



Review

# A critique of paradoxes in current advice on dietary lipids

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## Abstract

Beliefs about credible hypotheses of dietary causes of disease still need well-defined mediators to test for logical proof or disproof. We know that food energy causes transient postprandial oxidative insults that may not be fully reversible. Also, eating vitamin-like 18-carbon polyunsaturated fatty acids (PUFA) in foods maintains the 20- and 22-carbon highly unsaturated fatty acids (HUFA) in tissues. Tissue HUFA form hormone-like mediators that each amplify transient postprandial insults into fatal inflammatory, thrombotic and arrhythmic events in cardiovascular disease, a major preventable cause of death. Similar diet-based amplified events may also occur in other inflammatory proliferative disorders including cancer, dementia, arthritis and asthma. Puzzling paradoxes come from fragmented views of this situation which convey incomplete knowledge in oversimplified messages. Tools now exist to demonstrate successful prevention of two fatal food imbalances with credible dietary preventive interventions, but organizers and financiers to help gather the evidence remain unknown. The overall evidence accumulated about diet, disease and death may be nearing a paradigm shift in which prior observed facts remain while beliefs about their accepted interpretation change.

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**Keywords:** Heart attack; Highly unsaturated fatty acids (HUFA); Inflammation; Omega-6; Omega-3; Polyunsaturated fatty acids (PUFA); Prostaglandins

## Contents

1. Introduction . . . . .	78
2. The science of nature and the nature of science . . . . .	78
2.1. Simplicity vs. rigor . . . . .	79
2.2. More knowledge makes more ignorance . . . . .	79
2.2.1. Vitamin actions . . . . .	80
2.2.2. Hormone signals . . . . .	80
2.2.3. Enzyme regulation . . . . .	80
2.2.4. Gene expression . . . . .	81

**Abbreviations:** AHA, American Heart Association; CDC, Center for Disease Control and Prevention; CHD, coronary heart disease; CoA, coenzyme A; CPPT, Coronary Primary Prevention Trial; CVD, cardiovascular disease; DSHEA, Dietary Supplements Health and Education Act; GC, gas chromatography; HMG-CoA, hydroxymethyl glutaryl-coenzyme A; HUFA, 20- and 22-carbon highly unsaturated fatty acids (3 or more double bonds); KIM, “Keep It Managed” interactive personalized menu planning software; LDL, low density lipoproteins; LT, leukotriene; LXR, liver X receptor (a protein that binds responsive elements of genes); MRFIT, Multiple Risk Factor Intervention Trial; NEFA, non-esterified fatty acid(s); NIH, National Institutes of Health; NHLBI, National Heart, Lung and Blood Institute; NSAID, non-steroidal anti-inflammatory drug; PG, prostaglandin; PDAY, Pathobiological Determinants of Atherosclerosis in Youth; PPAR, peroxisome proliferator activated receptor; PUFA, polyunsaturated fatty acids (2 or more double bonds); SCAP, sterol responsive element binding protein cleavage activating protein; SRE, sterol responsive element (a DNA region influencing gene expression); SREBP, sterol responsive element binding protein (binds to SRE); TX, thromboxane; USDA, United States Department of Agriculture; VLDL, very low density lipoproteins.

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3.	Early health advice to the public . . . . .	81
3.1.	Food energy . . . . .	81
3.1.1.	Biomarkers of food energy imbalance . . . . .	81
3.1.2.	Popular diet plans . . . . .	83
3.1.3.	Managing appetite . . . . .	83
3.2.	Saturated fat . . . . .	84
3.3.	Polyunsaturated fat . . . . .	85
3.3.1.	Converting PUFA to HUFA . . . . .	85
3.3.2.	Ethnic diversity in tissue HUFA proportions . . . . .	86
4.	HUFA-based eicosanoid actions . . . . .	87
4.1.	Inflammation and prostaglandins . . . . .	87
4.2.	Thrombosis and aspirin . . . . .	87
4.3.	Immune disorders and leukotrienes . . . . .	87
4.4.	Arrhythmia and sudden death . . . . .	88
4.5.	Different $n-3$ and $n-6$ dynamics . . . . .	88
5.	Tools to monitor preventive interventions . . . . .	89
5.1.	Prevention trials need valid surrogate endpoints . . . . .	90
5.2.	Quantitative empirical relationships . . . . .	91
5.3.	Coupling to a food database (KIM2) . . . . .	92
5.4.	Making HUFA proportions visible (GC assay) . . . . .	92
5.5.	Comparing biomarkers . . . . .	93
6.	Priorities in primary prevention . . . . .	94
6.1.	Waiting for treatment? . . . . .	94
6.2.	Follow the money . . . . .	95
7.	Views in a different silo . . . . .	96
7.1.	Cholesterol suppresses lipid synthesis . . . . .	96
7.2.	The 1984 consensus conference . . . . .	97
7.3.	Alternate views . . . . .	99
8.	A take-home message . . . . .	100
	References . . . . .	101

## 1. Introduction

All truths are easy to understand once they are discovered; the point is to discover them. Galileo Galilei (1564–1642)

For half a century, I have seen diverse advice to the public about possible hazards or benefits of eating saturated fats, unsaturated fats, polyunsaturated fats or cholesterol. As a biochemist in lipid research, I wondered how the public messages could be so disconnected from what I knew about molecular mechanisms. The dissonance was amplified when powerful groups translated evidence from scientists into oversimplified public health messages. The translation involved appointed committees that vote for compromises among members' viewpoints, bureaucrats who build dominant positions for their agencies and marketing organizations that word public messages to enhance their corporate financial priorities.

None of the groups convey the controlled details of the science or hold any direct responsibility for the public's need to prevent disease. Citizens are left to choose among the oversimplified messages while managing their own health (for which they alone set a high priority). The fragmented knowledge and responsibility has provided many paradoxes over the years. This review examines the context

of misleading advice about two simple imbalances in voluntary food choices that can cause tissue damage, disease and death. The public needs more logical advice on how to prevent these two imbalances in their daily food choices.

## 2. The science of nature and the nature of science

Several terms set a context for how conflicts rise from the way that scientists use evidence to explain and predict events. The key event in a scientific adventure is one in which a fact is seen to be true by actual experience. In contrast, a fiction is an imaginative allegation that a fact exists when it is either known not to exist or not known to exist. The fiction may be either an untrue story invented to mislead or a speculation (hypothesis) to be tested. Sometimes a communicated fact is oversimplified to the point of causing erroneous misrepresentation or misconception. In this way, an oversimplified fact can seem fictional. Also, in a paradox, facts form a puzzle as one view of truth contradicts another. Paradoxes come easily from oversimplified stories that are missing key information. The apparent contradiction of two facts is often resolved by adding a third fact we need to know. In a sense, a paradox gives structure to what we do not yet know. It marks an area where more facts can

resolve the dissonance using logic (i.e., principles of reasoning to make valid inferences).

In science, a theory is an explanation of natural phenomena that predicts future observations and has survived multiple logical efforts to deny it. Confusion occurs when people use the word ‘theory’ as synonymous with hypothesis, conjecture, opinion, or speculation. Such lack of rigor blurs recognition of facts as different from fictions and hypotheses. The term ‘theory’ should not apply to explanations that are not sufficiently specific to test. Overall, interpreting the science of food and health provides an adventure, i.e. a hazardous enterprise of uncertain outcome that is an exciting experience.

People often use one of two associated events to predict the occurrence of the other (such as rain and thunder). Although such predictions may succeed some times, we should not use the correlation as a proof of cause. A proven cause would have mediators connecting cause to consequence in ways that permit logical tests of its validity. Making a theory from the hypothesis that thunder causes rain requires a testable sequence of mediators by which thunder causes rain. Alternatively, people could regard thunder as a surrogate marker (risk factor) for the likelihood or probability of rain without calling it a cause.

Surrogate markers are a major clinical concern when biomarkers are used as convenient endpoints instead of the primary clinical endpoints of saved lives or prevented disease, which need large, expensive, long-term efforts to collect sufficient statistical evidence [1]. Health experts see better efficacy from preventing the initial cause of a disease rather than merely treating associated signs or symptoms while leaving the primary cause unchanged to make continuing problems. Careful logic gives a proper inferential framework to recognize which biomarkers are causal mediators rather than merely associated biomarkers.

Epidemiologists describe the frequency with which a biomarker associates with clinical status, often alerting us to a probability (risk) of harm. However, oversimplified discussions of probabilities monitored by biomarkers (risk factors) sometimes imply (or let people infer) that the associated biomarker (risk factor) is actually causing the harm. A challenge for epidemiology is to convey statistical treatments of associated events in precise language that avoids misunderstandings. A challenge for molecular medicine is to identify and validate mediators that connect cause to consequence [1] and to describe logical interventions that the public needs to prevent harmful causes. This review notes some facts about lipids that can help design better public advice to prevent the current epidemic of diet-related diseases.

### 2.1. Simplicity vs. rigor

In the 14th century, William of Ockham noted that a good logical explanation of a phenomenon should make as few assumptions as possible and discard ideas that do not alter its observable predictions [2]. However, to avoid

misleading oversimplifications, Albert Einstein cautioned in the 20th century that “Everything should be made as simple as possible, but not simpler” [3]. Each new idea in science struggles in its early years to define its scope and boundaries. Thomas Kuhn described normal science in which scientists gather information that defines, expands and confirms an accepted idea, hypothesis or paradigm [4]. Normal science operates with a general suspension of skeptical disbelief in the central paradigm while confirming details are gathered to build acceptance of the idea. A collegial tradition (and the public’s sense) holds that proponents will eventually test the inferences in their hypothesis.

Failure of an experimental result to fit the prediction of a mature paradigm is often regarded as a mistake of the researcher rather than a refutation of the paradigm. However, when enough non-confirmatory evidence builds up, belief in the old paradigm reaches a crisis that promotes acceptance of new paradigms that reconcile both old and new evidence. The factual evidence remains, but the interpretations change. Kuhn termed this abrupt transition a ‘paradigm shift’ [4]. It is the hallmark of revolutionary science that follows a crisis in credibility.

Because of suspended disbelief, normal science often develops a form of ‘silo mentality’ [5] that makes it hard for adherents of one paradigm to appreciate the conceptual framework and terminology of a rival paradigm. As a result, bits of non-confirmatory evidence known in other “silos” may not be fully appreciated or used to challenge weak paradigms. Limited interactions among different ‘information silos’ [5] are common as each discipline focuses inward on its priorities with self-contained (vertical) communication of information and priorities. Separate disciplines (nutrition, physiology, pharmacology and cardiology) and subspecialties (vitamins, hormones, enzymes and genes) promote their own information silos that selectively nurture and support studies which add details that reaffirm established paradigms. A silo mentality in agencies and peer review panels deciding funding for new research proposals can significantly affect the maturation of paradigms as well as scientific careers.

In each ‘silo’, scientists busy gathering supportive data have little time or energy to examine alternatives that might refute the paradigms being confirmed. As a result, normal science might strengthen the *status quo* for a paradigm while it weakens the entry of revolutionary science. Such a dynamic also occurs in assembling fragmented advice for the public about food, health and prevention of disease. Advice favoring an established *status quo* is often promoted by more financial, political and public relations resources than new contradictory paradigms.

### 2.2. More knowledge makes more ignorance

In the 18th century, the study of nature had few ‘silos’ to impede one’s curiosity. Then, a well-informed individual could know most of what was known in many broad areas. Areas of study that we now call science took

shape during the 19th century. By the beginning of the 20th century, chemistry had tools to probe molecular aspects of hygiene and physiology. The studies then evolved over 100 years into the modern sub-disciplines of biochemistry, molecular biology, pharmacology and molecular medicine.

My own 50-year experience saw hundreds of rapidly growing paradigms interpret how food affects health. Now, the PubMed site of the National Library of Medicine (<http://www.ncbi.nlm.nih.gov/sites/entrez>) lists over 17 million biomedical papers. With no time to read and evaluate them all, we are increasingly ignorant of more facts every day! Readers of this review know that scientists (like the public) with limited time, energy and curiosity often defer scientific skepticism and suspend disbelief. By conditionally (temporarily?) accepting hypotheses that are accepted by others, scientists do not always act as careful logicians!

The absence of critical testing allows some claims and hypotheses to remain in a state of arrested maturation as normal science expands and reaffirms the paradigm without skeptical intellectual rigor distinguishing belief from logic. Temporary suspension of disbelief has an assumption that someone will eventually apply critical tests of logic (as the public believes scientists do all the time). However, some ‘mainstream’ beliefs of scientists may still not have fully tested logical inferences. To resolve the paradoxes that occur in the current misleading dietary advice to the public, the cumulative consequences of suspended disbelief, oversimplified hypotheses and silo mentality must be recognized. Four broad paradigms illustrate compartmented knowledge about mediators and surrogate endpoints that need to be integrated into public perceptions of how food affects health.

### 2.2.1. Vitamin actions

In 1900, foods were known as important sources of carbohydrates, proteins, fats and minerals. Then, discovery of ‘vital amines’, needed in very small amounts in food to support health, led nutrition researchers to isolate and identify previously unknown organic nutrients. The vitamin paradigm interpreted a 150-year-old fact that eating citrus fruit prevents scurvy. Nutritionists spent decades adding to the vitamin paradigm by adding small amounts of essential compounds to diets and measuring linearly increased growth of young laboratory animals (e.g., [6–9]). Quantitative vitamin nutrition had many successes between 1920 and 1960. The last-discovered essential amino acid, threonine, was identified and synthesized by my PhD mentor, Herbert Carter (reviewed in [10]).

Many water-soluble vitamins (e.g., pantothenic acid) act as necessary cofactors (e.g., coenzyme A) for enzymes that stimulate important metabolic reactions. The vitamin field now struggles with experimental designs to identify how essential nutrients affect health in older animals and humans. Requirements for vitamin D [11–13] and essential fatty acids [14] are now being re-assessed with hormone-mediated biological endpoints related to chronic disease

rather than growth. A silo mentality allows many nutritionists to deny vitamin status to nutrients like essential amino acids and essential fatty acids while accepting ascorbic acid (vitamin C). Also, arbitrary boundaries between micronutrients and macronutrients [15] further impair messages intended to advise the public (See Section 3.3.1).

### 2.2.2. Hormone signals

A debate over defining and naming the molecules we call hormones began in the early 1900s with two candidates: hormone (hormain = to excite or stir up) and autacoid (auto = self, akos = regulating or healing). Ego-centered battles over territorial names may seem inconsequential, but such events can change the dynamics of discovery. Both sets of mediators stimulate or diminish tissue actions, but many physiologists keep them in separate ‘silos’. Recognizing that many hormones, autacoids and neurotransmitters (histamine, adrenaline, serotonin, prostaglandins, leukotrienes, cannabinoids, retinoic acid, etc.) are formed in tissues from vitamin-like essential nutrients (amino acids and fatty acids) gives an opportunity to shift attitudes and paradigms in the public’s awareness of how food affects health. Also, a large class of hormone-like polypeptides and proteins mediate dynamic cellular interactions in regulating appetitive behaviors (melanocortins, orexins, leptin, insulin, etc.; [16,17]) and immune-inflammatory processes (cytokines, lymphokines, etc.; [18,19]). Whether these regulatory molecules are viewed as neurotransmitters, autacoids or hormones, their receptor-mediated actions must be included when telling the public how food affects health.

### 2.2.3. Enzyme regulation

In the early 1900s, scientists argued for years over the paradox of enzyme-catalyzed reactions having reaction kinetics both substrate-independent (zero order) and substrate-dependent (first order). The controversy eventually resolved after the revolutionary Michaelis–Menten paradigm described a catalytic site that becomes saturated with increased substrate availability. We now recognize thousands of proteins with saturable binding sites that affect food metabolism and hormone action. The 100-year-old “binding site” paradigm may help resolve current controversies about non-linear effects of dietary linoleic acid.

While studying for doctoral exams in 1953, I spent hours with large sheets of brown shelf paper drawing interconnected steps of enzyme-catalyzed pathways being discovered at that time. It was the ‘age of enzymes’ with new ones discovered every week. Drawing the arrays of connected pathways helped me learn connected sequences by which enzymes transform nutrients step-by-step into the diverse components of life. The upstream and downstream positions of the various metabolites give logical cause-and-effect contexts for how increasing one molecule stimulates increases of other molecules. Now, large professional charts of much more complex metabolic arrays are standard decorations in biochemistry offices and laboratories.

They help us avoid oversimplifying the cause-effect connectivity by which food may impair health.

#### 2.2.4. Gene expression

The recent explosive increase of information on how cellular environments influence gene expression provides many new ways to interpret the diversity of diet-disease relationships among individuals. Gene-coded DNA and RNA defines every enzyme-catalyzed step in the multi-step pathways connecting food to health and every hormone receptor that mediates our interactions with the environment. The genetic diversity of humans ensures that human responses to challenges are also diverse, and few individuals actually respond at the median intensity calculated for a population. A common comment is that fortunately healthy people “chose the right parents”.

However, the degree of expression of gene-coded RNA and protein is regulated by stimulating or suppressing signals from environmental molecules (including metabolites of essential nutrients). Thus, fortunate people may also have chosen the right environmental stimuli. Current excitement over the explosively expanding gene-related frontier might divert attention from the public’s need to develop stronger health promotion and disease prevention. We should not let enthusiasm for gene-related events that are not voluntary or preventable divert attention from helping the public manage the effects of food choices that are voluntary and preventable.

### 3. Early health advice to the public

In response to an emerging epidemic of fatal heart attacks 50 years ago, the American Heart Association (AHA) reviewed possible causes and advised the public to take four “prudent steps” to stop what was hopefully a preventable problem. The advised steps were:

1. Eat less calories.
2. Eat less calories as fat.
3. Replace saturated fat with unsaturated fat.
4. Eat some polyunsaturated fat.

Each had a rationale based on epidemiologic correlations. Each contained implicit hypotheses for which precise mechanisms were not yet proved at the time. There was no mention of the ‘cholesterol paradigm’ that dominated later discussions of health (see Section 7). The well-intended

AHA advice left scientists with the task of defining and testing specific hypotheses about the causal mediators and mechanisms of diet-induced death. Intervening decades have brought many detailed insights to the old advice.

#### 3.1. Food energy

The first two advised steps reflect epidemiologic associations of higher cardiovascular disease death rates with affluence and food excesses. A paradox appeared as much of the world’s population needed more food energy to survive while some might need less food energy to survive. The epidemiology-based hypothesis that ‘food energy kills’ had no logical mechanism or mediators. How could healthy food cause unhealthy people? Missing information about mediators connecting food energy to death made it difficult to design logical tests of this hypothesis. Food energy is mobilized after various nutrients (Fig. 1) are converted to the acetyl ester of coenzyme A (CoA; derived from the vitamin, pantothenic acid). The acetyl-CoA is oxidized by cellular mitochondria to  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . In this way, dozens of connected steps convert most of what we eat to  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . However, the flow of electrons is tightly coupled to the use of ATP in the work of exercise or chemical synthesis. Without expenditure of energy as work, each meal forms acetyl-CoA that cannot release its electrons and form  $\text{CO}_2$ . Instead, it follows alternate paths noted below.

##### 3.1.1. Biomarkers of food energy imbalance

Most meals have more food energy than will be burned by work, exercise and biosynthesis during the next hour or two. The excess acetyl-CoA forms malonyl-CoA and hydroxymethyl glutaryl CoA (HMG-CoA). However, the limited amount of the vitamin-based cofactor, coenzyme A, limits accumulating these metabolites and favors their polymerization to fatty acids and isoprenoids (Fig. 2). The liver makes dozens of connected intermediates as it converts the extra food energy of acetyl-CoA to fatty acyl-CoA and secretes it as triacylglycerol-rich very low density lipoproteins (VLDL). Thus, triglyceridemia is a convenient biomarker of body fuel management of excess food energy (Fig. 2), and it is a risk factor correlated with higher likelihood of death [20]. In the blood stream, lipoprotein lipase catalyzes remodeling of VLDL, cleaving triacylglycerols and releasing large amounts of non-esterified fatty acids (NEFA) that rapidly enter tissues and are

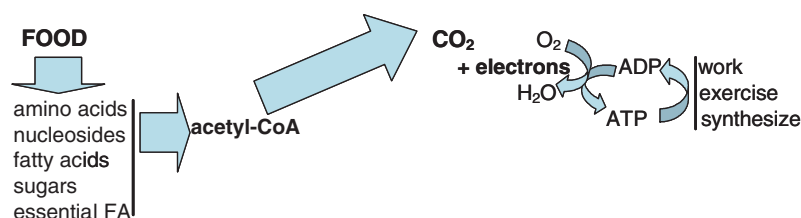


Fig. 1. Food energy intake and use.

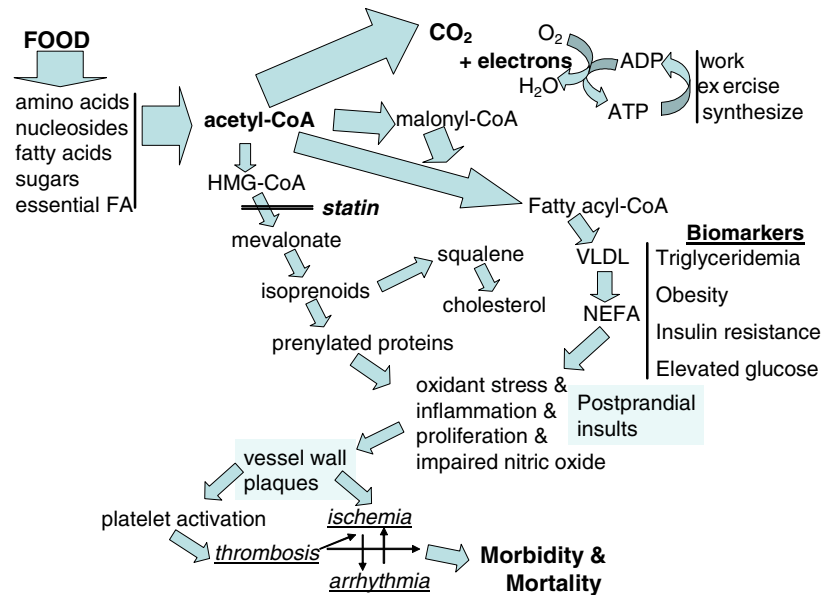


Fig. 2. Body fuel management connected to death.

accommodated by oxidation to  $\text{CO}_2$  or by esterification into fat. Limited energy expenditure favors fat accumulation. In this way, obesity is also a biomarker of food energy excess and body fuel management, and it might be a risk factor correlated with higher likelihood of death [21]. The transient postprandial NEFA actions add to insulin resistance [22], hyperglycemia [23] and oxidative stress [24]. All these transient biomarkers may also be risk factors correlated with death. The remaining low density lipoprotein (LDL) is a biomarker of the transient events that preceded its occurrence. It is the major lipid carrier in the circulation, and it is considered by many to be a major risk factor predicting likelihood of CHD death.

Discussions of LDL often neglect the harmful vascular impact of the toxic NEFA that must be accommodated whenever LDL is formed from VLDL. Also neglected is the fact that vascular injury occurs selectively at areas of the vasculature where hydrodynamics cause eddy currents with extended residence times [25,26]. This prolongs exposure of vascular cells to accumulated oxidants and inflammatory mediators that amplify the pathology (Fig. 2). In subendothelial spaces, the cells of inflamed vascular tissue generate many oxidants that oxidize phospholipids of lipoproteins [27–30], allowing oxidized-LDL to be another biomarker caused by excess food energy.

A major area of research demonstrates transient short-lived meal-induced oxidative insults [31–33] that sometimes amplify into long-term inflammatory vascular injury [34–36] associated with higher risk of thrombosis, ischemia, arrhythmia and death. Thus, Fig. 2 could show hundreds of transient mediators connecting reversible food energy insults to irreversible injury and death. Inadequate attention to early causal mediators in Fig. 2 has prevented developing a mature paradigm for preventing food energy toxicity, and it has delayed the design of effective primary

prevention interventions. Section 4 of this review considers how preventable eicosanoid actions that amplify oxidative insults into inflammatory, thrombotic injuries also depend on modifiable voluntary daily food choices.

Consequences of food energy excesses are common events. Less studied are biomarkers for calorie restriction, the condition at the other end of the spectrum of human fuel metabolism. Controlled clinical efforts to manage fuel metabolism and decrease excess body energy could benefit from knowing biomarkers and intermediates that are logical surrogate endpoints for successful and safe intervention outcomes [1]. The amounts of glucose and  $\beta$ -hydroxybutyrate (misleadingly called a ‘ketone’ body) circulating in the blood reflect the status of human fuel metabolism [37]. During adaptive changes to restricted energy intake, glucose levels fall a bit and  $\beta$ -hydroxybutyrate levels rise appreciably [38]. Paradoxically,  $\beta$ -hydroxybutyrate is regarded by many as a metabolic poison [37] when it is actually a potential biomarker of beneficial energy restriction. Its 50-fold rise from near non-existent to around 2 mM (while blood glucose approaches 4 mM) makes it a sensitive biomarker when managing food energy and fuel metabolism. Only much higher levels (20–50 mM) indicate harmful conditions.

A 150-year-old history stretches from the low carbohydrate diet of Banting to that of Atkins [39], but its controlled use in a general population study remains largely untested. Controlled evidence of effective use of body mass, blood glucose and  $\beta$ -hydroxybutyrate as surrogate endpoints in therapeutic trials might open a reassuring dialog about useful and safe ways to monitor energy restricted efforts to prevent excessive food energy imbalances. Perhaps some day, informed people will voluntarily adjust food energy intakes while maintaining these biomarkers at levels that fit their personal intervention strategy.

### 3.1.2. Popular diet plans

Knowing that fat has twice the energy density of protein and carbohydrate, people presumed that excess food calories could be easily lowered by lowering the proportion of fat in the food. This led to widespread promotion of low-fat foods and diet plans, but an epidemic of disorders continued for triglyceridemia, obesity, and insulin resistance (connected, in turn, with cardiovascular death; Fig. 2). Each popular diet plan was financed by the sales of books and materials which were marketed with oversimplified messages proclaiming merits of the plan. By 2004, the public could purchase more than 1000 diet books, some divergent from ‘mainstream’ medical advice [40].

To measure efficacy of diet advice with four of the more popular diet plans, a randomized trial evaluated them for 1 year [40]. The diets were defined as carbohydrate restriction without fat restriction [41]; fat restriction [42]; portion size and calorie restriction [43]; macronutrient balance and glycemic load [44]. After 1 year of comparably controlled conditions, each diet group had modest success. There was no association between diet type and weight loss. In fact, the strong curvilinear association between self-reported dietary adherence and weight loss was almost identical for each diet.

Regardless of diet type, participants in the top tertile of diet adherence reduced body weight by 7% in 1 year, and reduced some surrogate cardiac risk factors. The report did not test the hypothesis that food energy kills, but it confirmed that eating less food energy gives lower body mass. Another comparison of several popular diets showed that all gave modest weight loss at 12 months with greater loss on the diet regarded as “low carbohydrate, high protein, high fat” [45]. In contrast to these two reports, a summary of the limited weight loss with various dietary patterns considered that better results occurred with a ‘Mediterranean-type’ diet [46]. However, the biomarkers measured gave little insight into the mechanisms by which the diets caused compliant people to voluntarily decrease eating excess food energy.

### 3.1.3. Managing appetite

The old, oversimplified AHA advice, as well as recent AHA advice [47], to eat less calories and fats failed to address a more primary cause of eating: a sense of appetite or hunger and a lack of its converse, satiety. Those aspects affecting voluntary intake of food are mediated by dozens of neurotransmitters and hormones that were not well-developed concepts 50 years ago. Now they are an exciting set of rapidly maturing paradigms in research about mediators of appetitive behavior (e.g., [48,49]). A 2007 PubMed search (<http://www.ncbi.nlm.nih.gov/sites/entrez>) for only review articles identified 2683 on appetite, 1411 on hunger and 987 on satiety. Overall, there were 24,109 articles published on these three topics. We all have much to learn about the causes of ingesting excess food energy that might connect to cardiovascular disease [CVD] and its subset, coronary heart disease [CHD] (Fig. 2).

Epidemiological reports noted a paradoxical inverse association between alcohol consumption and body mass [50], supporting a hypothesis that food energy in the form of alcohol does not satiate appetite or contribute to body weight [51]. With no known mediators to clarify possible causal mechanisms involved, the hypothesis of disappearing calories remains inadequately tested. However, one insightful controlled study [52] suggests that alcohol is burned via acetyl-CoA as are all foods, but the traditional epidemiological use of average personal recall of ingested foods likely gives imprecise estimates of the calories actually ingested on drinking and non-drinking days. Poorly managed appetite for alcohol remains a serious unsolved clinical problem, and the ‘disappearing calories’ of alcohol provide a useful case history of the limits of epidemiological evidence.

One popular diet hypothesis is that the volume of food ingested creates satiety regardless of its caloric content [53]. This is reflected in advice from “Eat More, Weigh Less” [54] or “Volumetrics: Feel Full on Fewer Calories” [55]. However, a paradoxically alternate hypothesis is that larger portions contribute to voluntary over-consumption of energy and cause excess body weight [56]. A well-controlled test of this hypothesis with a 50% increase in portion sizes led to a mean increase in daily energy intake of 423 kcal ( $p < 0.0001$ ) which was sustained for 11 days [56] giving a mean cumulative increase in intake of  $4636 \pm 532$  kcal. Food marketing that encourages large (‘supersized’) meals likely diverts the public from limiting food energy intakes. Ironically, the stimulating effect of portion size was NOT evident for fruits (as a snack) or vegetables [56].

A variant of the calorie-limited meal approach is “The South Beach Diet” [57] which uses protein-rich snacks to help suppress hunger between meals. Smaller meal size might give less postprandial oxidant insults (Fig. 2). Also, eating protein might induce satiety with low-fat energy-restricted diets (15% or 30% energy as protein) that gave greater “diet satisfaction” with the high-protein diet, albeit weight reductions were similar [58]. The effects of high protein diets on thermogenesis, satiety and weight loss were reviewed [59]. A possible mechanism for protein-induced satiety might have peptide YY-mediated signals [60,61]. The role of protein, fat and carbohydrate in regulating body fuel management remains weakly defined.

The public needs better strategies to handle the primary processes and mediators of appetite that cause food energy intakes to exceed energy expenditures. For the present, the hypothesis that food energy can kill remains at an immature stage with poorly defined mechanisms for which surrogate endpoints need to be tested. Readers could logically infer from Fig. 2 that conditions that cause increased body fat might lead to death, even though elevated body fat might not [21]. Without stronger evidence of body mass being a valid causal surrogate endpoint connecting food energy intake with CVD death [1], the public will likely have poor motivation to control weight and resist the poorly understood appetitive signals that cause the imbalanced food energy intake associated with CVD.

### 3.2. Saturated fat

Advice to replace saturated fat with unsaturated fat stimulated my early experiments in lipid research. It made me ask by what mechanisms could saturated fats be “bad” and unsaturated fats “good”. Paradoxically, healthy human tissues continually make both saturated and unsaturated fats from metabolic fragments of foods. In humans, saturated fatty acids constitute half of the acids in membrane phospholipids and a third of the acids in triacylglycerols. The oversimplified advice failed to note that foods have saturated fatty acids ranging in length from 12 to 24 carbons. Are they all equally bad? Fifty years later, I still cannot cite a definite mechanism or mediator by which saturated fat is shown to kill people. The current advice to the public needs to identify logical causal mechanisms and mediators so we can focus logically on what food choices to avoid.

In the 1960s, my research looked for enzymes that selectively form phospholipids with saturated acids at the 1-position and unsaturated acids at the 2-position (reviewed in [62]). Using several sets of fatty acid structures, we saw that enzymes acting at the 1-position were NOT selecting saturated substrates by their absence of ethylenic bonds and their higher melting points (reviewed in [63,64]). The more data we gathered, the more dissonance developed around the belief that enzymes ‘recognized’ saturated and unsaturated fatty acids as we humans define them. The oversimplified saturated-unsaturated dichotomy needs revision. The complex mixture of membrane-bound enzymes being measured did not let us know how many different acyltransferases can discriminate between saturated and unsaturated acids. Hopefully, new molecular biology techniques will allow isolating pure acyltransferases [65,66] and characterizing specific molecular features [67] by which these enzymes might recognize ‘saturated’ or ‘unsaturated’ substrates.

The placement of fatty acids among the three positions of rat liver triacylglycerols has a strong preference for

palmitate (16:0) at the 1-position [68,69]. In contrast, oleate (18:1 $n$ -9) and linoleate (18:2 $n$ -6) are at the 2- and 3-positions. Interestingly, the 20- and 22-carbon highly unsaturated fatty acids (HUFA) abundant at the 2-position of phospholipids were not present very much at the 2-position of triacylglycerols. One might create an oversimplified ‘Just So’ type of story (see introduction of Ref. [63]) about how Nature puts ‘bad’ acids at the 1-position to control ‘fluidity’. However, human breast milk (regarded by some as Nature’s perfect nutrition) has saturated palmitic acid located mostly at the 2-position of triacylglycerols [70,71]. As a result, intestinal digestion of mothers’ milk ensures absorption of a saturated fatty acid over other acids. Nature seems to be taking no step to reject this ‘bad’ fatty acid from entry into newborn humans.

Quantitative studies with mutants of bacteria and yeast unable to form unsaturated fatty acids confirmed the popular paradigm that cell physiology is limited when high (>80%) proportions of saturated fatty acids cause low ‘fluidity’ of membranes [72]. However, specific structural features of acyl chains had unpredicted effects on cell growth. Some acetylenic acid isomers (8 and 10, but not 9 and 11) with low ‘fluidity’ had ‘good’ physiological action and some cyclopropane acid isomers (9 and 11, but not 10 and 12) with high ‘fluidity’ were ‘bad’ (details reviewed in [63,64]). The more data we gathered, the more dissonance developed around the idea that cellular enzymes and physiology respond to the features that we use to define saturated and unsaturated fatty acids.

Perhaps we had too readily suspended disbelief when accepting the epidemiology-based hypothesis of harm from saturated fats without knowing any mechanisms involved. Hindsight shows that millions of people eat palm oil and coconut oil as their major food oil without high mortality rates of cardiovascular disease (CVD). Those oils contain 85% saturated fat, whereas butter has 60% and lard has 40% (similar to that of human fat) (Table 1). Claims of health risks and health benefits from eating saturated fats create paradoxes.

Table 1  
Fatty acids in food oils

	Coconut oil	Palm kernel	Butter oil	Palm oil	Lard	Soy	Olive	Corn	
4:0	0.0	0.0	3.2	0.0	0.0	0.0	0.0	0.0	4:0
6:0	0.6	0.2	1.9	0.0	0.0	0.0	0.0	0.0	6:0
8:0	7.5	3.3	1.1	0.0	0.0	0.0	0.0	0.0	8:0
10:0	6.0	3.7	2.5	0.0	0.1	0.0	0.0	0.0	10:0
12:0	44.6	47.0	2.8	0.1	0.2	0.0	0.0	0.0	12:0
14:0	16.8	16.4	10.0	1.0	1.3	0.0	0.0	0.0	14:0
16:0	8.2	8.1	26.2	43.5	23.8	10.5	11.3	10.6	16:0
18:0	2.8	2.8	12.1	4.3	13.5	4.4	2.0	1.8	18:0
16:1 $n$ -7	0.0	0.0	2.2	0.3	2.7	0.0	1.3	0.1	16:1 $n$ -7
18:1 $n$ -9	5.8	11.4	25.0	36.6	41.2	22.6	71.3	27.3	18:1 $n$ -9
20:1 $n$ -9	0.0	0.0	0.0	0.1	1.0	0.2	0.3	0.1	20:1 $n$ -9
18:2 $n$ -6	1.8	1.6	2.2	9.1	10.2	51.0	9.8	53.5	18:2 $n$ -6
18:3 $n$ -3	0.0	0.0	1.4	0.2	1.0	6.8	0.8	1.2	18:3 $n$ -3
% Sat FA	86.5	81.5	59.8	48.9	38.9	14.9	13.2	12.5	

Data are from the USDA National Nutrient Database at <http://www.nal.usda.gov/fnic/foodcomp/search/>.

Re-assessment of the current public advice based on a belief that eating saturated fat can kill a person should identify definite causal mechanisms and mediators of harm. Without such logical links, scientists will properly continue providing skeptical reviews [73,74] of the oversimplified epidemiologic interpretation of ‘toxic saturated fat’. The pioneering Seven Countries Study [75] reported a strong correlation between saturated fat intake and CHD death rates that was confirmed by two studies but not by others (reviewed in [76]). In fact, recent analysis of 78,778 women followed for 20 years showed that intake of saturated fat was not a statistically significant predictor of CHD when adjusted for nondietary and dietary risk factors [77]. The public may reasonably wonder what is causing the hypothesis of saturated fat toxicity to have such a prolonged period of suspended disbelief. More healthy skepticism (disbelief) and curiosity might promote a constructive search for unknown mediators of the putative toxicity.

### 3.3. Polyunsaturated fat

The 50-year old advice to “eat some polyunsaturated fat” led to fascinating puzzles as we considered the diverse polyunsaturated fatty acids (PUFA) in foods and tissues. The acyl chains differ in length (18, 20 and 22), as well as positional location (*n*–3, *n*–6, *n*–7 and *n*–9) and number (2–6) of double bonds. The process of converting the vague AHA advice into scientifically rigorous hypotheses that could be tested had begun. The first steps were ‘normal science’, focused inward with confirmatory studies that defined the scope and boundaries of the field of PUFA metabolism. Fig. 3 shows various fatty acids that commonly occur in foods and in human tissues. Each tissue has very different proportions of triacylglycerols, phospholipids and cholesterol esters, and each lipid class has different proportions of the diverse fatty acids. The two major PUFA in foods (18:2*n*–6 and 18:3*n*–3) are vitamin-like in supporting growth of small mammals [6,9,78]. However they differ in maintaining physiological status [8,79]. Evidence suggests that the 20- and 22-carbon highly unsaturated fatty acids

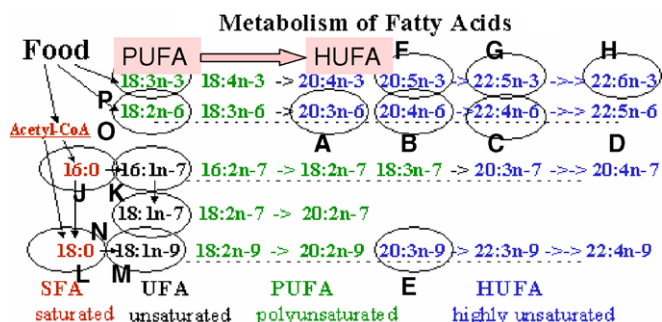


Fig. 3. Polyunsaturated (PUFA) and highly unsaturated fatty acids (HUFA). Modified from <http://efaeducation.nih.gov/sig/overview2.html>. Common tissue fatty acids are in ovals. The most abundant acids overall are the saturated palmitic acid, 16:0, and the monoenoic oleic acid, 18:1*n*–9.

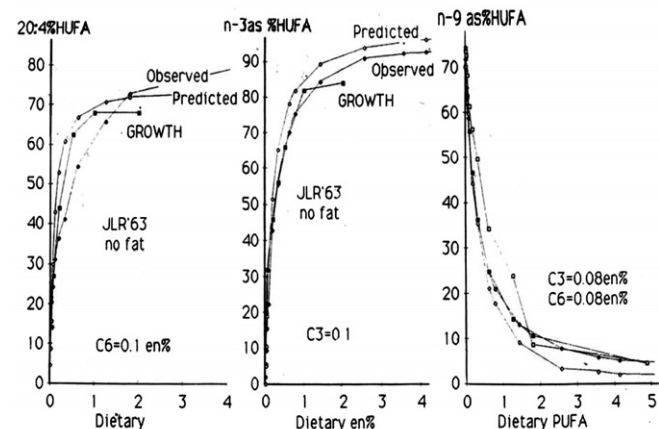
(HUFA) with three or more double bonds may be major mediators affecting diet-dependent diseases [14].

#### 3.3.1. Converting PUFA to HUFA

Major changes in tissue HUFA occur in the rat as linoleate intake is increased from zero to 0.14% of food energy (0.14 en%), and very little change occurs with intakes above 1 en% [80]. A pioneering set of studies showed how dietary PUFA maintain tissue HUFA [9,81–83]. The results with rats set a baseline for all HUFA studies that followed. Fig. 4 shows the important hyperbolic (saturable) competitive interactions among dietary *n*–3 and *n*–6 PUFA and the endogenous *n*–9 HUFA, 20:3*n*–9, as elongation and desaturation enzymes maintain diverse HUFA in tissue lipids [9]. In contrast, accumulation of dietary PUFA in tissue triacylglycerols has a linear dose-dependent response to diet abundance [84,85]. An important aspect in maintaining tissue HUFA is that dietary amounts of either type of essential PUFA (18:2*n*–6 or 18:3*n*–3) as low as 0.3% of daily food energy (0.3 en%) were adequate to prevent any physiological sign of nutrient deficiency in rats [9]. Eating more than 1 en% *n*–6 linoleate (or *n*–3 linolenate) did not appreciably increase the proportion of its derived HUFA in tissue HUFA (Fig. 4).

Importantly, results from studies of human infants [86] also showed that similar low dietary levels of linoleic (18:2*n*–6) maintained high HUFA levels and prevented signs of physiological deficit. In fact, Cuthbertson noted that minimum requirements for essential fatty acid (EFA) are “set far too high and are in fact less than 0.5% of calories” (<0.5 en%) [87]. Evidence from traditional quantitative nutrition studies might extend the vague AHA advice to “include some polyunsaturated fats” by advising an intake of about 1 en% PUFA divided equally between *n*–3 and *n*–6 PUFA (perhaps 0.5 en% each). We should skeptically examine the rationale of the Food and

Hyperbolic metabolic conversion of dietary PUFA to tissue HUFA



Very low intakes of n-3 or n-6 acids prevent EFA deficiency  
 Mohrhauer & Holman J. Lipid Res. 3: 151-159, 1963  
 ( <http://efaeducation.nih.gov/sig/dri.html> )

Fig. 4. Converting PUFA to HUFA. Results from Ref. [9].

Nutrition Board’s advice urging the public to eat amounts much higher than that [15]. The logic and rigor with which that appointed committee preferred ambient USA intakes of  $n-6$  PUFA much in excess of that needed for EFA function is not evident.

A useful biomarker of excess PUFA is shown in Fig. 4. Dietary intakes of either  $n-3$  or  $n-6$  PUFA near 1 en% prevented physiological deficits and caused the proportion of  $n-9$  in HUFA to be near 10%. In a similar way, human parenteral feeding studies confirmed the absence of deficiency signs whenever the  $n-9$  HUFA (20:3 $n-9$ ) was less than the  $n-6$  HUFA [88] and less than 30% of total HUFA. Paradoxically, the  $n-9$  HUFA is regarded by many as a metabolic poison when it actually is a biomarker of potentially beneficial restricted intake of  $n-6$  PUFA (similar to  $\beta$ -hydroxybutyrate serving as a biomarker of potentially beneficial restricted intake of food energy; noted in Section 3.1.1). A logical inference would be that values of %  $n-9$  in HUFA below 2% indicate excessive intakes of PUFA [89]. The near absence of 20:3 $n-9$  in most blood samples from Americans strongly suggests that current PUFA intakes in the USA may be far above that needed to prevent deficiency. The  $n-9$  HUFA biomarker might be useful when monitoring food intakes within the context of Cuthbertson’s assessment of human needs [87]. A rise from near non-existent to around 10% of HUFA could make it a sensitive biomarker when limiting PUFA intakes. It might become a valid surrogate endpoint in future efforts to avoid excessive PUFA intakes and excessive  $n-6$  eicosanoid actions (see Section 4).

3.3.2. Ethnic diversity in tissue HUFA proportions

Over the years, different ethnic groups have developed characteristic food habits that maintain widely different proportions of  $n-6$  in HUFA. Although traditional ethnic food choices occur, worldwide cultural exchanges are bringing rapidly changing food habits in Japan [103]. As a result, a predicted shift in tissue HUFA has occurred [104] with higher proportions of  $n-6$  in HUFA associated with an accelerating development of atherosclerotic vascular injury among young Japanese [105,106]. The changes in

tissue HUFA unfortunately run counter to what might provide desirable primary prevention (see Section 5). As a result, the current trend in the HUFA status of Japanese intensifies health concerns noted in 1990 [103].

Published values for the  $n-6$  biomarker in blood range from 20% to 80%  $n-6$  in HUFA (Table 2) with voluntary food choices [14,104,107]. These values are closely associated with observed CHD death rates for diverse populations worldwide [107]. Fig. 5 shows different CHD mortality rates associated with different ethnic food choices that maintain different proportions of  $n-6$  in HUFA. Three subgroups in Quebec had CHD death rates proportional to the measured biomarker values of 44%, 55% and 75%. Also, the quintile of MRFIT subjects with the lowest %  $n-6$  in HUFA had the lowest death rate [107]. The strong correlation ( $r = 0.98$ ) merits detailed studies to confirm likely causal mechanisms connecting the composition of tissue HUFA and disease.

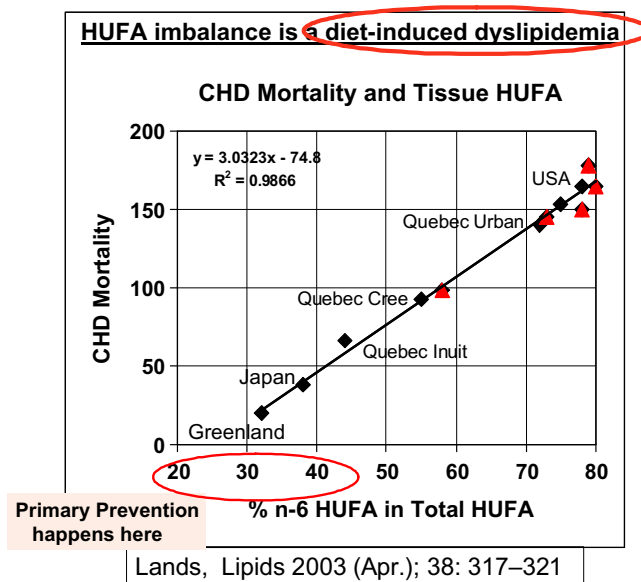


Fig. 5. Cardiovascular deaths correlate with the %  $n-6$  in HUFA. Modified from Ref. [107]. Voluntary primary prevention of the diet-induced dyslipidemia is happening with values between 20% and 40%  $n-6$  in HUFA.

Table 2  
Ethnic diversity in tissue HUFA proportions

Reference	Location	Sat	Mono	18:2 $n-6$	18:3 $n-3$	20:4 $n-6$	20:5 $n-3$	22:5 $n-3$	22:6 $n-3$	HUFA	% $n-6$ in HUFA
[90]	Greenland	43.6	19.2	14.0	0.1	5.2	4.9	1.6	7.9	21.3	32
[91]	Japan, 57-year-old	47.6	13.6	17.3	0.2	6.4	3.7	1.2	7.3	18.6	34
[92]	Quebec			22.2	0.0	6.2	3.0		5.0	14.2	44
[93]	Japan	30.4	21.3	31.7	0.2	6.6	2.6	0.7	5.4	16.3	47
[94]	Japan dietitians	32.1	21.0	27.8	0.9	4.9	1.7	0.4	3.2	10.9	51
[95]	England	30.7	20.1	33.3	0.8	7.2	1.5	1.2	3.0	15.1	62
[96]	Minnesota	40.9	12.3	21.6	0.2	12.2	0.7	1.0	4.5	18.4	66
[97]	Spain	47.1	19.7	15.9	0.1	6.8	0.6	0.4	5.5	22.7	72
[98]	James Bay			21.1	0.0	9.3	0.7		3.0	13.0	71
[99]	Quebec			22.1	0.0	6.4	0.5		1.3	8.2	78
[100]	ARIC study	49.3	9.2	22.0	0.1	11.5	0.6		2.9	14.9	77
[101]	Detroit, 25-year-old	31.1	24.1	26.5	0.4	7.6	0.2	0.3	1.8	12.0	82
[102]	Ohio	45.0	15.0	25.7	0.6	10.2	0.7	0.2	0.8	13.2	87

#### 4. HUFA-based eicosanoid actions

Polyunsaturated fatty acids (PUFA), especially the 20- and 22-carbon highly unsaturated fatty acids (HUFA), selectively accumulate at the 2-position of tissue phospholipids [62]. As a result, when the Karolinska group reported in 1964 that potent hormone-like prostaglandins form from HUFA [108,109], I suggested that a major tissue precursor for their formation would be the 2-acyl ester of membrane phospholipids. A collaboration in Stockholm quickly showed that phospholipase activity (PLA<sub>2</sub>) was needed to give non-esterified HUFA before the oxygenase could synthesize the hormone-like eicosanoid [110]. This study steered my research into an adventure of defining factors that affect formation of prostaglandins from HUFA [111,112] and relating the process to CHD mortality (reviewed in [14,113,114]).

##### 4.1. Inflammation and prostaglandins

Research on prostaglandin biosynthesis first showed us how hydroperoxides formed by fatty acid oxygenases stimulate faster oxygenation in an explosive amplified positive feedback process [115–118]. I also learned that this paradoxical aspect of downstream products causing more upstream actions occurs with many amplifying processes that occur in inflammatory diseases [14,31]. Amplification of inflammatory pathology by the oxidants produced by activated cells facilitates conversion of HUFA to active eicosanoids that amplify the chemotactic recruitment of more inflammatory cells to the region.

A 1971 report indicated that a major mechanism for aspirin action was inhibition of the oxygenase converting *n*–6 HUFA to prostaglandins [119]. The widely recognized benefits of aspirin action in suppressing prostaglandin-mediated inflammation and hyperalgesia stimulated me to study molecular mechanisms by which various non-steroidal anti-inflammatory drugs (NSAID) inhibit oxygenase-mediated pathology [120,121]. Some drugs sensitive to ambient peroxide tone were effective analgesic agents even though they were not effective inhibitors when inflammatory oxidant stress had amplified peroxides to high levels [122,123]. Our results also showed that *n*–3 HUFA competitively antagonized formation of prostaglandins from *n*–6 HUFA [124].

We had stumbled onto an interesting paradigm about how eating different dietary PUFA might affect health differently. That led 30 years ago [124] to the hypothesis that having more *n*–3 HUFA in tissue lipids might prevent pathology that was amplified by *n*–6 eicosanoid mediators. Many studies in many laboratories expanded and confirmed the paradigm over the next decade [14,125,126].

##### 4.2. Thrombosis and aspirin

In 1975, Hamberg and Samuelsson isolated and characterized thromboxane as the major mediator of thrombosis

[127]. Their report gave a dramatic example of how *n*–6 eicosanoids formed from *n*–6 HUFA can impair health and mediate disease processes. The active *n*–6 hormone, thromboxane A<sub>2</sub> (TXA<sub>2</sub>), is extremely unstable, lasting only a few seconds before forming inactive TXB<sub>2</sub>. In fact, all eicosanoid signaling occurs with a very short-lived active hormone that binds to its selective receptor while it also rapidly disappears [111]. As a result, the amplified eicosanoid-mediated signaling events quickly dissipate unless there is continued synthesis of new active hormone. An added aspect of eicosanoid action is that different cells have different amounts of enzymes converting the intermediate eicosanoid into diverse active prostaglandin hormones (e.g., PGD, PGE, PGF) with diverse physiological actions. One *n*–6 eicosanoid, prostacyclin (PGI), is formed by endothelial cells, and it diminishes thrombosis mediated by *n*–6 thromboxane (TXA<sub>2</sub>) formed by platelets [128, reviewed in 129].

To test which action might dominate in a dog model of heart attacks [126], diets were designed to give lower proportions of *n*–6 (and thus higher proportions of *n*–3) in tissue HUFA. Feeding *n*–3 HUFA led to lower degrees of thrombosis and tissue damage [126]. With human subjects, stimulated platelets formed much more TXB<sub>2</sub> than the 50 ng/ml TXA<sub>2</sub> that gives full aggregation [130]. Feeding *n*–3 HUFA decreased aggregation. A slowed rate of thromboxane formation may allow newly formed active TXA<sub>2</sub> to decompose before it binds and activates platelet receptors.

The instability of TXA<sub>2</sub> also makes accumulated TXB<sub>2</sub> a poor index of how much receptor-mediated signaling had occurred with the short-lived TXA<sub>2</sub>. Slowing the rate of conversion of *n*–6 HUFA to TXA<sub>2</sub> with either an NSAID or a competing *n*–3 HUFA seems to be a feasible preventive tactic. Recently, 85% of patients discharged after acute myocardial infarction were using aspirin for cost-effective secondary prevention of thrombotic events [131]. Unfortunately, data have not been assembled to estimate the cost-effective benefits of a simple primary prevention tactic of eating more *n*–3 HUFA.

##### 4.3. Immune disorders and leukotrienes

Discovery of lipoxygenase-derived leukotrienes [132] gave yet another set of mediators by which food-based HUFA affect health and disease. The biological activity of the *n*–6 leukotriene B<sub>4</sub> (LTB<sub>4</sub>) was at least 30 times greater than that of the *n*–3 LTB<sub>5</sub> in aggregation of rat neutrophils, chemokinesis of human PMN, lysosomal enzyme release from human PMN and potentiation of bradykinin-induced plasma exudation [133]. The *n*–3 HUFA, eicosapentaenoic acid (EPA), affected functions of several types of leukocytes critical to inflammation and immune-related events in asthma [134]. Four grams per day for 8 weeks caused a >50% decrease in the LTB<sub>4</sub> production and chemotaxis of neutrophils accompanied by increased formation of the biologically less active LTB<sub>5</sub> [134].

The amount of LTB<sub>4</sub> and LTB<sub>5</sub> produced by stimulated rat leukocytes closely resembled the changes in arachidonic acid and EPA content of leukocyte phospholipids [135]. Thromboxane production in stimulated leukocytes from the EPA-fed animals was also decreased compared with the control group.

The ability of *n*-3 HUFA to decrease production of the vigorous amplifying *n*-6 eicosanoids, LTB<sub>4</sub> and TXA<sub>2</sub>, supports the hypothesis that eating foods with more *n*-3 and less *n*-6 fats might attenuate leukocyte activity and have useful anti-inflammatory effects. Fish oil supplementation was protective with exercise-induced bronchoconstriction and asthma [136]. A fish oil diet improved pulmonary function with a concurrent reduction in bronchodilator use. Compared to results with normal and placebo diets, the induced sputum differential cell count percentage and the concentrations of LTC<sub>4</sub>-LTE<sub>4</sub>, PGD<sub>2</sub>, interleukin-1beta, and tumor necrosis factor-alpha were all significantly reduced before and following exercise with the fish oil diet [136]. The powerful chemotactic actions of the *n*-6 LTB<sub>4</sub> during leukotriene-enhance immune-inflammatory events are often underappreciated [137].

#### 4.4. Arrhythmia and sudden death

Pioneering studies of dysrhythmia showed less susceptibility for tissues from rats fed *n*-6-rich sunflower or *n*-3-rich tuna oil [138] compared to tissues from rats fed sheep fat or standard laboratory diet. Susceptibility of the latter sets of animals seemed due to eicosanoid actions that were abrogated with the NSAID, indomethacin. Tuna oil supplementation gave a lower incidence and severity of ventricular fibrillation during both occlusion and reperfusion, whereas sunflower oil gave lower arrhythmias during occlusion but not in reperfusion [139]. Rats fed the standard diet had a 46% incidence of ventricular fibrillation on occlusion, greater than for tuna (6%) and sunflower (21%) groups and lower than the 68% for rats fed saturated fat. Apparently, the fibrillation during reperfusion of acutely ischemic myocardium has different limiting signaling mechanisms from the fibrillation developed during occlusion, suggesting that different eicosanoid patterns have important consequences on pathophysiology [140].

During reperfusion of a previously ischemic heart, rats supplemented with palm oil had ventricular premature beats comparable to those with PUFA-rich sunflower oil [141]. In addition, the incidence of severe ventricular fibrillation was much less after feeding palm-oil than it was after feeding a saturated animal fat. Clearly, not all saturated fats have the same physiological impact. Fish oil gave significantly lower reperfusion arrhythmias independent of antecedent ischemic arrhythmias. Fatal ventricular fibrillation was significantly lower after supplementation with fat rich in *n*-6 (8%; *n* = 25) and *n*-3 (0%; *n* = 24), but not with monounsaturated (36%; *n* = 25) or saturated fat (42%; *n* = 24) [142]. Results with isolated myocytes (see

below) show that *n*-6 eicosanoids may produce paradoxical helpful and harmful actions.

A canine model of sudden death confirmed that fish oil supplementation can prevent cardiac arrhythmias [143]. To study the mechanism for this effect, Kang and Leaf observed beating rates of cultured cardiac myocytes, which were consistently decreased with added *n*-3 HUFA [144]. However, the *n*-6 HUFA, arachidonate, sometimes gave either faster, slower or unchanged rates. Interestingly, treatment with oxygenase inhibitors allowed the *n*-6 HUFA to consistently decrease the rates [144]. This effect illustrates an important difference between *n*-6 and *n*-3 HUFA that is likely mediated by eicosanoids. This likelihood was confirmed in direct tests with eicosanoids which showed that almost all *n*-3 metabolites were not arrhythmogenic with cardiac myocytes, whereas the *n*-6 eicosanoids, PGE<sub>2</sub> and PGF<sub>2</sub>, were [145].

Complex interactions of *n*-6 and *n*-3 eicosanoids in affecting signals of the sympathetic system [146] make a tangled mixture of possible consequences from different mechanisms during ventricular and atrial fibrillation following ischemia and reperfusion. These events are modulated, in turn, by sympathetic tone influencing heart rate variability, which is also influenced by HUFA proportions [147]. A recent review noted that years of research left the hypothesis of anti-arrhythmic effects of fish oil 'unproved but still viable' [148]. A recent workshop noted that *n*-3 HUFA affect ion channels, exchangers and moderators, and more research is needed to form evidence-based public policy statements [149].

#### 4.5. Different *n*-3 and *n*-6 dynamics

An early event in an eicosanoid-mediated response is release of the HUFA precursor from the 2-position of membrane phospholipids [110]. The hydrolytic release seems indiscriminate with regard to *n*-3 or *n*-6 structures [150]. However, in Americans, the *n*-6 arachidonate is the dominant HUFA because of voluntary food choices rather than gene-defined enzymatic selectivity. High amounts of *n*-6 linoleate in the USA diet compete with much smaller amounts of *n*-3 linolenate in forming tissue HUFA, creating an oversimplified impression that the *n*-3 PUFA is less efficient than the *n*-6 PUFA in the elongation-desaturation pathway.

Prostaglandin formation by oxidation of HUFA, however, does proceed more rapidly with the *n*-6 arachidonic acid than the *n*-3 eicosapentaenoic acid [151,152]. This selectivity may provide more rapid and intense amplified responses with *n*-6 than *n*-3 eicosanoids [14]. Early evidence that tissue receptors might discriminate between *n*-6 and *n*-3 eicosanoids in transmitting hormone-like signals came from studies with the newly discovered prostacyclin [153]. The *n*-6 and *n*-3 homologs of anti-aggregatory prostacyclin (PGI<sub>2</sub> and PGI<sub>3</sub>) had equal potency, whereas the *n*-3 homolog of aggregatory thromboxane (TXA<sub>3</sub>) seemed less potent than the *n*-6 homolog, TXA<sub>2</sub>. This

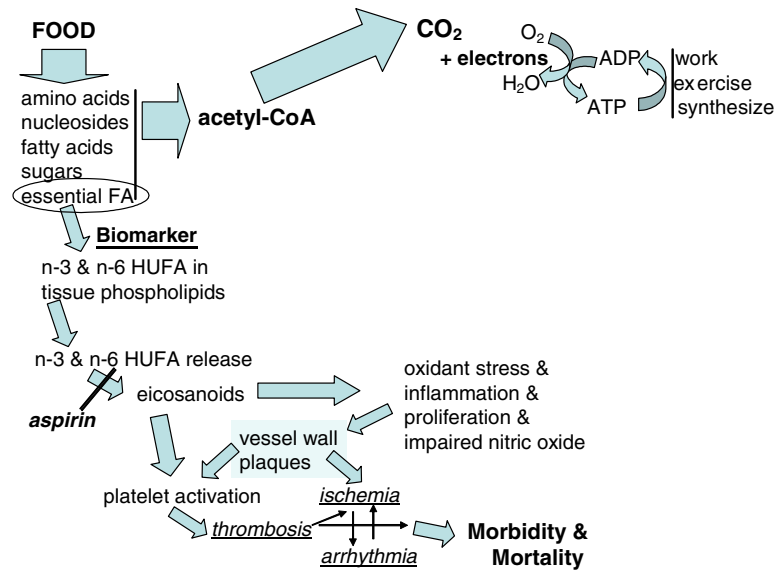


Fig. 6. Eicosanoids mediate effects of food on health.

observation led to a hypothesis for how eating seafood might lower tendencies for thrombosis and atherosclerosis [153].

More refined tools of molecular biology are starting to give evidence of many different enzyme and receptor selectivities that favor  $n-6$  over  $n-3$  actions [154]. The evidence confirms ways in which eating  $n-3$  fatty acids might give less intense eicosanoid-mediated responses (and less pathology) through various tissue receptors. For example, signals from prostaglandin E acting at the EP4 receptor appeared five-fold greater with  $n-6$  than with  $n-3$  homologs. Similarly, the  $n-6$  PGF is much more active than the  $n-3$  PGF at the FP receptor.

Surprisingly, one PGD receptor, DP1, appeared to be the only gene product that acts more strongly with the  $n-3$  than the  $n-6$  homolog [154]. The investigators hypothesized that this preference might help explain anti-aggregatory actions of  $n-3$  HUFA. More knowledge of the detailed discriminations during selective receptor signaling will likely strengthen the framework for logical inferences about ways that the current typical (i.e., statistically ‘normal’) levels of  $n-6$ -rich foods in the USA cause excessive proportions of  $n-6$  eicosanoids that may exacerbate serious chronic diseases like cardiovascular disease, a major preventable cause of death. Similar diet-based amplified events may occur in some inflammatory proliferative disorders including cancer, dementia, arthritis and asthma [14,114,155]. Evidence for this hypothesis is slowly accumulating from early clues from the MRFIT trial [156] to more recent trials with controlled tissue biomarkers confirming the hypothesis [157].

Like all hypotheses, it may yet be proved incorrect, but the paradigm continues to be strengthened and extended by the ‘normal science’ of the past 30 years. Another concept recently extending and expanding the hypothesis of opposing effects of  $n-3$  and  $n-6$  HUFA describes new anti-inflammatory derivatives, resolvins and protectins [158].

These hormone-like mediators are formed from tissue  $n-3$  HUFA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Specific members of this new class of mediators appear to moderate directly the magnitude and duration of inflammation in ways that complement the inhibitory action of EPA and DHA on  $n-6$  eicosanoid formation.

By 1985, the dynamic aspects of forming and removing active eicosanoids from tissue HUFA [111,112] were known, and many pharmaceutical companies had initiated drug development programs to design, patent and market agents that could diminish excessive  $n-6$  eicosanoid actions. Knowledge of the intense  $n-6$  eicosanoid actions mediating health and disease had increased greatly, and the benefits of increasing dietary  $n-3$  fats were becoming well recognized [14]. The importance of so many powerful HUFA-based hormones affecting inflammation, thrombosis, atherosclerosis, asthma and arthritis led to the 1982 Nobel award. The biomedical community was beginning extensive use of aspirin (an NSAID) for secondary prevention of  $n-6$  eicosanoid-mediated heart attacks.

The time seemed right in the mid-1980s to begin designing a primary prevention trial to test the paradigm that altering food choices to raise tissue levels of  $n-3$  HUFA and lower  $n-6$  HUFA could beneficially diminish excessive  $n-6$  eicosanoid actions and decrease the need for lifelong medications. The paradigm includes voluntary food choices diminishing known mediators in pathways that connect food choices to undesired clinical outcomes of inflammation, thrombosis, ischemia, arrhythmia and death (Fig. 6).

## 5. Tools to monitor preventive interventions

Some biomarkers noted in Figs. 2 and 6 may be useful surrogates in early detection of CVD disease before more serious clinical signs and symptoms appear [1]. However,

the transient nature of the short-lived eicosanoid mediators does not allow suitable measurements to predict clinical risks for individuals in the general population. After years of exploring eicosanoid formation and anti-inflammatory mechanisms, I received an unrestricted Pfizer Biomedical Research Award. The funds let me measure quantitative effects of dietary PUFA on tissue HUFA which we used to design tools for nutritional primary prevention interventions.

Altered intakes of essential fatty acids predictably alter tissue levels of HUFA, making tissue HUFA status useful for monitoring compliance during dietary interventions. In addition, the HUFA precursor proportions are useful for predicting the likely intensity of  $n-6$  eicosanoid actions and subsequent pathology. Thus, results in Fig. 5 and in Section 4 suggest that the desired relationship between treatment, tissue HUFA biomarker (surrogate endpoint) and clinical endpoint [1] could likely be arranged in a clinical trial of primary prevention. Recognizing that the ultimate priority of clinical interventions will remain obtaining proof of improved signs, symptoms and survival [1], studies evaluating tissue HUFA status as a surrogate endpoint for primary prevention of CVD need to be discussed, debated and developed.

### 5.1. Prevention trials need valid surrogate endpoints

Confirming the pioneering results of Mohrhauer and Holman [9] (see Fig. 4), my group developed an empirical hyperbolic equation to predict the quantitative effects of diet on the proportions of HUFA in rat tissues [159]. Considering how diverse PUFA contribute to tissue HUFA (Fig. 3 in Section 3.3), we grouped four types of acid with different metabolic properties (SFA, UFA, PUFA and HUFA) to define quantitative effects of dietary PUFA on the composition of fatty acids in rat tissues [159].

For example, triacylglycerols of plasma, liver and adipose linearly accumulated higher levels of PUFA with higher amounts in the diet [159]. In contrast,  $n-3$  and  $n-6$  HUFA accumulated in tissue HUFA in a competitive hyperbolic manner, displacing  $n-9$  HUFA (Fig. 4). As a result, when diets have more than 5% of energy ( $>5$  en%) as PUFA, the  $n-9$  HUFA abundance is negligible [8], and tissues accumulate only  $n-3$  and  $n-6$  HUFA. In this way, the percent of  $n-6$  in HUFA becomes simply 100% minus the percent of  $n-3$  in HUFA, and the absence of  $n-9$  HUFA is an indicator of relatively high PUFA intakes

[89]. Also, the presence of more than 50%  $n-6$  in HUFA is an indicator of relatively low intakes of  $n-3$  PUFA and/or  $n-3$  HUFA.

Lipid chemists traditionally report the content of a fatty acid as a percent of all fatty acids in the sample analyzed. However, some tissue samples contain mixtures of phospholipids, triacylglycerols and cholesterol esters, each with different patterns of fatty acid composition. Thus, the percent of  $n-6$  HUFA in total erythrocyte fatty acids is influenced by the HUFA-rich phospholipids which are the major lipids in this tissue. However in plasma, HUFA are a lower percent of total fatty acids because of variable amounts of triacylglycerols and cholesterol esters which contain more SFA, UFA and PUFA than HUFA and increase the denominator in the calculation. Fig. 7, using symbols from Fig. 3, shows how the additional acids (J + K + L + M + N + O + P) decrease the calculated value.

The effect of the non-HUFA acids is evident in reports of fatty acids in plasma and erythrocytes (which have similar proportions of  $n-3$  and  $n-6$  in their HUFA). Plasma fatty acids of some Americans had 2.2 wt%  $n-3$  HUFA and 9.9 wt%  $n-6$  HUFA, and relative proportions of the HUFA were 18%  $n-3$  and 82%  $n-6$  [160]. Erythrocyte fatty acids in these people had higher values of 5.6 wt% of  $n-3$  HUFA and 19.3 wt%  $n-6$  HUFA, but the proportions of HUFA (22%  $n-3$  and 78%  $n-6$ ) were close to those of plasma. In a similar way, plasma fatty acids of some Japanese had 5.0 wt% of  $n-3$  HUFA and 5.6 wt%  $n-6$  HUFA, whereas the proportions of HUFA were 47%  $n-3$  and 53%  $n-6$  [161]. Again, erythrocytes had higher percentages of HUFA (12.0 wt% of  $n-3$  HUFA and 12.3 wt%  $n-6$  HUFA) although the proportions of HUFA (49%  $n-3$  and 51%  $n-6$ ) were close to those in plasma.

Results with rats showed that the quantitative relationships between dietary PUFA and the %  $n-6$  in HUFA were similar for liver, plasma and erythrocytes [159]. The use of erythrocyte total fatty acids in clinical studies has been reported [162]. However, knowing the similar proportions of HUFA in plasma and erythrocytes makes it rational to simply measure the proportions of HUFA in whole blood without expending the time and effort in centrifuging samples [163]. In fact, the proportion of  $n-3$  in HUFA may be measured in a drop of blood [164] to simplify the logistics of monitoring dietary interventions or assessing an individual's likely risk of CHD.

### **Biomarkers of HUFA status in tissues are:**

$$\%n-3 \text{ in HUFA} = 100 \cdot (F+G+H) / (A+B+C+D+E+F+G+H)$$

$$\%n-3 \text{ HUFA in FA} = 100 \cdot (F+G+H) / (A+B+C+D+E+F+G+H+J+K+L+M+N+O+P)$$

$$\%n-6 \text{ in HUFA} = 100 \cdot (A+B+C) / (A+B+C+D+E+F+G+H)$$

$$\%n-6 \text{ HUFA in FA} = 100 \cdot (A+B+C) / (A+B+C+D+E+F+G+H+J+K+L+M+N+O+P)$$

Fig. 7. Calculating biomarkers of tissue HUFA status. Symbols are from Fig. 3.

5.2. Quantitative empirical relationships

The quantitative diet studies with rats [159] were extended to describe how dietary PUFA affect tissue HUFA in humans [165]. The linear accumulation of dietary PUFA in triacylglycerols [84,165] was confirmed. In addition, the empirical hyperbolic equation and constants were expanded from the rat studies [159] to fit daily intakes of HUFA as well as PUFA. The relationship published in 1992 (Fig. 8) fit results with rats, mice and humans, but it was initially based on a limited number of human subjects [165]. Later access to carefully controlled data from hundreds of Japanese [104] allowed revision of three of the eight constants to fit data shown in Fig. 9. The competition between *n*–3, *n*–6 and *n*–9 acids for accumulation in tissue HUFA is reflected in the terms of the empirical hyperbolic equation that has aspects that parallel the Michaelis–Menten formula noted in Section 2.2.3.

The diet-dependent biomarker (% *n*–6 in HUFA) ranges from 20% to 80% (Fig. 9), and it is associated closely with CHD mortality (Fig. 5). The biomarker predicts

the likely intensity of *n*–6 eicosanoid-mediated inflammatory, thrombotic and arrhythmic events, provides an estimate of absolute CHD risk, and monitors each individual’s success in making desired dietary changes during primary prevention [166]. The empirical equation for the *n*–6 biomarker (and its complementary *n*–3 biomarker) helps clinicians design dietary interventions that would likely change tissue HUFA proportions enough to favorably alter physiological status (see Fig. 5). In this way, the empirical diet-tissue relationship supports a general strategy for the public to lower dietary intakes of *n*–6 fats and raise intakes of *n*–3 fats. Such changes might decrease causal tissue mediators and provide life-long effective primary prevention of the current epidemic of CHD medication, morbidity and mortality.

Knowing dietary intakes of four types of acids (*n*–3 and *n*–6 PUFA plus *n*–3 and *n*–6 HUFA) allows estimates of likely tissue proportions of *n*–3 and *n*–6 HUFA. When colleagues complained that the empirical equation (Fig. 8) was intimidating and awkward to use, we embedded the equation and constants into a small spreadsheet to permit estimates without seeing the calculations involved (Fig. 10). The calculator can be accessed at the website <http://efaeducation.nih.gov/sig/dietbalance.html>.

Fig. 10 has examples of how dietary intakes of PUFA and HUFA expressed in terms of the percent of food energy (en%) relate to the proportion of *n*–6 that would likely be maintained in tissue HUFA. The calculator helps estimate a likely efficacy for dietary *n*–3 PUFA in decreasing the % *n*–6 in tissue HUFA (i.e., elevating % *n*–3 in HUFA). For example, increasing the intake of 18:3*n*–3 by 0.5% of food calories (0.5 en%) in the USA diet of Fig. 10 gives an estimated decrease in % *n*–6 HUFA in tissue HUFA from 80% to 77%. In contrast, adding 0.5 en% *n*–3 HUFA that does not need to compete in the elongation-desaturation pathway gives an estimated 57% *n*–6 in HUFA. Unexpectedly, once the planning ‘calculator’ was available, it generated requests from investigators for help

**Tissue HUFA are maintained by dietary PUFA**  
an empirical hyperbolic metabolic relationship

$$\frac{20:3+20:4n-6}{\text{in HUFA}} = \frac{100}{1 + \frac{HC_6}{en\%H6} \left(1 + \frac{en\%H3}{HC_3}\right)} + \frac{100}{1 + \frac{PC_6}{en\%P6} \left(1 + \frac{en\%P3}{PC_3} + \frac{en\%H3}{HI_3} + \frac{en\%O}{C_0} + \frac{en\%P6}{Ks}\right)}$$

$HC_3 = 3.0$      $HC_6 = 0.70$      $PC_3 = 0.0555$      $PC_6 = 0.0441$   
 $HI_3 = 0.005$      $HI_6 = 0.040$      $C_0 = 5.0$      $Ks = 0.175$

Lands et al, BBA 1180: 147-162 (1992)  
[constants revised in 2002]

( <http://efaeducation.nih.gov/sig/hufacalc.html>)

Fig. 8. Empirical estimates of tissue HUFA maintained by food. The equation and revised constants are at <http://efaeducation.nih.gov/hufacalc.html>.

From Lands (2003) Curr.TopicsNutraceut.Res.1: 113-120

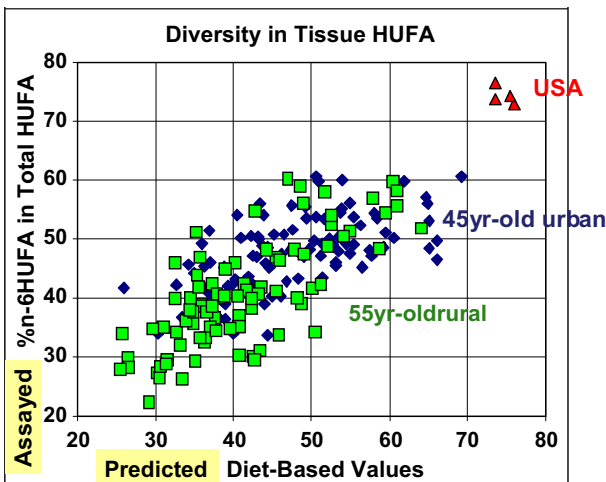


Fig. 9. Observed proportions of *n*–6 in HUFA fit predicted proportions. Figure is modified from Ref. [104].

A handy calculator for planning trials is at:  
<http://efaeducation.nih.gov/sig/dietbalance.html>

**DIET PREDICTS HUFA PROPORTIONS IN PLASMA**

	Trial Diet	Typical diets			
		USAM	edit	Japan	
AVERAGE DAILY DIETARY INTAKES					
en% 18:3 <i>n</i> -3 >>>	0.30	0.85	0.50	0.76	(short 3)
en% 18:2 <i>n</i> -6 >>>	1.00	6.82	2.30	5.04	(short 6)
en% <i>n</i> -3 HUFA >>	0.50	0.03	0.09	0.54	(long 3)
en% <i>n</i> -6 HUFA >>	0.08	0.08	0.08	0.08	(long 6)
PREDICTED					
% <i>n</i> -6 in HUFA	25	80	63	51	

An equation predicting HUFA dyslipidemia is at:  
<http://efaeducation.nih.gov/sig/hufacalc.html>

Fig. 10. A planning calculator for estimating intervention outcomes. Available at <http://efaeducation.nih.gov/dietbalance.html>.

in identifying foods that provide the needed daily intakes. Few people know how much  $n-6$  fat they eat per day.

### 5.3. Coupling to a food database (KIM2)

To inform people about the amounts of the four types of PUFA and HUFA in their daily foods and to manage the hundreds of detailed arithmetical calculations involved, the empirical equation (Fig. 8) was combined 7 years ago with the US Department of Agriculture (USDA) nutritional database to give personalized interactive software called KIM (Keep It Managed). An upgraded version, KIM-2, can be downloaded free from the website <http://efaeducation.nih.gov/sig/kim.html>. The interactive program helps people discover desirable food choices that meet their own personal taste preferences and sense of risk aversion. The interactive program shows the milligrams of  $n-6$  and  $n-3$  PUFA and  $n-6$  and  $n-3$  HUFA in selections from nearly 12,000 servings of food, arranging the selections either by mealtimes or by food groups (Fig. 11). The software combines the amounts of food energy and milligrams of PUFA and HUFA in foods selected for the day's menu plan, expressing the sum as en%  $n-6$  and  $n-3$  PUFA and  $n-6$  and  $n-3$  HUFA.

These four sums enter the empirical equation (Fig. 8) to estimate a likely balance of eicosanoid precursors that may result in tissue HUFA after extended use of the daily meal plan (Fig. 12). KIM-2 also stores, retrieves and prints prepared meal plans and recipes which can be further modified to meet personal preferences of people being counseled by dietitians and clinicians. With knowledge of nutrient contents, individuals can make informed voluntary food choices needed to maintain a HUFA status desired for primary prevention of excessive  $n-6$  eicosanoid-dependent

pathology. The interactive software permits rapid 'tailoring' of pre-formed meal plans to fit individual personal tastes and desires which can be further reinforced with intermittent diet counseling. The design goal was to develop and print a personalized daily meal plan during a 15-min consultation.

### 5.4. Making HUFA proportions visible (GC assay)

The discussion of food energy in Section 3.1 noted major failures of compliance during efforts to alter people's food habits. In general, clinical trials to modify people's behavior toward food have a dismal history of non-compliance, causing misleading messages about whether the intervention itself actually works. Results with compliant individuals show success (e.g., [40]), but failure of compliance creates a general sense of overall failure with the intervention. The cause-effect connection between food energy and body mass allows people to monitor success with food energy balance as they maintain a healthy weight. A similar sort of information feedback is needed to monitor the success of food choices that maintain a healthy proportion of tissue HUFA.

When people know their HUFA biomarker status (and its relation to risk), they may be better motivated to choose foods that increase  $n-3$  and decrease  $n-6$  intakes and maintain their  $n-6$  biomarker at a level that fits their personal sense of risk aversion. A convenient low-cost measurement of the  $n-6$  biomarker could help each person monitor [166] and maintain personal success during large scale primary prevention interventions. In addition, correlating an individual's biomarker status with observed physiological and clinical measures over time can let statistical analyses manage varied individual compliance levels and

The screenshot shows the KIM software interface. At the top, there are menu options: MAIN MENU, Tutorial, Mealtimes, Food Groups, Client Info, Report. The client name is 528 Bill Johnson 2112. Below this, there are buttons for 'Save Plan', 'Retrieve Plan to be Changed', 'Household Measures', 'Retrieve Exchanged Plan', and 'Delete this Plan'. A summary bar shows: 2342 Kcal, %Cal: 1.57%, 0.47%, 0.04%, 1.13%, and 16% predicted % long 6 in total long in your body. The main area is divided into food groups: Cereals, Grains & Breads; Vegetables; Fruits; Dairy & Egg; and Meats, Fish & Legumes. Each group has a table of food items with columns for Serving Size, gm, Servings, kcal, short 6, short 3, long 6, and long 3. A yellow callout box points to the 'mg' column in the 'Cereals, Grains & Breads' section, with the text 'See milligrams of EFA per serving'.

Food Group	Serving Size	gm	Servings	kcal	short 6	short 3	long 6	long 3	
Cereals, Grains & Breads	Bread, mixed-grain, toasted (includes)	1 slice	24	1	65	226	15	0	0
	Bread, cracked-wheat	1 slice	25	2	130	324	17	0	0
	Cereals ready-to-eat, KELLOGG,	0.75 cup (1	29	1	92	297	21	1	0
	Spinach, raw	1 cup	30	1	7	7	35	0	0
Vegetables	Mushrooms, raw	0.5 cup pieces	35	1	9	47	0	0	
	Potatoes, boiled, cooked in skin, flesh,	1 potato	136	0.5	59	22	7	0	
	Lettuce, looseleaf, raw	0.5 cup,	28	1	5	13	32	0	
Fruits	Apples, raw, with skin	1 medium	138	1	81	12	0	0	
Dairy & Egg	Butter, with salt	1 pat (1" sq,	5	1	36	92	59	0	0
	Milk, reduced fat, fluid, 2% milkfat, with	1 cup	244	1	122	105	68	0	0
	Cheese, cottage, nonfat, uncreamed,	4 oz	113	1	96	12	5	0	0
	Cheese, gruyere	1 slice (1 oz)	28	1	116	364	121	0	0
Meats, Fish & Legumes	Fish, salmon, coho, wild, cooked, dry	0.5 fillet	178	1	247	100	190	39	1885

Fig. 11. An interactive computerized menu planning program. Available free at <http://efaeducation.nih.gov/sig/kim.html>.

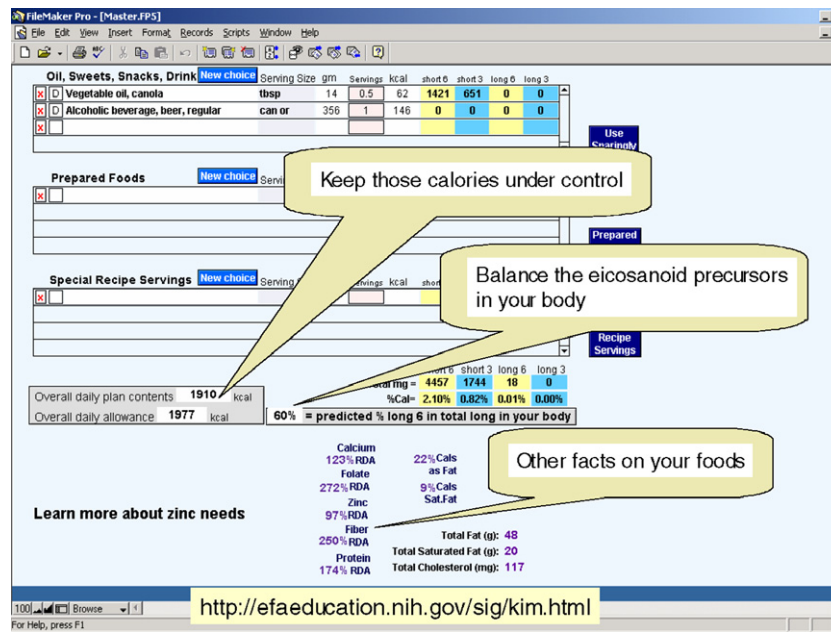


Fig. 12. Using nutrient data and keeping it managed (KIM-2). Available free at <http://efaeducation.nih.gov/sig/kim.html>.

confirm or deny the efficacy of the biomarker as a surrogate endpoint in an open voluntary dietary intervention. Diverse food servings, compliance levels and gene patterns that modulate accumulated biomarker levels are influences that can be tested separately after the biomarker is validated as a surrogate endpoint correlated with CVD events.

An early approach to wide-scale monitoring of the biomarker in individuals used 50  $\mu$ l finger-tip blood samples and robotic injectors for gas chromatography (GC) to measure the HUFA status of 20 individuals per day [167]. However, recent technology has faster GC separations [168,169] and automated robotic derivatization [170,171] that can form a high-throughput laboratory system that might analyze HUFA biomarker status for 100,000 samples per year, possibly at an average cost below \$10 per sample. The GC assay will likely prove easier, cheaper and more valid than the tedious and imprecise diet interviews using personal recall which are currently used in epidemiological studies.

In an era of rapidly increasing health care costs, health professionals in Northern Europe and the USA might beneficially assay the  $n-6$  biomarker at any stage in the life course of cardiovascular disease (CVD) for which they want to confirm an estimated likely risk (see Fig. 5). Knowing personal  $n-6$  biomarker values may help focus public attention on effective life-long food selections for primary prevention of a causal factor in the current epidemic of diet-induced CVD morbidity and mortality.

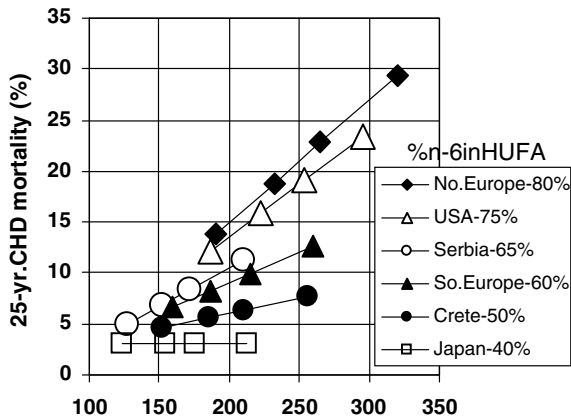
### 5.5. Comparing biomarkers

Blood cholesterol is often a target of secondary prevention in people with observed clinical signs and symptoms

for whom statins are used to slow the synthesis of cholesterol from unburned metabolites of ingested foods (see Fig. 2). However, extensive attention to the blood cholesterol biomarker over the past 20 years has not provided an effective focus for primary prevention of imbalances in food energy and body fuel management. Also, cholesterol is only indirectly connected (see Fig. 2) with known causal mechanisms for inflammation, thrombosis and arrhythmia [113,114]. In the USA and Northern Europe, blood cholesterol abundance associates with risk of death from CHD, although it has no clear association with CHD risk in Japan [172].

A 25-year prospective follow-up of the Seven Countries Study [172] showed that blood cholesterol alone did not predict the absolute CHD death rates observed for different groups worldwide (Fig. 13). Absolute death rates differed at each cholesterol level, and the authors considered factors affecting inflammatory oxidative processes and thrombosis to be “of great importance” [172]. Indeed, combining the likely magnitude of the inflammatory and thrombotic  $n-6$  biomarker with the total cholesterol (TC) biomarker in the following way,  $\text{CHD deaths} = 3 + 3 \times (\% n-6 \text{ in HUFA} - 40) \times (\text{TC} - 100)/1000$ , closely predicts the observed absolute death rates (Fig. 13).

Estimates of likely values for the average percent of  $n-6$  in HUFA for the six diverse mixed populations (see also Fig. 5) are listed in the insert of Fig. 13. This biomarker has its highest average values in Northern Europe and the USA. The close fit of observed and predicted values of absolute mortality for all of the diverse subsets suggests, but does not prove, that increased amounts of food metabolites (which form mevalonate and isoprenoids noted in Fig. 2) may NOT cause death when the  $n-3$  and  $n-6$  HUFA in tissues are balanced.



Food energy imbalances which elevate blood cholesterol may be fatal only to the degree that omega-6 (n-6) exceeds omega-3 (n-3) in tissue HUFA.

Fig. 13. Predicting outcomes observed in the Seven Countries Study. Modified from data in Ref. [172]. Predicted absolute death rates are based on an empirical equation,  $\text{CHD deaths} = 3 + 3 \times (\% n-6 \text{ in HUFA} - 40) \times (\text{TC} - 100)/1000$ , that combines TC values with an estimated value for the likely average percent of n-6 in HUFA of blood lipids.

Fig. 13 also suggests that the blood cholesterol biomarker of imbalanced fuel metabolism (Fig. 2) may predict CHD risk ONLY to the extent that the pro-inflammatory and pro-thrombotic n-6 biomarker exceeds its complementary anti-inflammatory and anti-thrombotic n-3 biomarker (Fig. 6). This idea may be important when planning effective primary prevention of CVD. In fact, Fig. 13 suggests that balancing the n-3 and n-6 HUFA in human tissues might give more effective primary prevention of CVD than decreasing the formation of cholesterol. Tests of these hypotheses can be done with tools now on hand. However, agencies willing to support tests of a nutritional preventive intervention are less readily identifiable.

## 6. Priorities in primary prevention

### 6.1. Waiting for treatment?

Is it ethical (or even economical) to withhold community-wide preventive nutrition efforts and only wait until after disease signs appear in individuals who may then be treated to remove the signs (surrogate biomarkers) but not the initial causes of disease? Without prevention of primary causes, each generation faces a growing prevalence of disease. Without proper knowledge, people can only wait until they accumulate enough signs and symptoms to qualify for expensive treatments that must then continue for life. Informed forecasts indicate that unprecedented reductions in recognized risk factors will likely be needed to prevent a rise in prevalence and incidence of CVD among Americans as the 'baby boom' generation matures [173]. More than one in five Americans will likely develop definite symptoms of CVD before the age of 60, and more than one

in three will die from CVD [173,175]. Thus, the evident pride of the biomedical profession in expensive treatments that prevent death has no corresponding pride in preventing conditions that cause the need for the expensive treatments.

Histological measurements of 3000 young Americans [176,177] confirm earlier evidence from soldiers in Korea [178] and emphasize that true primary prevention of the dietary causes of CVD must begin early before significant vascular damage accumulates, and the efforts must be maintained throughout the life span. The diet-induced disease begins before adolescence and progresses until treatment or death intervenes (Fig. 14). Dozens of articles from the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group give conclusive evidence of the inexorable progress of vascular damage for which early prevention procedures need to begin with children and adolescents [176].

Autopsy showed fatty streaks in abdominal aortas of about 20% of 15- to 19-year-old subjects and nearly 40% of 30- to 34-year-old subjects. Raised fatty streaks were present in the right coronary arteries of nearly 10% of 15- to 19-year-old subjects and approximately 30% of 30- to 34-year-old subjects [176,177]. All subjects in the study had atherosclerosis by the age of 15 [176]. Progression of vascular damage is usually well established before the age of 40.

A similar pattern of vascular pathology in Japan is becoming increasingly severe as the Japanese lifestyle becomes westernized [105,106]. In addition, an increasing prevalence of 'western-type' cancers in Japan accompanies increased eating of n-6 fats [179]. Many eicosanoid-linked diseases might be lessened with more dietary n-3 HUFA [14,180]. To estimate a healthy dietary allowance for n-3 HUFA, Hibbeln et al [181] defined a deficiency in n-3 HUFA as the attributable risk from 13 morbidity and mortality outcomes, including all causes, CHD, CVD, homicide, bipolar disorder, as well as major and postpartum

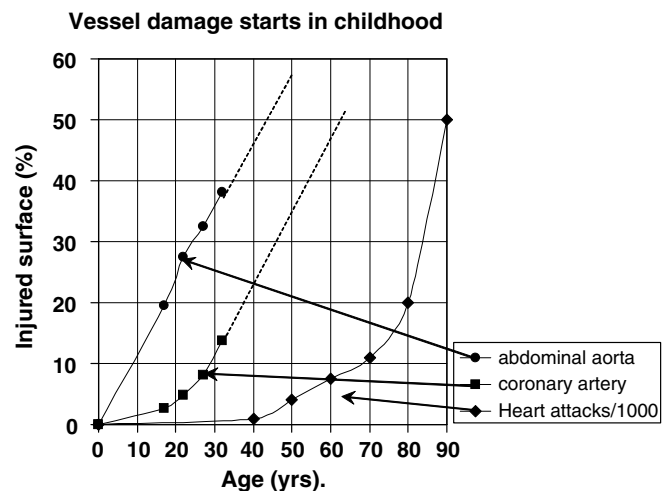


Fig. 14. Primary prevention should begin in childhood. Results from Ref. [176]. Death rates are from [113,114].

depressions. This led to estimates of the amount of dietary  $n-3$  HUFA that could likely maintain tissue HUFA proportions at levels associated with 98% of the population being free of risks attributable to deficient intakes of  $n-3$  HUFA.

Higher intakes of competing dietary  $n-6$  fats make it necessary to eat more  $n-3$  HUFA to attain this goal [181]. Thus, the needed allowance for USA might be much less if ingested  $n-6$  fat was closer to 1 en% rather than the current 8%. Some trial-and error estimates with the calculator in Fig. 10 might allow better understanding of the phenomenon evident in Fig. 4. Reducing intakes of 18:2 $n-6$  will still maintain high proportions of  $n-6$  in tissue HUFA unless competing  $n-3$  HUFA are available to displace  $n-6$  HUFA from tissue phospholipids.

Nutritional imbalances that alter composition and function of tissue HUFA (and likely cause CVD and many chronic diseases of the elderly) can easily remain in lifestyle habits of individuals even after drug treatments have suppressed diagnostic clinical signs and symptoms. Importantly, causal dietary imbalances act chronically over decades before clinical signs become easily observed and before the person becomes a patient to be treated. As a result, even before people become patients, omega-3 enriched foods may become important tools for primary rather than secondary prevention [104]. Preparing and marketing such foods is a rapidly growing financial venture.

Improved health care procedures have cut death rates of CVD and CHD. However, the incidence of hospitalization for myocardial infarction during 1987–1994 was stable or slightly increasing [174], and the prevalence of CVD remains very high [175]. More than 70 million Americans (about 1 in 3 adults) have some form of CVD. About 1.2 million Americans have a heart attack each year. CVD will cost the nation an estimated \$403 billion in 2006, including health expenditures and lost productivity [175].

## 6.2. Follow the money

Most people develop traditional food habits as they grow up within families and communities. Differences in these habits are evident in the diet-dependent  $n-6$  biomarker of %  $n-6$  in HUFA, which monitors early upstream conditions that precede events causing tissue pathology and death (Fig. 6). The magnitude of this biomarker alerts people to take preventive personal dietary actions rather than waiting for eventual downstream signs of pathology to accumulate. Modern methods of food processing and marketing have neglected the information in Fig. 6 while they appreciably alter the types of food oils that are eaten in America [181,182].

Global market dynamics create worldwide shifts in food availability which are controlled by corporate priorities of the food industry (e.g., shelf-life stability and profit margins) rather than a priority for better health [183]. Large international corporations are now managing the entry of

the saturated fats of palm oil into processed foods being marketed in the USA, shifting financial flows from USA producers toward Asian palm oil producers. Few scientific messages appear with logical inferences that have skeptical reasoning about evidence of likely health outcomes of this shift. Dr. Nestle provides detailed authoritative insights with rueful hindsight of her personal encounters with the well-entrenched forces controlling our nation's nutrition [183]. Readers can learn much from her insightful review of the sharp disparity in ethical standards between corporate executives, members of Congress and staff members in regulatory agencies.

Details of the battles over infant formulas and school lunches inform us how paradoxical messages evolve from the separate priorities of corporations and government legislative and administrative units [183]. Their struggle for power and dominance in defining scientific integrity led to the 1994 DSHEA legislation (Dietary Supplements Health and Education Act) that shifted much responsibility for dietary supplements from government to the public (whose information would then come mostly from corporate marketing advertisements). In the process of relaxing the rigor of inferences and implications, the government opened a door to new expanding markets for functional foods (called 'technofoods' by Nestle). She also describes the legislated system of check-off programs that move \$millions for promotion of products based on certain commodities. In fact, a complex set of government subsidies ensures that some agricultural commodities are financially favored to go into mass-produced prepared foods [184]. The large subsidies paid from the public to large profit-making corporations are not widely recognized by the public. The benefit/cost estimate for such public subsidies have puzzling financial aspects with regard to the public's expenditures for health.

The ethics of preventing unwanted diseases with more logical food use has little societal support compared to the large financial incentives for treating diseases after they occur. Primary prevention would involve the food industry marketing preventive foods and lifestyles (using profits from the public), whereas treatments of disease involve the pharmaceutical industry marketing medications (using profits from the public). In each case, one-sided messages from marketers are the major form of public advice on dietary lipids. In describing how the food industry escapes accountability as it undermines health, Simon [185] describes the imperative for corporate profit growth driving sophisticated efforts to control public perceptions and government regulations. He notes that fragmented responsibilities and accounting processes allowed corporations to divert \$3.5 trillion of externalized costs (for health care expenses, unsafe products and pollution) to other groups funded by the public while it made \$822 billion in profits.

The fragmented responsibility for maintaining skeptical scientific accountability and the fragmented responsibility for conveying valid public messages have created seriously incomplete knowledge and incorrect public awareness of

how imbalanced food hurts health. For example, the Japanese population is currently responding to marketing forces in an experiment of voluntary ‘open label’ intervention with food choices that increase the proportion of  $n-6$  in tissue HUFA from its prior 30% to 70%, which is associated with greater CVD and its consequent health expenses [103–106]. Readers might wonder if any marketing force might support the American population experimenting with a voluntary ‘open label’ intervention that lowers the proportion of  $n-6$  in HUFA from the current 80% to 30% associated with much lower incidence of CVD and its consequent lower health expenses. Who could profit from such an experiment?

Ironically, preventing a disease provides little cash flow to attract the powerful ‘medical-industry complex’ corporations or even smaller entrepreneurs or tax-supported governmental agencies. Apparently, the neglect can only be cured by re-directing the flow of funds from its ultimate origin, the public. However, our society is in a general state of denial that any prevention is needed. Even the major government agency with a prevention mission, the Centers for Disease Control and Prevention of the Department of Health and Human Services, elects to delete ‘P’ (for prevention) from its logo (CDC) and website address ([http://www.cdc.gov/dhdsp/about\\_program.htm](http://www.cdc.gov/dhdsp/about_program.htm); [175]).

The public might hope that this large tax-supported program would provide more benefit/cost evaluations of primary prevention nutrition programs that decrease the prevalence of disease and decrease the need for expensive treatments and medications. Gaps in the incomplete and diverse public messages create and sustain many paradoxes in the public’s knowledge about food and health. The ethics of preventing undesired diseases must confront the financial incentives in treating them. The market makes the message.

Incomplete understanding of food energy consequences noted in Section 3.1 were explored in great detail by Taubes [39], who confronts the conventional wisdom on diet, weight control and disease. He notes the public’s need for scientific knowledge that has been properly tested and validated with rigorous skeptical logic. His book cites a history that makes readers wonder what forces kept the weight-loss diet developed by Harvey for Banting in 1862 from being rigorously tested even by modern biomedical researchers. Section 3.1 notes that mediators that cause higher body weight might also cause CVD even though higher body weight itself may not do so. Body fat may not be in the direct chain of events connecting food to death. Readers could wonder whether any controlled tests have shown weight loss to be a valid and effective surrogate endpoint for successful intervention in diet-induced CVD (e.g., [21]).

Much evidence points to a causal role in CVD for two important nutritional imbalances: expenditure/intake of energy (Fig. 2) and omega-3/omega-6 essential fatty acids (Fig. 6). This review provides biochemical details for how the %  $n-6$  in HUFA might be an effective surrogate end-

point for intervention in diet-induced CVD. Extending this paradigm, a recent book by Tribble [186] provides a readable story for the public (compared to the detailed biochemistry in [14]). It considers applications of the paradigm in a style suited to steering the broader public toward using recipes and menus to make desired lifestyle alterations that might help them achieve a desired tissue balance of  $n-3$  and  $n-6$  HUFA. Complementing the books described in this section [14,39,183–186] is a recent one by Allport [187] that gives an entertaining history of the people involved in interpreting the current ‘Western’ diet with regard to maintaining balanced tissue HUFA.

This review article describes two preventable dietary imbalances for which specific clinical trials can be designed, discussed, debated and developed. A nutritional primary prevention study should use a biomarker that is a proved mediator connecting food choices to clinical endpoints of CVD morbidity and mortality [1]. In the case of energy homeostasis, Fig. 2 shows biomarkers that are increased by food choices, but the biomarkers may not mediate the fatal chain of CVD events (even though they are indirectly associated with them).

In the case of polyunsaturated fatty acids, tissue HUFA can only come from food choices. Fig. 9 shows that the %  $n-6$  in HUFA is modified by food choices. Tissue HUFA are direct precursors of the hormones mediating the fatal chain of CVD events (Fig. 6). With this valid surrogate endpoint [1], long-term measures of CVD morbidity and mortality can be tested for their fit to the surrogate endpoint (as in Fig. 5). An almost dogmatic view holds that the ‘gold standard’ of clinical trials for patented drugs is a double-blind placebo-controlled crossover trial. However, such a protocol without variable daily voluntary food choices is not suited to compliance in a long-term voluntary nutrition trial. The solution for a primary prevention trial is to measure how well the biomarker value of intervention compliance for each person (which is a surrogate endpoint) fits the eventual long-term longitudinal data on clinical outcome for that person. Results from Japan [103–106] suggest that the correlation seen in Fig. 5 will be confirmed in the USA.

## 7. Views in a different silo

### 7.1. Cholesterol suppresses lipid synthesis

While the Nobel prize-winning insights of hormone-like eicosanoids derived from vitamin-like HUFA were recognizing important amplifiers in inflammation and thrombosis [109,129,188], other Nobel prize-winning insights were recognizing receptor-mediated endocytosis to be suppressing gene expression. Brown and Goldstein [189] showed that people with familial hypercholesterolemia have defective LDL receptors with impaired endocytosis that hinders uptake of circulating cholesterol. As a result, inadequate cholesterol suppression in the cell allows high rates of HMG-CoA reductase expression and rapid formation of

mevalonate associated with heart attacks early in life [189]. As with the amplifying signals of eicosanoids, the suppressing signals of cholesterol involve multi-step chains of causal mediators which we need to understand.

The incidence of familial hypercholesterolemia in populations worldwide is about 1 in 500 (0.2%). Blood cholesterol levels alone are not sufficient for a diagnosis of familial hypercholesterolemia because other mediators in the complex signaling pathway connecting blood cholesterol to gene expression might also diminish cholesterol's ability to suppress gene expression (e.g., [190]). Epidemiological studies with groups having different proportions of familial hypercholesterolemia will give incorrect predictions for the general population [191]. Most importantly, for the 99.8% of the population NOT having defective LDL receptors, we need to identify other mediators and mechanisms for the vascular injury and CHD mortality that cause nearly a third of all deaths in the USA [173].

During the past 20 years, the research program of Brown and Goldstein moved beyond beneficial effects of cholesterol uptake to provide hundreds of reports about the important network of signaling proteins that mediate those effects. They open new views of food energy management. For example, a recent report [192] described a mechanism for Schoenheimer's 72-year earlier demonstration of feedback suppression of cholesterol synthesis. Suppression by cholesterol is mediated by proteins called Insigs that interact with sterol response element-binding proteins (SREBP) and SREBP-cleavage activating protein (SCAP). The proteins interact in ways that regulate the activity of sterol response elements (SRE) of genes coding many enzymes that convert food energy into tissue lipids. In this way, feeding more cholesterol decreased hepatic sterol synthesis by 93% in normal mice, but not in the mice lacking Insig (which also had a five-fold higher rate of synthesis, [192]).

A recent review [193] summarized important SREBP actions, listing over 20 genes for lipid synthesis which are controlled by steroid response element (SRE) domains in their promoters. Insulin enhances expression of the SREBP-1c gene by increasing the activity of liver X receptors (LXR) at a nuclear hormone response element that acts as a co-regulator with the SRE domain [194]. Chronic LXR activation induces apoptosis in pancreatic islet cells, and glucose synergistically enhances the loss of these insulin-producing cells [195,196]. In this way, the SREBP-LXR system also provides a rationale for different metabolic effects observed during food energy management [37], which can be modulated with saturated and unsaturated fats (Section 3.2). It also supports new paradigms for how glucose and insulin might induce harmful metabolic signals that insult or injure vascular endothelial cells [23]. A 2007 search of PubMed (<http://www.ncbi.nlm.nih.gov/sites/entrez>) gave 1641 reports about SREBP, SCAP or LXR. We have much to learn about the network of signals regulating lipid synthesis and food energy management.

Unsaturated fatty acids act as competitive antagonists of LXR in cultured rat hepatoma and human HEK-293 cells and in cell-free assays that reflect LXR activation [197]. This antagonism may explain, at least partially, the ability of unsaturated fatty acids to lower the levels of mRNA for SREBP-1c, the transcription of which depends on an endogenous LXR ligand. The lowered SREBP-1c, in turn, leads to a fall in mRNAs for enzymes that synthesize unsaturated fatty acids, thus completing a suppressing feedback loop. Unsaturated fatty acids (but not saturated acids) inhibit expression and action of SREBP-1 but not SREBP-2, whereas sterols inhibit SREBP-2 [198].

Knowing about the suppressive signals of non-esterified cholesterol may prompt curiosity about whether cholesterol in LDL is actually 'good' or 'bad'. For example, cells with defective lysosomal acid lipase have impaired release of cholesterol from cholesteryl esters [199]. These cells, and others with signaling pathway defects (e.g., [192]), mimic familial hypercholesterolemia in having lower than normal LDL-mediated feedback suppression of HMG-CoA reductase and other enzymes of lipid synthesis. A carefully assembled framework of logic about beneficial suppressive actions of cholesterol might provide a useful alternative to current oversimplified views of 'bad' cholesterol.

In this regard, normal lysosomal action can lead to cholesterol crystals after uptake of cholesterol-rich membrane fragments and oxidized lipoproteins by phagocytic cells [200]. This is a logical consequence of lysosomal action when digesting scavenged membrane fragments and damaged lipoproteins at inflammatory sites. However, the easily observed and long-recognized appearance of cholesterol crystals in vascular plaques has been used in an oversimplified way to 'confirm' the hypothesis that elevated blood cholesterol is a major cause of coronary artery disease (rather than being a misunderstood suppressor of lipid synthesis).

Cholesterol suppression of SRE-regulated genes expressing mRNA for enzymes for fatty acid synthesis may slow the movement of excess acetyl-CoA into plasma triacylglycerols. Knowing the connectivity of the signaling mediators that regulate lipid metabolism helps set an important framework of logic to design and test several hypotheses of how food might cause disease. Such tests should address the fact that an appetite-driven voluntary ingestion of large amounts of acetyl-CoA precursors creates questions: How will the body dispose of ingested but unburned carbon and electrons? Will that disposal be harmful? What decrease of % *n*-6 in HUFA will prevent transient post-prandial oxidative insults from becoming chronic inflammatory injuries? Will that decrease have harmful consequences?

## 7.2. The 1984 consensus conference

Prior to the 1984 conference sponsored by the National Heart Lung and Blood Institute of the National Institutes

of Health (NHLBI/NIH), a 10-year study of heart disease with 12,866 participants, the Multiple Risk Factor Intervention Trial (MRFIT), had cost \$115 million and produced equivocal results with no difference in mortality for treated and control groups [201]. A second study, the Coronary Primary Prevention Trial (CPPT), spent 5 years and \$150 million showing that cholestyramine treatment of men in the upper 5 percentile of blood cholesterol levels produced about 8% lower cholesterol levels and 24% fewer deaths [201].

To avoid weaknesses of the earlier trials, the next study was planned to have 55,000 older men and women in the 60–85 percentile of blood cholesterol, half of whom would take a HMG-CoA reductase inhibitor (see Fig. 2) for 5 years [201]. Prior to that trial, a Consensus Development Panel met at a Cholesterol Consensus Conference and declared blood cholesterol levels to be a causal factor [202], allowing it to be considered suitable as a surrogate endpoint for treatment interventions in cardiovascular disease. A unique five-part set of articles detailing the history surrounding this pivotal conference was provided recently by the conference chair, Steinberg [203–207]. His interpretive history discusses in an authoritative and charming way his personal participation with events and people associated with testing the validity of the ‘lipid hypothesis’.

The hypothesis proposes that hypercholesterolemia is a quantitatively significant causative factor in human atherosclerosis [207]. Several aspects of the history are noted here to show readers ideas they might beneficially explore in more detail. The pathway for converting food energy to cholesterol has more than 30 gene-defined enzyme-catalyzed steps. Steinberg noted that one early unsuccessful effort to decrease plasma cholesterol by blocking activation of free acetate to acetyl-CoA (see Fig. 2) was at a step too early and not needed in cholesterol synthesis, whereas two other efforts inhibited later steps in the pathway to cholesterol which Steinberg regarded as too late to avoid accumulation of atherogenic sterols [207].

The CPPT trial had screened almost 500,000 men to recruit the full cohort which was followed for an average of 7.4 years. Overall compliance was disappointing and the treatment group had no statistically significant difference in total mortality compared with those not treated. Tensions among the research units, the profession and the press are poignantly recalled by Steinberg [206]. He also cites one logical critique that merits evaluation. The trial results applied only to men with very high cholesterol values [208] and did not settle the diet-heart problem of the general population in which one of three adults have CVD [175]. Another critique was that “the managers of NIH have used Madison Avenue hype to sell this failed trial in the way the media people sell an underarm deodorant” [209]. This aspect was evident in the Science report [210] that one expert said “I think they made an unconscionable exaggeration of all the data”, and another said “none of this excuses misrepresenting the evidence. Our first obligation is to be honest and forthright”. The comments illus-

trate the frustration following two large expensive trials that produced no clear solution to the general population’s diet-heart problem.

Because of doubts whether the association between CHD and cholesterol was sufficient evidence to prove a cause-and-effect relationship [203], the NHLBI/NIH sponsored the 1984 Consensus Conference. The fourteen panel members appointed by the Office of Medical Applications of Research agreed to declare that “elevated blood cholesterol level is a major cause of coronary artery disease”. Consistent with earlier AHA diet advice (see Section 3), the Conference report [203] urged that the first step in treatment should be caloric restriction and weight loss, and “even when use of drugs seems appropriate, it is important to stress that maximal diet therapy should be continued”. Clearly, the committee regarded CHD as a diet-induced disease caused by imbalanced food energy. Unfortunately, the subsequent public information programs (e.g., [213]) focused on cholesterol levels in ways that diverted attention from the instruction, motivation and encouragement needed for effective preventive management of food energy. Since then, two decades of poor attention to diet priorities have fostered widespread use of statins and an obesity epidemic.

In describing a 1978 European questionnaire, Steinberg wrote that “even 6 years before the NIH Consensus Conference, >90% of the experts in the field found the evidence connecting blood cholesterol causally to heart attacks already strong” [206]. Two items in the questionnaire were: “Do you think there is a connection between plasma cholesterol level and the development of coronary heart disease?” (189, yes; 2, no) and “Do you think that our knowledge about diet and coronary heart disease is sufficient to recommend a moderate change in the diet for the population in an affluent society?” (176, yes; 16, no). These can be compared to questions unanimously answered yes by the 14-member NIH Panel: “Is the relationship between blood cholesterol levels and coronary heart disease causal?” and “Should an attempt be made to reduce blood cholesterol levels of the general population?” [206]. Readers might usefully explore differences in the logic and meaning of the two sets of questions. The consensus conference report did not describe mediators or mechanisms by which associated blood cholesterol levels were logically inferred to be causal. Majority votes (even when unanimous) are not a logical framework for valid inference of a causal relationship.

Drug company money had become the “newest twist” in arranging and funding clinical trials – as succinctly summarized [201] by Kolata, a knowledgeable, accurate and, justifiably, widely respected reporter from Science [206]. The NHLBI director noted that “Today, if you don’t call on the private sector, you are a lousy manager” (control of financial resources is also noted in Section 6.2). The director also confirmed that “surrogate endpoints are a big issue” (see Section 2.1) in the compromises made to study the diet-heart question [201]. Steinberg’s intriguing history

[207] tells how 5 years prior to the consensus conference, Merck entered a “knock-down, drag-out race to see who would be first to successfully bring a statin to market”. He notes that the 1984 Consensus Conference report “figured very large” in the FDA decision to justify approval of cholesterol-lowering therapy without requiring the manufacturers to submit at the time of application clinical trial data demonstrating efficacy [206]. The shortened time-table from declaring a hypothesis (fiction) to be a fact likely had a positive impact on pharmaceutical corporations’ financial benefits.

Five investigators at the conference offered to make themselves available to answer questions about the newly introduced Merck inhibitor of HMG-CoA reductase which they were already studying [206]. This apparently created misunderstanding among conference participants and Steinberg [206] regarding the committee’s continuing emphasis on dietary actions “even when use of drugs seems appropriate” [203]. In spite of the declared consensus [203] and Steinberg’s assertion of final agreement [207], the logical status of blood cholesterol as a cause of CVD remains a debated topic (although it is clearly associated indirectly with CVD occurrence). As with obesity (see Section 3.1), factors that increase the biomarker may cause disease even though the biomarker itself may not do so. Objections by skeptical individuals concerning the logic of the inferences made during the conference [39,206,211,212] have been dwarfed by a continual stream of clinical and epidemiological reports confirming an association of blood cholesterol with disease and death rather than skeptically evaluating logical inferences about possible mediators and mechanisms by which cholesterol might cause death. The goal of good clinical interventions is to remove causes rather than indirect signs or symptoms.

Public concern for cholesterol as a cause of CVD has been amplified for twenty years with financial and administrative resources of the NIH National Cholesterol Education Program [213] and extensive publicity by well-funded pharmaceutical marketers. Other public messages from American health agencies and diverse marketers have also created an illogical sense of danger from dietary cholesterol (a concept not adopted in Canada; [214]) as well as circulating cholesterol. Attention shifted over time from total cholesterol to ‘bad cholesterol’ and ‘good cholesterol’ in blood without public recognition that the proteins and phospholipids in the two types of lipoprotein complexes (LDL and HDL) exert different actions during vascular oxidative inflammatory events (Section 3.1.1). Something about food choices certainly seems to be working through complex pathways (Figs. 2 and 6) to cause disease and death. However, many clinical studies leave the mechanisms connecting cause to consequence weakly defined and weakly tested. Thus, valid surrogate endpoints for clinical intervention [1] still remain a concern as noted long ago by the NHLBI director [201].

### 7.3. Alternate views

Evidence of statin-induced benefit [215] shows that slowing excessive formation of mevalonate (with its many downstream isoprenoid products other than cholesterol; see Fig. 2) can benefit some people. As noted in earlier reviews [113,114], the benefit of statins does not prove that cholesterol (many steps downstream from mevalonate) is a causal agent in CVD. The cholesterol-independent (pleiotropic) actions of statins that improve endothelial function, stabilize atherosclerotic plaques, decrease oxidative stress and inflammation, and inhibit thrombogenesis [216–218] may involve decreasing isoprenoids [farnesyl pyrophosphate and geranylgeranyl pyrophosphate] that regulate the actions of Rho, Ras and Rac [219–221].

A 2007 PubMed search (<http://www.ncbi.nlm.nih.gov/sites/entrez>) identified hundreds of articles on ‘statin AND inflammation’ (975), ‘statin AND proliferation’ (642), and ‘statin AND pleiotropic’ (510). Overall, there were 16,615 articles on statins. We have much to learn about the biological actions of mevalonate derivatives. Curiosity about possible benefit or harm in decreasing these intermediates is leading to more drug development research to block other stages of the cholesterol synthetic pathway (e.g. [222]). If the mediators connecting acetyl-CoA to CVD (see Fig. 2) are prenylated forms of Rho, Ras or Rac, then efforts to block squalene metabolism [222] may alert us to an alternate to Steinberg’s view of the earlier drug candidates that acted “too late to avoid accumulation of atherogenic sterols” [207]. We may learn whether squalene and sterols are beyond the branch point where isoprenoids are causal mediators of vascular pathology (Fig. 2).

Although secondary prevention with statins and nitroglycerin is saving lives, primary prevention would focus on preventing the voluntary dietary (‘upstream’) imbalances that cause the need to use such medications. Evidence for benefits of increasing  $n-3$  proportions in tissue HUFA comes from many studies. An evaluation of randomized controlled trials [223] with 97 studies of 137,140 individuals in intervention and 138,976 individuals in control groups showed risk ratios for overall mortality of 0.87 for statin interventions and 0.77 for  $n-3$  fatty acid interventions (see Table 3). For cardiac mortality, risk ratios were 0.78 for statins and 0.68 for  $n-3$  fatty acids. Results from this meta-analysis support the hypothesis noted in Section 5.5 that preventing the amplifying imbalances of  $n-6$  eicosanoids in the pathway of Fig. 6 may give better health benefits than preventing the body fuel imbalances in the pathway of Fig. 2.

Efforts to make valid inferences by combining results from all relevant published reports in a meta-analysis format seem desirable and rational, but they can have weaknesses. The criteria used for inclusion and exclusion of reported data may sometimes follow a framework of logic that creates bizarre biases. For example, one ‘systematic’ review assessed the rather ambiguous primary hypothesis

Table 3  
Summary of meta-analysis of randomized controlled trials

*Efficacy of clinical interventions* [223]

Meta-analysis of 97 randomized controlled trials with 137,140 people in intervention & 138,976 in control groups

*Risk ratios for overall mortality*

0.77 for *n*-3 fatty acids

0.84 for resins

0.87 for statins

0.96 for niacin

0.97 for “diet advice”

1.00 for fibrates

*Risk ratios for cardiac mortality*

0.68 for *n*-3 fatty acids

0.70 for resins

0.78 for statins

*Risk ratios for non-cardiovascular mortality*

1.13 for fibrates

Results are from [223].

that “reduction or modification of fat intake affects mortality, cardiovascular morbidity and cardiovascular events” [224]. However, the analysis excluded any trials that aimed to alter omega-3 intake because “the method of action (if any) is probably different from any action caused by the reduction in intake of total or saturated fat – that is it does not primarily lower low density lipoprotein cholesterol”. The authors opined that “it is unlikely that further resources will now be forthcoming to perform a large trial of dietary modification”. They concluded that the evidence “pooled in this review contributes all that is available to guide clinical practice and health policy on dietary manipulation”. Readers may recognize the value of skeptical logic when testing the inferences made in such conclusions. Paradoxes occur in much current advice about dietary lipids because so much public advice occurs in oversimplified messages from marketers trying to increase sales rather than from skeptical logical inferences of health scientists trying to prevent disease.

## 8. A take-home message

Curiosity is the beginning of understanding. It opens escape routes from the silo mentality that paradoxically impairs scientific discovery while supporting scientific expansion. Scientists behave in human ways, using dreams, wishful thinking, imprecise inferences and careless terminology in early stages of a project. However, the training of a disciplined scientist involves recognizing and controlling these human weaknesses. In the process, reading Karl Popper’s stern admonitions about the use of logic is not a popular activity! Nevertheless, ‘mainstream’ scientific thought is usually portrayed with a belief that its logic and inferences have been rigorously tested. However, belief and trust do not have the same framework of logic as a skeptically tested rigorous inference.

Fortunately, the public can recognize the difference between simple belief and logical inference. This ability is

important in recognizing the limits of certainty when hearing advice that relates aspects of an individual person to median aspects calculated for a population. Most people differ from the exact median value. Logic also sharpens awareness that epidemiology assembles information about associations of events for which the details of causal mediators are inferred from other methods and measurements. Although many associated risk factors may predict a possibility of harm, only a limited number of connected factors are the actors that harm.

Unfortunately, epidemiological messages frequently contain careless (and even reckless) use of terms that imply causal events rather than merely associated events that were the observed facts. Such messages may reflect a human desire to project greater importance than is logically merited. Certainly, misunderstanding can occur when the greater of two values is said to be increased over the other when no time-dependent measures were monitored. There are hundreds of examples of ‘allocating’ the likelihood of an event to various associated events (risk factors) in which the description of the probabilistic allocation carelessly becomes an attribution of cause (using terms like “risk due to”) rather than simply an estimated probability of co-occurrence. Over-dependence on epidemiological correlations may be neglecting balanced use of other controlled evidence of *n*-6 eicosanoid actions which mediate disease and are profitable targets for drug treatment.

The AHA continues to advise the public about food and health with incrementally changed messages [225,226] that respond to a growing awareness of likely benefit from increasing *n*-3 HUFA proportions in tissues [14] and antagonizing harmful actions of *n*-6 HUFA. The old advice to “eat some polyunsaturated fat” has matured over 50 years into a sophisticated set of evidence about how *n*-3 HUFA benefits may occur and what people might beneficially eat [226]. In contrast, advice to limit intake of saturated fats remains with no clear evidence of mediators of their harm. In fact, it has been joined by advice to avoid *trans* fats that also have no clear mechanism or mediator connecting them causally to CVD.

The evident association of food energy with disease and death needs still stronger evidence of mediators that actually connect food to death. Diverse biomarkers of energy management associated with CVD currently receive much attention and concern, while the primary cause of unmanaged appetitive behavior has poorly recognized mediators. Evidence cited in this review suggests that correcting imbalances in food energy management (related to blood cholesterol levels) might be less effective for primary prevention of cardiovascular death than correcting HUFA imbalances from eating excessive amounts of *n*-6 and insufficient amounts of *n*-3 polyunsaturated fatty acids (as noted in earlier reviews [113,114]). Similar logic may also apply to the diet-based amplifying events that occur in other inflammatory proliferative disorders including cancer, dementia, arthritis and asthma [14]. Proposals to balance tissue HUFA seem as beneficial now to individuals in increasing

health and decreasing health expenses as they did before promotion of statins diverted attention from it over 20 years ago.

Identifying the ways that causal factors connect to consequences is the business of good science. New paradigms not discussed in this already-too-long review may yet connect energy homeostasis to appetite by signals involving malonyl-CoA, SREBP [227] and PPAR [228]. Another paradigm may connect the current high proportion of  $n-6$  in HUFA to responses of the cannabinoid receptor (CB-1) that is more sensitive to  $n-6$  than  $n-3$  endocannabinoids [229] and might stimulate SREBP-enhanced fat synthesis [230] and also enhance appetite [231]. Curiosity as to whether these processes related to  $n-6$  HUFA in tissues might also cause CVD death will promote more adventures that will likely be told by other reviewers.

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