MINIREVIEW

Nutritional Significance and Metabolism of Very Long Chain Fatty Alcohols and Acids from Dietary Waxes

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Very long chain fatty alcohols obtained from plant waxes and beeswax have been reported to lower plasma cholesterol in humans. This review discusses nutritional or regulatory effects produced by wax esters or aliphatic acids and alcohols found in unrefined cereal grains, beeswax, and many plant-derived foods. Reports suggest that 5–20 mg per day of mixed C24–C34 alcohols, including octacosanol and triacontanol, lower low-density lipoprotein (LDL) cholesterol by 21%–29% and raise high-density lipoprotein cholesterol by 8%–15%. Wax esters are hydrolyzed by a bile salt–dependent pancreatic carboxyl esterase, releasing long chain alcohols and fatty acids that are absorbed in the gastrointestinal tract. Studies of fatty alcohol metabolism in fibroblasts suggest that very long chain fatty alcohols, fatty aldehydes, and fatty acids are reversibly interconverted in a fatty alcohol cycle. The metabolism of these compounds is impaired in several inherited human peroxisomal disorders, including adrenoleukodystrophy and Sjögren-Larsson syndrome. Reports on dietary management of these diseases confirm that very long chain fatty acids (VLCFA) are normal constituents of the human diet and are synthesized endogenously. Concentrations of VLCFA in blood plasma increase during fasting and when children are placed on ketogenic diets to suppress seizures. Existing data support the hypothesis that VLCFA exert regulatory roles in cholesterol metabolism in the peroxisome and also alter LDL uptake and metabolism. Exp Biol Med 229:215–226, 2004

Key words: very long chain fatty acids; policosanol; octacosanol; wax esters; cholesterol

The alternative health care market has formulated a variety of dietary supplements that purportedly lower cholesterol. Among these formulations is a product called policosanol, which is a mixture of very long chain fatty alcohols and other constituents that is usually isolated from sugarcane wax, spinach wax, or beeswax by solvent extraction and saponification. The composition of policosanol is about 90% aliphatic alcohols (1) but varies depending on the source of material and the method of preparation. Although the C28 primary alcohol, octacosanol, is the most abundant constituent, the starting material also contains very long chain aliphatic alkanes, fatty aldehydes, and fatty acids that typically range from 24–34 carbons in length. Very long chain fatty acids (VLCFA) inhibit cholesterol synthesis in cell culture (2), and present data do not indicate whether the cholesterol-lowering effect of policosanol requires conversion of fatty alcohols to fatty acids. Because evidence suggests that very long chain fatty alcohols, aldehydes, and acids are interconverted in a metabolic cycle (3, 4), the abbreviation VLCFA3 will be used in referring to these constituents.

The presence of waxes in the diet and increased worldwide consumption of policosanol provide impetus to learn more about uptake, metabolism, and mechanism of action of very long chain fatty alcohols and acids. Although human diets have contained waxes for millennia, possible metabolic effects in humans are not well understood. For example, the use of policosanol to lower cholesterol is based on a limited number of clinical trials, and no metabolic studies yet explain why wax constituents might produce this effect. Therefore, this article describes the distribution of waxes in common foods, wax digestion and absorption, and the metabolism of very long chain fatty alcohols and fatty acids in mammals. Among the questions raised is whether wax esters represent naturally occurring cholesterol-lowering agents that may explain why cholesterol tends to be elevated in people who consume prepared foods and lower in people whose diets are primarily vegetarian.

This work was supported by grants from the U.S. Agricultural Experiment Stations, Hatch project GEO00922, U.S. Energy Partners, LLC, and the College of Family and Consumer Sciences, University of Georgia.

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Received July 15, 2003.
Accepted October 17, 2003.

1535-3702/04/2293-0001$15.00

Early Studies Concerning Wax Esters and Octacosanol

Possible nutritional effects of wax esters were suggested by Cureton in studies on wheat germ oil and athletic performance (5). The subjects were men, women, and children who volunteered to participate in various regimens of physical training while consuming either wheat germ oil, octacosanol prepared from wheat germ oil, or a placebo. The studies suggested that consumption of several grams per day of wheat germ oil or equivalent amounts of octacosanol improved endurance, resistance to physical stress, and recovery after training. More recently, it has been reported that octacosanol and policosanol improve reaction time, which possibly indicates a neurological effect (6).

Wheat germ oil contains about 5% wax esters, which precipitate if the oil is chilled (winterized). A chapter in Cureton's book indicated that wheat germ oil or octacosanol lowered the concentration of cholesterol in the liver of rabbits fed 1% cholesterol in the diet (7). The chapter concluded, "Results indicate an active principle, probably a combination of polyunsaturated fatty acids and octacosanol, that aid in the degradation and elimination of excess cholesterol" (p. 299). An earlier abstract stated that wheat germ oil lessened the increase in liver cholesterol caused by cholesterol feeding in rabbits (8) and that the effect was not attributable to vitamin E. Therefore, Levin and Collins (9) obtained a U.S. patent for the production of very long chain alcohols including octacosanol from wheat germ oil. However, the supporting studies were not conducted according to a double-blind, placebo-controlled protocol, and the primary work was not published in peer-reviewed journals. In view of the limitations to the design of the early studies, a recent review of the use of nutritional supplements by athletes concluded that evidence for a beneficial effect of octacosanol on human performance is weak (10). Nevertheless, the flaws in study design do not necessarily mean the outcomes were false. Better-designed studies concerning effects of octacosanol on neuromuscular function or human performance are needed.

Effects of Policosanol on Blood Lipid Profiles. Independent confirmation of an effect of octacosanol on cholesterol was provided when sugarcane rinds or wax extracted from sugarcane was found to lower cholesterol in rats (11, 12). The effect was noted when wax was added to the diet at a level of 0.5 g/100 g, and purification of the active principles led to the identification of long chain alcohols as cholesterol-lowering agents (13). The ability of policosanol to lower cholesterol or cholesterol synthesis has now been demonstrated in preclinical studies in rats (14, 15), rabbits (16, 17), dogs (18), and monkeys (19). However, a cholesterol-lowering effect of feeding policosanol was not observed in hamsters (20).

Policosanol has been reported to decrease total cholesterol and low-density lipoprotein (LDL) cholesterol in male and female patients with elevated cholesterol. Outcomes of over 60 clinical trials have been reviewed in detail, and reports of cholesterol lowering are very consistent (1, 21). In patients with high cholesterol, dosages of 5–20 mg/day lower LDL-cholesterol from 10%–30% (21). The decrease appears to be maintained for up to 2 years of treatment (22). Subjects have included middle-aged patients with high cholesterol, patients with non–insulin-dependent diabetes mellitis (23–25), postmenopausal women (26, 27), and the elderly (28, 29). Reports suggest that the plasma concentration of high-density lipoprotein cholesterol (HDL) increases at a slower rate than LDL decreases, so that the LDL:HDL ratio significantly improves within 1 year (22). Triglycerides either do not change significantly or slowly decrease (21). The main limitations to the clinical studies that have been conducted to date are that (i) policosanol is a poorly defined mixture, (ii) the mechanism of action remains unknown, and (iii) most of the studies have been conducted by one cardiovascular center with populations from Cuba and Central or South America (21). Although policosanol has been treated as a drug, it is a mixture of long chain alcohols that exists in many human foods. There is clearly a nutritional perspective in addition to a pharmaceutical perspective.

Occurrence and Composition of Waxes in Foods. True waxes are defined chemically as esters formed between long chain fatty acids and long chain alcohols. In addition to wax esters, plant waxes and honeycomb wax contain a variety of very hydrophobic compounds that include nonesterified very long chain hydrocarbons, alcohols, aldehydes, and acids. Some plant waxes contain a variety of branched lipids, secondary alcohols, diols, ketones, and other metabolites (30, 31). Because waxes are relatively abundant in cereal grains, bran, and germ, as well as in leaves, seeds, nuts, and unrefined oils (30), the human diet has always contained modest levels of waxes. In addition, honey was formerly consumed with honeycomb, which is primarily wax (Table 1). Before it became customary to remove the comb from honey around 1900, people who used honey as their primary sweetener undoubtedly consumed as much as a gram of wax per day. Some U.S. suppliers still sell intact honeycomb and bottles of honey that contain a large piece of comb, or "chunk," honey.

Because the alcohols in wax have only a single hydroxyl group available for esterification, waxes differ from triacylglycerols in having only one fatty acid esterified to a single alcohol functional group. The fatty acids used in the synthesis of wax esters may range from C12–C24, and the alcohols in plant waxes tend to be very long (C24–C34). Octacosanol is a C28 saturated alcohol, and triacontanol is a C30 alcohol. Two key chemical features of the waxes are their relatively low melting points and very poor solubility in water. Because much plant wax is epicuticular, boiling melts the wax and removes it from the plant. It is likely that several methods of cooking would remove some wax from cereals and other foods. Wax constituents are very hydrophobic, and it is interesting that cholesterol is often described as a waxy compound. The very long chain

Table 1. Major Wax Components of Typical Human Foods[a]

Source	% dry weight	Wax esters	Primary alcohols	Free acids	Aldehydes	Alkanes
Honeycomb	93	35–45	—	8–12	—	14–23
Sugarcane	—	6	26	10	50	8
Wheat	0.4–0.7	9	17	3	36	9
Oats	0.38–0.9	7–21	5.6–14	8.8–14	—	8.8–15
Maize	—	14–62	14	14	9	17
Rice	0.1	35	40	0	10	15
Sorghum	0.2	4	34	24	32	1
Apple	—	18	6	20	2	20
Grape	—	9	40	7	12	1
Cabbage	—	4–22	2–9	2–9	—	36–40

[a] References are cited in the text.

aliphatic alcohols and acids do not dissolve in water and are isolated chemically using warm organic solvents, such as hexane or chloroform. It is extremely unlikely that non-esterified aliphatic wax constituents would be transported in the blood plasma or cytoplasm in the absence of carrier systems.

Much plant wax is epicuticular, and the yield of extracted waxes is often presented as weight of wax per unit of leaf surface. For example, greenhouse-grown cabbage contains about 40 $\mu g/cm^2$ (32), and sorghum leaves contain about 113 $\mu g/cm^2$ of wax (33). Approximate yields range from 0.1–0.9 g/100 g in many plants (Table 1). Evidence suggests that lipases are involved in both synthesis and hydrolysis of the ester bond (30, 31). The plant waxes are found in intracellular oil droplets and also on the surface of plants. For this reason, oils prepared by cold pressing of plants typically contain primarily oils, but those prepared by solvent extraction contain higher levels of waxes (34).

Beeswax. The main sources of commercial very long chain fatty alcohols are saponified beeswax and sugarcane wax. Humans have consumed wax in the form of honeycomb for thousands of years, and there is little question that this source has accounted for a significant amount of the VLCFA3 in the food chain. Unhydrolyzed beeswax produced by the honeybee, *Apis mellifera*, contains about 23% hydrocarbons, 45% wax monoesters, 6% diesters of long chain alcohols with palmitic acid, 1% free alcohols, and 12% free acids (Table 1). Approximately 6% of beeswax is not identifiable (35, 36). The chain length of the predominant hydrocarbons ranges from 25–33, whereas the esterified alcohols are 24–34C in length. Palmitic acid is the major acid found in the ester fraction. The wax also contains about 3% diols and 13% hydroxy acids. Bumblebee wax from *Bombus* species is similar, with about 37% hydrocarbons, 29% monoesters, and 34% of other, more polar constituents. Evidence suggests that bees synthesize these constituents rather than acquiring them from plants (36).

Cereal Grains (Family Gramineae, Tribe Triticeae). Waxes are found in the leaves and stems, bran, and germ of all major grain crops, such as wheat (genus *Triticum*), oats (*Avena*), barley (*Hordeum*), and rye (*Secale*). Wheat grain contains primarily the C26–30 alcohols, hexacosanol, octacosanol, and triacontanol (37, 38). The amount of wax varies from about 0.4–1.0% of dry weight (depending on what part of the plant is in question), the type of grain, the stage of maturation, and the growing conditions. Cereal grain waxes contain relatively high percentages of free alcohols that are usually either 26C or 28C. *Triticum* species contain 15%–35% octacosanol, along with 4%–15% hydrocarbons, 4%–25% esters, and 15%–40% diketones. Hexacosanol is the major VLCFA3 in *Secale* and *Hordeum* and represents 15%–70% of the waxes in these genera. Hydrocarbons range from 2%–10%, esters range from 5%–20%, and diketones range from 10%–25%. Oats (*Avena sativa*) contain primarily hexacosanol with a smaller amount of octacosanol (39).

Grasses (Family Gramineae, Tribe Poaceae). Economically important plants in this tribe include sugarcane, rice, maize, sorghum, and millet. Most commercial policosanol is presently extracted from sugarcane and either saponified or obtained by supercritical fluid extraction under conditions that release free alcohols and acids from waxes. Sugarcane wax contains a mixture in which 50%–72% of the alcohols, acids, and aldehydes are 28C in length (37), and the waxes can be recovered commercially after sugars have been extracted. Rice surface wax contains about 15% alkanes, 35% esters, 10% aldehydes, and 40% primary alcohols, of which octacosanol comprised 90% (40). Only trace amounts of free acids were found. Rice bran contains 12% lipid, of which waxes are less than 10% (41). The esterified acids range from 16–24C, and the alcohols range from 22 to 36. The dominant alcohols include tetra-, hexa-, octa-, and triacontanol plus tetra- and hexatriacontanol. Sorghum grain wax has been reported to have relatively little wax ester and an unusually high amount of free acid or aldehyde, which readily oxidizes to form acid (42, 43). Maize, in contrast, contains 42%–62% wax esters when mature but primarily free alcohols when immature. Triacontanol is the major alcohol, followed by hexacosanol (44, 45).

Other Food Sources. VLCFA3 are found in all plant leaves, fruits, seeds, and nuts, and 26–30C alcohols and wax

esters can be extracted from various foods obtained from local markets (46). These include hazelnuts, cashews, peanuts, almonds, pine seeds, sunflower seeds, maize, wheat, rice, raisins, plums, and apples. A major commercial source of octacosanol is spinach; however, we have been unable to locate published references or patents concerning its preparation. Wax is a minor constituent of grape skin and contains about 10%–12% wax esters and higher proportions of free alcohols and acids (47). Only 2.8 g of wax could be recovered from 2 kg of raisins. Apples contain primarily alkanes and secondary alcohols with a smaller amount of primary alcohols (48). Cabbage wax contains primarily hydrocarbons (paraffins) rather than wax esters and primary alcohols (32, 49). The major alcohol is hexacosanol. Kale wax is similar to cabbage and is unusual in having a very high proportion of branched constituents (50).

Estimated Human Intakes of Wax Esters and VLCFA. Intake of hexacosanoic acid has been evaluated because this is a key VLCFA that accumulates in the blood of patients with X-linked adrenoleukodystrophy. It is important that the concentration of this minor fatty acid be assessed to verify responsiveness to dietary therapy (51). A survey of American foods that were preferred by patients with X-linked adrenoleukodystrophy (X-ALD) suggested that typical U.S. intake of hexacosanoate is 12–40 mg/day (52). Plant oils are the richest sources, and at 208 mg/100 g, peanut oil had the highest content. A tablespoon of peanut oil would contain about 12 mg of hexacosanoic acid. Most fish, meats, poultry, and eggs contain much less then 1 mg/100 g. Most of the foods analyzed were refined or prepared, and it may be noteworthy that whole-grain or bran cereals and pita bread had the highest contents among starchy foods. Peeled fruits and vegetables contain significantly less VLCFA than unpeeled fruits and vegetables, which is consistent with waxes being principal sources. Dietary content of hexacosanoate was measured in 42 Japanese foods (53), and it was estimated that daily intake was 12–36 mg. Because this analysis included a variety of processed foods and did not include any VLCFA3 except hexacosanoate, it seems likely that intake of VLCFA3 can be much higher than these estimates. For example, beeswax contains about 12% free acids (47% C24, 12% C26, and 12% C28 acids). An analysis of beeswax would yield only about 10 mg hexacosanoate per gram wax, but the wax contains about 35% monoesters of C24–34 fatty alcohols and C16–20 fatty acids. Thus, about 25% of beeswax could generate long chain fatty acids if the wax can be digested and the fatty alcohol cycle is operative.

Because cereal waxes are epicuticular or present in oil bodies, any processing that removes the bran and germ also eliminates most of the wax esters. Consumption of grain products decreased in the United States from approximately 300 lbs per person per year in 1910 to 136 lbs per year in 1970 and has risen again to about 200 lbs per year. However, most Americans consume less than one serving of whole grain per day (54). A bushel of wheat weighs 60 lbs and yields about 60 lbs of whole-wheat flour or 42 lbs of sifted white flour. The discarded 18 lbs of wheat includes the bran and germ, along with most of the wax esters and other compounds that may lower cholesterol. Beeswax or honeycomb is the richest natural dietary source of long chain aliphatic alcohols. With the introduction of processes to remove honeycomb from honey and substitution of artificial sweeteners for honey in the diet, this potential source of waxes and long chain alcohols has been removed. Consumption of honey can represent a significant amount of the calories consumed in hunter-gatherer and agricultural societies. For example, adult male Ache of Paraguay consume approximately 3700 kcal/day when foraging, and honey constitutes an average of 18% (range, 6%–30%) of daily calories (55). At a caloric density of 3 kcal/g, this would represent over 200 g. Beekeepers obtain about 2 lbs of wax per 100 lbs of honey, so wax intake may average 4 g/day in certain populations.

If one assumes that whole grains contain 0.2–0.5 g of wax per 100 g, then 1 lb (454 g) of whole grain could potentially yield more than 1 g of wax. A significant fraction of grain wax is unesterified, so digestion is not required to release the VLCFA3. As shown in Table 1, more than half the wax is potentially available to be converted to long chain fatty acids in the fatty alcohol cycle. It is reasonable to assume that early farmers may have consumed up to 1000 kcal or over 200 g of whole grain per day, so that average intakes of waxes from grain could have exceeded 1 g. Similarly, rice bran is a source of VLCFA3, much of which is available as free alcohol (41), which is more bioavailable than esterified VLCFA3. It is likely that consumption of unmilled rice could provide amounts comparable to whole wheat. Even if bioavailability were as low as 10%, it is reasonable to estimate that absorption of VLCFA3 from whole foods could range from negligible levels in societies that rely primarily on animal food sources to more than 0.2 g/day in societies that consume whole-grain staple foods, honey, and a variety of other fruits and vegetables. This amount is calorically trivial but may be functionally significant if it indeed lowers LDL cholesterol as suggested by clinical studies. If these considerations are correct, the actual intake of long chain compounds from waxes is probably 10-fold higher than the estimates based solely on hexacosanoate.

Digestion and Bioavailability of Wax Constituents. Adaptations in many organisms permit efficient utilization of waxes and their constituent VLCFA3 as sources of metabolic energy. It has been estimated that as much as 35% of all energy fixed in oceanic ecosystems is converted to wax esters (56). Like other lipids, waxes yield about 9 kcal/g when oxidized and are important sources of metabolic energy for many marine organisms and seabirds (56, 57). Wax esters are synthesized abundantly in marine organisms, such as copepods and krill, where waxes provide an energy density equivalent to glycerol lipids and also afford a means to regulate buoyancy. Efficient digestion and

absorption of these hydrophobic compounds by fish and seabirds require adaptations that include secretion of high concentrations of bile salts and mechanisms for remixing of gastrointestinal contents (58). The relative lack of adaptation to using waxes as energy sources in humans is evident in the leakage (keriorrhea) noted after consumption of marine fish that contain a high amount of wax esters (59).

Several studies of comparative digestibility and bioavailability of waxes and their constituents have been conducted. Many seabirds readily hydrolyze wax esters and assimilate the released fatty acids and long chain alcohols with approximately 85% efficiency. Terrestrial birds show lower efficiencies; for example, yellow-rumped warblers absorb only about 40% of the alcohol but over 85% of the fatty acid. An efficiency of aliphatic alcohol assimilation of about 40% has been reported in rats, but the value is less than 25% in dogs (58).

Few terrestrial organisms are specialized for efficient use of wax esters. However, the effect of waxes on rat growth has been tested by feeding diets with a defined wax, oleyl palmitate, or oleyl alcohol at either 4 or 15 g/100 g diet for 2–4 weeks (60). Absorption of the wax was about 50%, and the animals fed at this level developed steatorrhea. Interestingly, wax esters were excreted in feces of animals that received either the wax or the free alcohol, and tests *in vitro* showed that pancreatic lipase synthesized waxes from the free alcohol and available fatty acids. The data suggest that intact wax esters are not absorbable. For uptake to occur, the esterified fatty alcohol must be released by a lipase or other carboxyl esterase (Fig. 1). Efficiency of VLCFA3 uptake decreases as chain length and hydrophobicity increase.

Kolattukudy and Hankin (61) prepared a radioactive 29C alkane, *n*-nonacosane, in broccoli. When isolated and fed to rats, it was found that less than one-quarter of the alkane was absorbed and metabolized. However, much of the absorbed radioactivity was found in n-17–chain length liver lipids that included hydrocarbons, cholesterol esters, tri- and diglycerides, and phospholipids. When nonacosane was mixed with corn oil, the majority was excreted in feces unchanged (61). A cytochrome P450 from rabbit intestinal microsomes oxidizes hexadecane to cetyl alcohol in the presence of an NADPH-regenerating system (62, 63). Different cytochrome P450 enzymes hydroxylate alkanes at several positions (64). The efficiency with which alkanes might contribute to a cholesterol-lowering effect of waxes appears to be limited by poor absorption and the need for hydroxylation. Kolattukudy and colleagues also reported that randomly tritiated octacosanoic acid is readily metabolized to C16 and C18 compounds in rats (65). It was suggested that this conversion occurred by limited β-oxidation or by chain splitting. Similarly, a fraction of [^{14}C]-octacosanol is absorbed after oral administration in rats and is distributed to several organs (66–68).

Absorption and metabolism of nonesterified octacosanol have been studied relative to the question of whether very long chain alcohols support physical performance. When

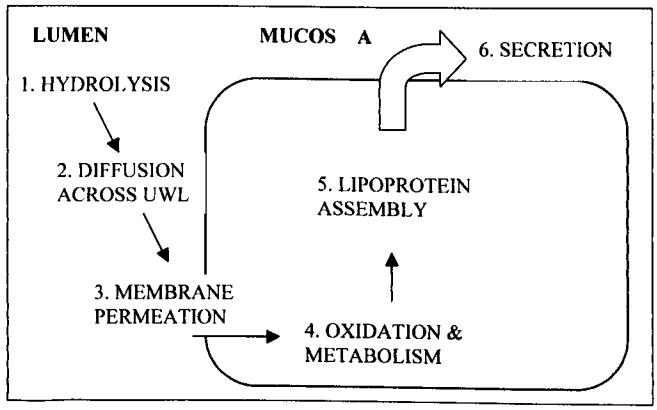

Figure 1. Processes involved in absorption of wax esters. (1) Hydrolysis by lipase or carboxyl esterase, not showing re-esterification. (2) Diffusion across unstirred water layer (UWL). (3) Passive or carrier-mediated absorption into intestinal epithelial cell. (4) Metabolic activation, possibly including oxidation to form long chain acids. (5) Assembly into lipoprotein particles. (6) Secretion into blood plasma (modified from Ref. 58).

small amounts of [^{14}C]-octacosanol were administered to rats through a stomach tube with a vehicle of tricaproyl glycerol, about half the dose was metabolized during the following week, whereas a third was excreted in the feces. The highest concentration in plasma was observed 1 hr after the dose was administered (66). Liver, brown adipose, and white adipose accumulated more than 1% of the administered dose per gram, whereas the concentrations of octacosanol in spleen, kidney, heart, lung, brain, and muscle remained less than 0.4% of the dose. The maximum amount in brain was 0.05% per gram. In a separate experiment, rats were fed diets containing 0.06 g/100 g of octacosanol along with [^{14}C]-octacosanol as a tracer and were trained to exercise or remain sedentary. The rats that were fed octacosanol were reported to exercise on a tread wheel significantly more than the control group fed diets without octacosanol (67). Significantly more octacosanol was found in the muscle of exercised rats fed octacosanol than in the nonexercised group that was fed octacosanol or in the exercised rats fed a control diet. The data suggested a role for octacosanol in muscle metabolism, although the identity of the radioactive compounds in muscle was not stated. Oxidation of fatty acids in muscle was elevated in rats fed a high-fat diet containing octacosanol (69). The addition of octacosanol to rat diet at 10 g/kg led to a decrease in peri-renal adipose lipid and a decrease in plasma triglycerides. The effect of serial administration of octacosanol to rats showed that liver initially contained more radioactivity than muscle, but the tracer was eliminated from liver at a higher rate. After 9 days, muscle contained a higher percentage of the original dose. The information was interpreted to mean that the liver may be converting octacosanol to long chain fatty acids, which were subsequently taken up by muscle (68). A subsequent study found that a fraction of the administered octacosanol was found in sterols and triacylglycerol, which indicated conversion to fatty acids and esterification (70). This is consistent with the

finding that saturated and polyenoic VLCFA are present in neutral lipids, including cholesterol esters in fibroblasts from patients with peroxisomal deficiency and from patients who are metabolically normal (71).

Enzymes for Digestion and Metabolism of Wax Esters and Fatty Alcohols. Wax esterase enzymatic activity appears to be attributable to lipases or related carboxyl ester hydrolases. Studies of mammalian wax digestion by partially purified pancreatic lipase have used the wax ester cetyl oleate. Activity increased in the presence of colipase and bile salts such as sodium taurocholate (58). However, di-isopropyl fluorophosphate blocked hydrolysis of cetyl oleate by pancreatic extracts from seabirds, chickens, or pigs. Because this compound inhibits carboxyl ester hydrolase (E.C.3.1.1.13) but not pancreatic lipase (E.C. 3.1.1.3), it was suggested that the relative activities of these enzymes may explain adaptive differences in the ability of various seabirds and animal species to assimilate energy from wax esters. DNA sequencing indicates that carboxyl ester hydrolase appears to be the same protein that has been named sterol esterase, retinyl ester hydrolase, bile salt–activated lipase, and pancreatic lysophospholipase. This widely distributed enzyme is synthesized in the pancreas, milk glands, liver, and various organs in birds, mammals, and humans (72, 73). The crystal structure of bile-stimulated lipase has been reported. The enzyme is a homodimer that binds taurocholate and cholesterol linoleate in the active site (74). Because cholesterol is a planar, largely aliphatic alcohol that contains 26C, a cholesterol ester is similar to a wax ester. It is plausible that cholesterol esterase and bile salt–stimulated lipase are different names for the same enzyme.

Hydrolysis of wax esters releases a free fatty acid and an alcohol, both of which are readily absorbed by the intestinal epithelium. It appears that the rate of wax hydrolysis is the limiting step. Thus, the question of whether long chain aliphatic alcohols released from dietary waxes might be of functional significance in humans depends on the ability of the intestinal milieu to solubilize and cleave these very hydrophobic substances. Studies in fish, birds, and mammals suggest that secretion of bile acids, colipase, and a carboxyl esterase would be necessary. Most workers in this field have suggested that bioavailability of the long chain alcohols in mammals is on the order of 10%–40% of the consumed waxes (21, 58). Beeswax contains approximately 25% long chain aliphatic alcohols. Therefore, one might speculate that at least 0.1 g of natural wax would need to be consumed to obtain an effective dose of policosanol from a food. Other factors that require investigation include the effects of biliary and pancreatic secretagogues, lecithin, and competition by preferred substrates for the hydrolytic enzymes. Pancreatic lipase hydrolyzes triacylglycerol at approximately 10 times the rate of waxes, so the presence of dietary fats may stimulate secretion of bile and pancreatic enzymes yet inhibit wax hydrolysis.

Until very recently, fatty acid uptake has been assumed to take place by passive diffusion into the enterocyte followed by translocation by way of fatty acid–binding proteins. However, fatty acid transporters have now been cloned, and it is postulated that intestinal and adipocyte uptake of long chain fatty acids is carrier mediated (75, 76). There is presently no information concerning the uptake of very long chain aliphatic acids and alcohols into cells, but metabolism of very long chain fatty acids requires a specific peroxisomal transport protein that does not depend on carnitine, unlike mitochondrial fatty acid transport (77, 78). One of the key questions concerning the reported cholesterol-lowering effect of aliphatic alcohols is why the effect takes place at doses of 5–20 mg/day. The presence of membrane-bound carriers for long chain fatty acids makes it possible that uptake is more selective than has been assumed.

The two major pathways for utilization of very long chain alcohols are (i) synthesis of ether phospholipids and (ii) oxidation to VLCFA. One function of the oxidation pathway for fatty alcohol metabolism is utilization of products released during turnover of ether lipids. For example, fatty alcohols such as hexadecanol are rapidly incorporated into brain phospholipids that have half-lives of 15–36 mins in rats (79). The oxidative pathway is important because very long chain fatty alcohols and fatty acids are incorporated into all brain lipid fractions, including phospholipids, proteolipids, gangliosides, glycolipids, glycerolipids, and myelin, and must be metabolized during turnover of these lipids (80, 81).

Inherited Disorders of Fatty Alcohol and Fatty Acid Metabolism. Impetus for studies on the metabolism of VLCFA3 has derived from analysis of a variety of inherited conditions in humans that include X-ALD, Sjögren-Larsson syndrome, and other peroxisomal diseases. This work indicates that very long chain alcohols and acids originate both from endogenous synthesis and from dietary sources and that aberrant metabolism impairs a number of neurological and immunological functions. In addition, the information indicates a number of points at which VLCFA and cholesterol metabolism intersect, particularly in regard to regulation of peroxisomal fatty acid oxidation and activation of the PPARα transcriptional system.

Owing to a deficiency in fatty aldehyde dehydrogenase (part of a complex known as fatty alcohol:NAD+ oxidoreductase) in patients with Sjögren-Larsson syndrome, long chain aldehydes are poorly oxidized and are reduced to alcohols that accumulate in tissues (82, 83). Evidence suggests that long chain fatty acids and alcohols up to at least C24 are reversibly interconverted (4). Indeed, a fatty acid cycle has been proposed (4, 64, 84). Enzyme systems exist in liver (85, 86), fibroblasts (4), and brain (87) that convert fatty alcohols to fatty acids. In some tissues, fatty acids can be reduced back to alcohols. However, when fatty alcohols are incubated with the oxidoreductase, fatty aldehydes are not released from the enzyme. Instead, the fatty

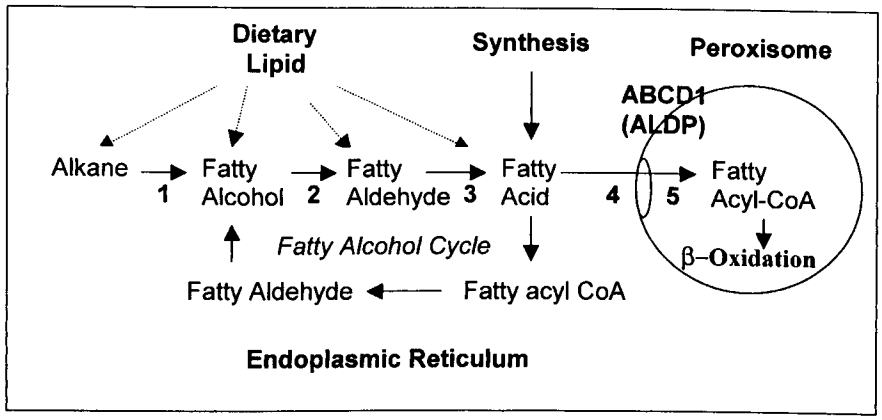

Figure 2. Postulated pathway for interconversion of very long chain fatty alcohols and acids by the enzymes described in Table 2. Reaction 1: alkane 1-mono-oxygenase. Reaction 2: fatty alcohol:NAD^+ oxidoreductase. Reaction 3: fatty aldehyde: NAD^+ oxidoreductase. Reaction 4: uptake *via* ABCD1 (the fatty acid transporter, ATP-binding cassette protein D1, which is also called ALDP). Reaction 5: very long chain fatty acyl CoA ligase. In the fatty alcohol cycle, VLCFA are converted to fatty acyl CoA and reduced to fatty aldehyde and fatty alcohol in NADPH-dependent reactions (4). If the mode of action in cholesterol lowering results from peroxisomal interactions, it is likely that very long chain fatty acids are the active constituents. Net flow of substrates toward oxidation would prevent accumulation of precursors.

alcohols are fully oxidized to long chain fatty acids that are released from the enzyme system (88). Uncertainty concerning the fate of very long chain compounds stems from their insolubility, which makes it increasingly difficult to monitor interconversion of C24–C30 compounds in tissue culture or in cell-free systems. Evidence suggests that the dominant route of metabolism is oxidation of fatty alcohols to fatty acids followed by β-oxidation. For example, very long chain fatty alcohols accumulate only when the fatty alcohol oxidoreductase is defective, and fatty acids accumulate only when peroxisomal metabolism is defective.

Peroxisomal metabolism of very long chain fatty acids is defective in X-ALD, and accumulation of VLCFA is one of the diagnostic criteria. VLCFA are synthesized in liver and neurons, and lignoceric acid (24:0) and nervonic acid (24:1 n-9) are components of sphingomyelin. Both fatty acids are synthesized in nerve tissue by elongation and/or desaturation of palmitic or oleic acid. VLCFA levels were increased in all patients homozygous for Zellweger syndrome, neonatal adrenoleukodystrophy, and infantile Refsum's disease and in patients with deficiencies of peroxisomal acyl-coenzyme A oxidase, bifunctional enzyme, and 3-oxoacyl-coenzyme A thiolase (89). In chromatograms from brain tissue of metabolically normal individuals, fatty acids longer than 24C are difficult to detect. However, fatty acids of up to 30C are detectable in patients with X-ALD, and the level of hexacosanoic acid is one of the diagnostic criteria (89).

In X-ALD and Sjögren-Larsson syndrome, the marker compounds are thought to originate partly by endogenous synthesis and partly from the diet (81). Evidence for dietary origin includes a patient with X-ALD who accumulated radioactive C26:0 in brain tissue when fed radiolabeled hexacosanoic acid by nasogastric tube (90). Endogenous synthesis of very long chain fatty acids is suggested by the observation that patients given D_2O accumulate deuterium in C26:0 at about 80% of the level observed in stearic acid but do not accumulate deuterium in phytanic acid (91). Thus, phytanic acid is assumed to be solely of dietary origin, whereas some hexacosanoic acid is synthesized. Endogenous synthesis can also be shown by incubation of fibroblasts from patients with Zellweger's syndrome with ^{14}C-acetate. This leads to incorporation of label into saturated, monoenoic, and polyenoic VLCFA (71). Biosynthesis of VLCFA appears to be a function of a widely distributed family of microsomal enzymes that elongates fatty acids longer than 16 carbons (92, 93). Accumulation of long-chain alcohols when fatty aldehyde oxidoreductase is deficient suggests that the normal metabolic pathway involves conversion to the long chain fatty acid and beta oxidation (Fig. 2). Therefore, it is feasible that long chain fatty alcohols and acids obtained from plant waxes or beeswax are interconverted and ultimately metabolized in peroxisomes.

Table 2 indicates some of the enzymes that are postulated to be responsible for uptake and metabolism of wax constituents according to the scheme shown in Figure 2. After hydrolysis of wax esters in the gastrointestinal tract by a carboxyl esterase, uptake may occur either by passive membrane permeation or potentially by mediation of a fatty acid carrier. Transport through the cell is expected to be limited by the very low solubility of these very hydrophobic compounds and may be mediated by proteins such as fatty acid–binding proteins. Metabolic interconversion of alkanes, alcohols, aldehydes, and acids takes place in the endoplasmic reticulum, where a mono-oxygenase converts alkanes to primary alcohols. Fatty alcohols are then oxidized by an enzyme complex that contains a fatty alcohol dehydrogenase and a fatty aldehyde dehydrogenase. This complex requires NAD^+ and releases very long chain fatty acids. A fatty alcohol cycle has been proposed in which the reverse reaction is initiated by an ATP-dependent fatty acyl-CoA synthetase and a fatty acyl-CoA reductase that utilizes NADPH (3, 84). Oxidation of VLCFA predominantly occurs after uptake into the peroxisomes. The adrenoleuko-

Table 2. Putative Enzymes of Wax Ester and Very Long Chain Fatty Acid Metabolism in Mammals[a]

Substrate	Enzyme or protein	EC number	Location	Representative tissues
Wax ester	Carboxyl ester lipase	3.1.1.1 or 3.1.1.13	Secretory	Pancreas, liver
VLC-fatty acid	Fatty acid transport protein	None assigned	Plasma membranes	Intestine, heart, adipose
VLC-alkane	Alkane 1-monoxygenase	1.14.15.3	Microsomes	Intestine
VLC-fatty alcohol	Fatty alcohol oxidase	1.1.3.20	Microsomes	Liver, fibroblasts
VLC-fatty aldehyde	Fatty aldehyde dehydrogenase	1.2.1.48	Microsomes	Liver, fibroblasts
VLC-fatty acid	Fatty alcohol dehydrogenase	1.1.1.192	Microsomes	Liver, fibroblasts
VLC-fatty acid	Peroxisomal channel ALDP1	None assigned	Peroxisomes	Liver
VLCFA and CoA	VLCFA:CoA ligase	6.2.1.3	Peroxisomes	Brain, liver

[a] ALDP1, adrenoleukodystrophy protein 1.

dystrophy protein (ALDP) was identified as a membrane-bound carrier produced by the ABCD1 gene that facilitates uptake of VLCFA into peroxisomes (77). The latter protein is a member of the ATP-binding cassette gene family. ABCD2 is a related fatty acid transport protein that was first identified by cloning procedures. After uptake, a VLCFA: coA ligase synthesizes VLCFA-acyl-CoA to initiate beta oxidation (94). In brain, a lignoceryl CoA ligase has been identified (95). Peroxisomal fatty acyl:CoA ligases such as lignoceryl:CoA ligase are specific for longer-chain fatty acids than the corresponding mitochondrial ligases (96, 97). It is assumed that β-oxidation proceeds to the 18–20C level and then may continue in the peroxisomes, or the substrates may be shuttled to mitochondria for complete oxidation.

VLCFA and Peroxisomal Cholesterol Metabolism. The variable composition of policosanol that has been used in clinical studies makes it difficult to attribute cholesterol lowering to a specific constituent. Although most reports deal with mixtures of very long chain alcohols, inhibition of cholesterol synthesis in fibroblasts by VLCFA from sugarcane has also been reported (98). In addition, the mixture of VLCFA inhibits lipoprotein oxidation *in vitro* (99). These studies suggest that long chain fatty alcohols may inhibit cholesterol synthesis after being converted to VLCFA. This hypothesis is consistent with information derived from studies with cells from patients with peroxisomal disorders.

Peroxisomes are capable of synthesizing cholesterol, and patients with defective peroxisomal activity have abnormally low LDL cholesterol. The rate of cholesterol synthesis is significantly reduced, and the rate of LDL uptake and degradation is 3- to 5-fold elevated in fibroblasts from peroxisome-deficient patients (100). When normal fibroblasts were enriched with tetracosanoic acid (C24:0), LDL binding and degradation were 4-fold higher than in non-treated cells. This suggests that a reciprocal relationship between cholesterol accumulation and metabolism of VLCFA may exist. Altered LDL uptake clearly indicates that VLCFA may exert effects that are not restricted to peroxisomal function. A SREBP regulatory element has been identified in the promoter for ABCD2, which is one of the transporters for VLCFA. Cholesterol depletion up-regulates ABCD2 and increases VLCFA metabolism in cultured human fibroblasts and monocytes from patients with X-ALD (101). Other ATP-binding cassette proteins are also closely linked to cholesterol metabolism and particularly to HDL-mediated reverse cholesterol transport. Up-regulation of ABCA1 in liver and macrophages of transgenic mice is associated with increased plasma HDL cholesterol levels, increased net flux of cholesterol to the liver, and reduced diet-induced atherosclerosis. Other members of the ATP-binding cassette protein superfamily participate in intestinal cholesterol metabolism (102).

HDL (or apolipoprotein A1) synthesis is increased by PPARα, and it is feasible that metabolites of VLCFA3 could activate either PPARα or another transcription factor, such as LXR, RXR, or HNF4, that is related to lipid control of gene expression (103–108). Very long chain fatty acids are known to be ligands of PPARα (109). If the cholesterol-lowering effect of VLCFA3 does take place *via* a peroxisomal mechanism, the mechanism of action would be expected to be similar to fibrates, which lower LDL cholesterol (110), raise HDL cholesterol (111), and enhance reverse cholesterol transport. This is consistent with effects of other fatty acid derivatives that lower cholesterol partly as a consequence of altered peroxisomal metabolism (112). It is now evident that different dietary lipids interact with a network of gene regulatory elements that are involved in metabolism of cholesterol, phospholipids, and sphingolipids. It is highly likely that this is true of VLCFA, and the linkage between defects of peroxisomal metabolism and various neuropathies suggests that exploration of tissue-specific effects of VLCFA will be a productive area for research.

Summary and Prospects

All major cereal crops contain true waxes and very long chain alcohols and acids, and the Dietary Supplement Health and Education Act permits the sale of these natural compounds in the United States. Far from being metabolically inert, very long chain fatty alcohols and acids appear

to provide essential functions but can produce toxicity at abnormally high plasma concentrations. Human diets in which whole grains and honeycomb are staple foods probably provide 1–2 g of wax constituents per day, but intake is expected to be much lower when diets lack whole grains and honeycomb. This is a trivial source of calories. However, many waxes contain more than 25% VLCFA3 in the form of free alcohols, aldehydes, and acids as well as wax esters. A number of clinical studies report that mixtures of very long chain alcohols and possibly very long chain fatty acids lower LDL cholesterol and raise HDL cholesterol in humans. No toxicity has been observed except in subjects with inherited metabolic defects, and some evidence suggests that octacosanol or policosanol may improve aspects of muscular performance. If true, this differs from the statin drugs, which can produce liver and neuromuscular toxicity.

Extensive work on the enzymology of peroxisomal disorders has already demonstrated the main pathways for metabolism of VLCFA3 (Table 2). In this pathway, fatty alcohols and aldehydes serve as precursors that are converted to VLCFA in the endoplasmic reticulum, whereon the VLCFA are taken up into peroxisomes and oxidized. It is interesting that disordered metabolism of VLCFA is nearly always associated with neurological or neuromuscular symptoms. Why this is the case has not been answered, and it seems certain that normal VLCFA metabolism is important for normal nerve function. The role of peroxisomes relative to these long chain fatty acids appears to be prevention of toxicity associated with elevated levels. Whereas there is no evidence that wax constituents produce toxicity at ordinary levels of intake, it is clear that carriers of inherited peroxisomal disorders do not metabolize these compounds at normal rates (113).

A number of questions remain concerning possible effects of very long chain aliphatic acids and alcohols on cholesterol levels. First, based on the methods of preparation used to extract the VLCFA3 from plants or beeswax, it is not clear whether all chain lengths are equally effective in lowering LDL cholesterol. Preparations referred to as policosanol are not well defined and vary in composition of fatty alcohols and acids. It is also not certain whether long chain alcohols, aldehydes, and acids all have the capacity to lower cholesterol. Another problem results from the dual origin of VLCFA by way of diet and endogenous synthesis. The relative contribution of these two sources of VLCFA to the total pool remains to be assessed, and it is not known whether endogenously synthesized VLCFA might also lower cholesterol. Clinical studies have not reported cholesterol lowering by intact wax esters. It appears that bioavailability of wax constituents is limited by inefficient intestinal hydrolysis, by resynthesis of wax esters from free alcohols and fatty acids, and potentially by a need for metabolic activation (probably oxidation) of long chain alcohols. The low water solubility of wax constituents also raises the question of whether specific transport systems exist and the anatomical site at which effects on LDL and HDL occur. As pointed out earlier (21), the intestine participates in HDL metabolism and should be evaluated as a site of action. At present, the clinical trials are consistent but have been done by a fairly small number of investigators, so more comprehensive trials are needed. A recent study of policosanol and phytosterols in cholesterol-fed hamsters failed to show an effect on LDL or HDL cholesterol (20). It is not clear whether natural waxes in which esters predominate are equally effective at lowering cholesterol. Wax hydrolysis may be partly due to pancreatic lipase, but a variety of carboxyl esterases could potentially cleave wax esters. If functional foods are to be produced with waxes, optimal delivery systems to ensure hydrolysis and absorption of the active VLCFA3 must be identified. It seems likely that the nonesterified VLCFA3 found in many human foods are consumed at levels that could lower cholesterol, but proof is lacking. Results of many studies that have tested efficacy of bran, germ, or plant fibers to lower cholesterol could be confounded by presence of wax constituents in the fractions tested. It is interesting that loss of wax constituents during milling and food processing may be one more reason why refined diets tend to produce elevated cholesterol.

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