Like mice and rats, hamsters typically have a high percentage of HDL-C. However, Western-type diets high in cholesterol (~0.5% w/w) and SF (15 – 20% butter fat w/w) can elevate plasma TC and LDL-C and promote appreciable atherosclerosis (i.e. fatty streaks, foam cells) without the use of cholic acid in as little as 6 weeks (26). Hamsters don't require cholic acid because dietary cholesterol (even as high as 1% w/w) has little influence on their bile acid synthesis pathways (20), allowing excess dietary cholesterol to enter the blood circulation rather than being converted to bile acids and excreted in the feces. Furthermore, these animals possess cholesteryl ester transfer protein (CETP) like humans (but not mice and rats), which allows the transfer of cholesterol from HDL to LDL particles in plasma. Certain SFs (relative to unsaturated fats) and cholesterol both have some ability to promote this pathway (27), and this, in combination with their ability to reduce LDL-C uptake by the liver (28) allows for significant elevations in LDL-C and eventual atherosclerosis induction. These animals are highly sensitive to particular saturated fatty acids as a Western-type diet high in potent cholesterol-raising SF from hydrogenated coconut oil (lauric and myristic acids) can cause even more aortic cholesterol accumulation than a high cholesterol (0.15%) diet with a more cholesterol neutral fat such as cocoa butter (palmitic, oleic and stearic acids) (29). Additionally, dietary protein type can also influence LDL-C levels and atherosclerosis as hamsters fed casein and lactalbumin had higher levels than those fed an equal amount of soy protein, and like humans, males may be more susceptible than females (11).

Guinea Pigs

Unlike other rodents, guinea pigs have a cholesterol profile similar to most humans (higher proportion TC as LDL-C) when maintained on a low fat/low cholesterol diet. Like hamsters, they possess CETP (30) and do not require cholic acid for atherosclerosis induction and cholesterol elevation (31, 32). They are highly sensitive to changes in dietary fatty acid composition as Western-type diets high in SF (i.e. palm kernel oil, ~80% SF) without dietary cholesterol can elevate TC and LDL-C levels relative to those containing less SF (i.e. palm oil, beef tallow, ~50% SF) (31). The type of protein (i.e. high casein to soy protein ratio) (32), or carbohydrate (i.e. high sucrose) (33) can exacerbate the condition. The use of a Western-type diet with added cholesterol (at least up to 0.3%, w/w) can cause further elevations in TC and LDL-C and induce atherosclerotic lesions (i.e. fatty streaks) after 12 weeks (34-36). The carbohydrate to fat ratio also has been found to have some importance in promoting atherosclerosis as high cholesterol diets (0.25%, w/w) that are high in carbohydrate (42 kcal%) and moderately high in fat (35 kcal%) are more capable of promoting atherosclerosis than those low in carbohydrate (11 kcal%) and high in fat (55 kcal%) (37).

Genetically Modified Mouse Models

More recently, mouse models have been developed with genetic mutations causing them to be more atherosclerosis prone, promoting atherosclerotic plaques similar to those found in humans. Some well known examples of genetically modified mice used in atherosclerosis research include the LDL receptor (LDLr) null and apolipoprotein E (apoE) null mouse which have mutations that hinder removal of

circulating cholesterol by the liver. The use of Western-type diets with added cholesterol (0.15% - 1.25% w/w) which are cholic acid free can induce significant atherosclerotic lesions after 12 weeks in LDLr null mice (38-40) (FIG. 1, 38). Lesion development is very dramatic in apoE null mice fed a Western-type diet and beginning stages of atherosclerosis (i.e. fatty streak lesions) can be found at 6 weeks (41). With these mouse models, the main influence on atherosclerosis has been found to be dietary cholesterol rather than the level of fat (42-44), but certain threshold levels of dietary cholesterol may exist at least within the context of a low-fat purified diet (45). While very high fat diets (i.e. 60 kcal% fat) are capable of inducing some atherosclerosis, the addition of cholesterol to such diets promotes more atherosclerosis in LDLr null mice (46). Furthermore, the fatty acid profile and even the carbohydrate form (i.e. fructose, sucrose) can be manipulated to modify the atherosclerosis phenotype to the researcher's advantage (39, 42, 47).

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