Invited Commentary

Dietary fatty acids, cholesterol, and the lipoprotein profile

When evaluating clinical studies to establish a 'single truth' about saturated, monounsaturated, or polyunsaturated fat on the one hand, or dietary cholesterol on the other, how does one account for the relative importance of individual fatty acids or dietary cholesterol (Hayes, 1995; Khosla & Sundram, 1996). The statement that: 'saturated fatty acids (SFA) raise cholesterol' ignores the fact that 12:0+14:0-rich fats are more potent than a 16:0-rich or 18:0-rich fat in this regard. Further, the idea that 'polyunsaturates lower cholesterol' does not reveal that they do so effectively only in individuals or populations with limited available polyunsaturated fat, and that polyunsaturated fat effectiveness is substantially diminished above a certain 'threshold' intake.

In the same vein, several clinical studies suggest that dietary cholesterol minimally impacts human lipoproteins. Yet that conclusion belies the fact that chronic intake of even a low level of cholesterol probably exerts a major 'priming' influence on whether or not a 16:0-rich fat raises LDL, and whether more 18:2 is thus required to achieve total plasma cholesterol (TC) lowering. One needs only to consider the potent LDL lowering afforded by phytosterols blocking cholesterol absorption to appreciate the importance of dietary cholesterol (Law, 2000). Like the response to 18:2, the TC (and LDL) response to dietary cholesterol is also apparently non-linear, a plateau in TC developing between 400–600 mg cholesterol intake per day (Hopkins, 1992). These conclusions are derived from experiments in carefully selected animal models, i.e. hamsters, gerbils, and monkeys, coupled with similarly designed studies in human subjects (Hayes *et al.* 1995).

Meta-analysis of human clinical trials begs the question of the individual host response, including the fine points of regulatory physiology, so it is still important to conduct animal experiments where individual fatty acid and cholesterol interactions might be evaluated in situations that reflect the human experience. When modelling these relationships, remember that hamsters and gerbils have roughly ten times the metabolic rate of man and thus consume about ten times as much energy per kg body weight. This means that for a typical human cholesterol intake, e.g. 36 mg/MJ, the hamster would eat and process ten times the human equivalent per kg body weight. Since the hamster is also more sensitive to dietary cholesterol, intakes of 5–24 mg/MJ (0·1–0·5) g/kg diet) would seem an appropriate range for modelling these relationships in such species if we are to decipher where the fatty acid component ends and cholesterol intake becomes an overbearing distortion.

Which saturated fatty acids are key?

Despite the knowledge that saturated fats raise TC and polyunsaturated fats lower it (Grundy & Denke, 1990), controversy persists concerning specific dietary fatty acids, and more importantly, their underlying mechanism of action on LDL and HDL dynamics. Dietary fatty acid modulation of lipoproteins is important because the LDL:HDL ratio affects atherogenesis. It is conceivable that a modestly improved balance in dietary fats (fatty acids) would greatly improve the circulating lipoprotein profile. Historically, we were taught that saturated fats containing 12:0, 14:0, 16:0 (lauric, myristic and palmitic acid respectively) raised the plasma cholesterol and LDL, whereas those containing less than 12:0, as well as 18:0 (stearic acid), had minimal effect. Furthermore, monounsaturated fats rich in oleic acid (18:1) had no effect on TC when exchanged for carbohydrate, but exerted a cholesterol-lowering effect (both TC and LDL) when exchanged for SFA. Similarly the major polyunsaturated fatty acid, linoleic acid (18:2), was found to be cholesterol-lowering (mainly LDL) when exchanged for dietary saturated fatty acids or, in the case of hypercholesterolaemia, when simply added to the diet without removing other fats (Bronte-Stewart *et al.* 1956). However, linoleic acid (18:2) also lowers HDL at high intakes (>20 % energy).

18:2 and the lipoprotein profile

The largest fluctuations in TC and LDL-cholesterol result from major changes in SFA consumption. However, dietary 18:2 is arguably the most influential fatty acid because the absolute mass of available 18:2 (that consumed plus that stored in adipose tissue) dictates whether the impact of a specific SFA will be apparent (Hayes, 1995). When 18:2 intake is sufficiently large (e.g. >12 % of energy or 25–30 g/d), it is extremely difficult to demonstrate a SFA or dietary cholesterol effect. When 18:2 represent <3 % of energy, however, the effect of certain SFA or dietary cholesterol can be observed readily and even exaggerated. Most populations in the world consume 3–6 % of energy as 18:2.

The importance of 18:2 is conceptualized by the '18:2 threshold' which implies that a certain intake of 18:2 is needed to adequately metabolize other fatty acids and dietary cholesterol (Hayes, 1995). With progressive 18:2 depletion 'below one's threshold', TC and LDL tend to rise in a non-linear fashion when stressed by dietary SFA and cholesterol, whereas 'above threshold' most fatty acids tend to exert a minimal, often comparable, effect on TC and LDL. The curvilinear, logarithmic response to 18:2, and the '18:2 threshold' concept, have greatly altered our perception of plasma cholesterol modulation by dietary fatty

acids. For example, the effect of 18:2-deprivation can be seen as distinct from the SFA elevation of cholesterol, at least in a diet-sensitive gerbil model, where decreasing 18:2 intake (below threshold, approaching essential fatty acid depletion) raised plasma cholesterol independent of TC elevation by certain SFA (Hajri *et al.* 1998).

The 'conditionally' cholesterolaemic aspect of palmitic acid

Differences in LDL production and LDL receptor activity (clearance) presumably dictate how dietary fatty acids affect LDL pool size and lipoprotein metabolism (Hayes *et al.* 1997; Hajri *et al.* 1998). For example, in both monkeys and gerbils the SFA increase in LDL was primarily attributable to overproduction of LDL, not to LDL receptor down regulation, which mainly reflects cholesterol feeding and its accumulation in the liver (Spady *et al.* 1993). In normolipaemic animals and human subjects (TC <2 g/l) fed cholesterol-free diets, only 14:0-rich natural triacylglycerols consistently elevate TC (Hayes & Khosla, 1992; Pronczuk *et al.* 1994). In polygenic hypercholesterolaemic individuals (i.e. >2·25–2·50 g/l), LDL receptor activity is presumably reduced (Vega & Grundy, 1987) and adding more dietary cholesterol (>400 mg/d) in the presence of a fat with a low polyunsaturated:saturated ratio (saturated fat) apparently keeps this activity depressed, as discussed elsewhere (Hayes & Khosla, 1992). Accordingly, a 16:0-rich fat as a prime producer of VLDL seems to add to the LDL pool in human subjects when LDL receptors are depressed. Added 18:2 reduces elevated TC apparently by depressing hepatic triacylglycerol and VLDL secretion and LDL production while alleviating depressed LDL receptor activity to enhance clearance (Hayes & Khosla 1992; Spady *et al.* 1993). Whether SFA or monounsaturated fatty acid exert an opposite effect on LDL receptor activity independent of the 18:2 threshold and diet cholesterol in man is not clear. Another consideration in this discussion is the source of saturated fat, i.e. whether the structure of the saturated fat triacylglycerol is synthetic or natural may be important. For example, piglets fed palm oil with 16:0 in the *sn*-1,3 positions had lower plasma cholesterol values than piglets fed an equivalent mass of 16:0 in the *sn*-2 position from a synthetic lard (Innis *et al.* 1993).

Hamster and gerbil models

In this issue of the *British Journal of Nutrition*, Billett *et al.* (2000) model the interaction between individual SFA and dietary cholesterol in hamsters by feeding pure monoacyl triacylglycerols of tri-14:0, tri-16:0, and tri-18:0 at three cholesterol intakes (0, 1·2, or 2·4 g/kg diet). As LDL receptors are essentially down regulated in hamsters at intakes of 0·4 g/kg, these cholesterol intakes would be somewhat extreme, as alluded to earlier. In addition, food intake was about half the norm for hamsters this size, and all were losing weight slightly, suggesting that low 18:2 intake or the structured triacylglycerols were problematic. As the authors note, malabsorption of tristearin increased food and cholesterol consumption by 20 % in that group. Thus, the equivalency of cholesterol intakes and steady state dynamics needed for comparative lipoprotein evaluation were not optimal. Under these circumstances observations relating diet cholesterol interaction with specific saturated fatty acids were inconsistent and difficult to interpret with confidence. For example, the 14:0 × cholesterol interaction induced the greatest TC elevation at 1·2 g cholesterol/kg, but 16:0 × cholesterol and 18:0 × cholesterol interactions proved equally cholesterol-elevating at 2·4 g cholesterol/kg, and both surpassed that attributed to 14:0 × cholesterol.

By comparison, Fig. 1 captures the unique 16:0 × cholesterol interaction in gerbils fed more modest cholesterol intakes (0·2, 0·4 or 0·8 g/kg diet) mixed into natural fat blends (Pronczuk *et al.* 1994). The fats provided natural triacylglycerol structure while emphasizing specific fatty acid exchanges in purified diets. Gerbils were studied because they respond more to fatty acids and less to dietary cholesterol than hamsters. The figure depicts the change in TC (relative to the pure fatty acid effect without added cholesterol) induced by increments of dietary cholesterol representing about 50 100, and 200 mg/d human equivalents. Palmitic acid (16:0) elicited the most striking rise in

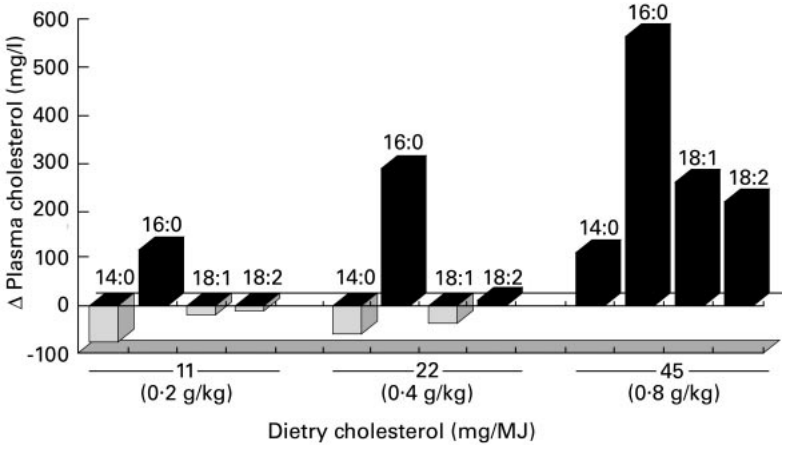

Fig. 1. Relationship between type of dietary fat and cholesterol intake and change in plasma cholesterol in gerbils (after Pronczuk *et al.* (1994)).

TC when cholesterol was added, but this uniqueness was dissipated by 0·8 g cholesterol/kg, when LDL receptors would be severely down regulated and the cholesterol effect dominant.

In a second study (Hayes *et al.* 1998), however, hamsters were fed several natural, cholesterol-free fats including medium-chain triacylglycerol (8:0+10:0), cholesterol-stripped butter (14:0+16:0+18:0), palm stearin (16:0+18:0+18:1), or olive oil (18:1), each adjusted with safflower oil to provide 4–5 % of energy from 18:2. These four diets were compared with safflower (high 18:2) or a blend of palm stearin with fish oil (16:0+18:1+*n*-3 highly-unsaturated fatty acids), where only 2·5 % of energy as 18:2 was present along with 4 % of energy as *n*-3 highly-unsaturated fatty acids in a 16:0+18:1-rich fat. Dietary cholesterol was not present. Under these circumstances the 14:0-18:0 SFA from these natural fats had only modest effects on plasma lipids, lipoprotein kinetics, and hepatic mRNA abundance for apolipoprotein B, A1 and E, hydroxymethylglutaryl-CoA reductase, and the LDL receptor. By contrast, a striking rise in TC was accompanied by a decrease in HDL with appropriate changes in related variables when *n*-3 highly-unsaturated fatty acids replaced much of the 18:2 in the presence of palm stearin. Again, the combination of low 18:2 plus high 16:0 was most impressive.

In summary, these reports collectively demonstrate that nutritional modelling of the diet fatty acid × cholesterol interaction on lipoprotein metabolism is greatly influenced by the animal model and nutritional paradigm (fatty acid balance and cholesterol burden) selected as the stressor(s).

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