Nutrition and genetics: an expanding frontier[1–4]

Robert E Olson

ABSTRACT The age of molecular biology began in 1953 with the discovery of the structure of DNA. By 1961 the genetic code for the translation of the sequence of bases in DNA to amino acids in proteins was underway, and a model for the genetic regulation of protein synthesis was proposed. My interest in the genetic regulation of nutrient metabolism began in that year during my sabbatical leave in the laboratory of Sir Hans Krebs at Oxford University. In the present article, I describe 2 episodes in my career during which I used genetic concepts to explain a nutritional phenomenon; the first episode occurred before doing the experimental work, and the second occurred after the experimental work was completed. My first brainstorm, which occurred in 1961, was to investigate the hypothesis that all of the fat-soluble vitamins act by the regulation of a cluster of genes. Unfortunately, I selected vitamin K as my model and discovered that it is the only fat-soluble vitamin that does not work in full or in part by the regulation of a set of genes. In 1967 I undertook a second problem, which was to determine the mode of action of polyunsaturated fatty acids in lowering plasma lipid concentrations in humans. We discovered that linoleic acid reduced the storage and enhanced the oxidation of fatty acids. The genetic interpretation of this study has come only recently: polyunsaturated fats have been shown to down-regulate enzymes that accomplish storage of fatty acids and to up-regulate genes that enhance fatty acid oxidation. *Am J Clin Nutr* 2003;78:201–8.

KEY WORDS Fat-soluble vitamins, polyunsaturated fatty acids, fatty acid storage, fatty acid oxidation, transcription factors, genes, obesity, serum lipids

INTRODUCTION

I am greatly honored to have received the Herman Award for 2002. My friendship with Bob Herman began in the 1970s as a result of our common activities within the American Society for Clinical Nutrition and through review panels of the National Institutes of Health. I remember that Bob was Editor of The American Journal of Clinical Nutrition from 1975 to 1980 (1) and President of The American Society for Clinical Nutrition from 1979 to 1980 (2) just before his untimely death in December 1980. We should all remember in these troubled times that Bob was a soldier as well as a physician and that he obtained most of his clinical training at the Walter Reed Army Hospital and served as the Chief of Medicine at a mobile army surgical hospital in Korea in 1959. I last saw Bob in San Francisco, where he was the Chief of Medicine at the Letterman Army Institute. His research dealt primarily with the digestion and metabolism of carbohydrates in both animals and humans. I was impressed at the time with the extent to which Bob integrated basic science concepts into his clinical investigations, as shown in **Figure 1**, and this integrative approach has guided my own research ever since. My clinical investigations have dealt with the study of the effect of dietary protein, amino acids, and polyunsaturated fatty acids (PUFAs) on plasma lipid concentrations and lipid turnover (3), the study of the pathogenesis of protein-energy malnutrition in children in northern Thailand (4), and the measurement of the body pool of phylloquinone and its turnover in adults who consume diets that are adequate or deficient in vitamin K (5).

MOLECULAR BIOLOGY AND THE FAT-SOLUBLE VITAMINS

Because I have had no formal training in genetics, many will believe that I am presumptuous in selecting the topic of nutrition and genetics for my Herman Award Lecture. My early graduate education was in classic biochemistry and nutrition, and my early research was devoted to the study of the effect of changes in nutritional status on the metabolism of nutrients in both animals and humans. When Avery, MacLeod, and McCarty (6) published their paper on transformation of pneumococci by DNA in 1944, I was just completing work for my PhD degree at St Louis University on the bioassay of adrenal cortical steroids (7, 8). Then in 1953, when Watson and Crick (9) published their classic paper on the structure of DNA, I had just completed my medical education and had moved to Pittsburgh to become Professor and Chairman of the Department of Biochemistry and Nutrition in the Graduate School of Public Health at the University of Pittsburgh (10).

In 1961 Nirenberg and Matthaei (11) began their studies on the genetic code, and Jacob and Monod published their insightful paper "Genetic Regulatory Mechanisms in the Synthesis of Proteins" (12) in which the induction of β-galactosidase by lactose in Escherichia coli was used as a model (**Figure 2**). During that year, I was on sabbatical leave from the University of Pittsburgh

[1] From the Department of Pediatrics, College of Medicine, University of South Florida, Tampa.

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[4] Address reprint requests to RE Olson, Department of Pediatrics, College of Medicine, University of South Florida, 12901 Bruce B Downs Boulevard, Tampa, FL 33612. E-mail: rolson@hsc.usf.edu.

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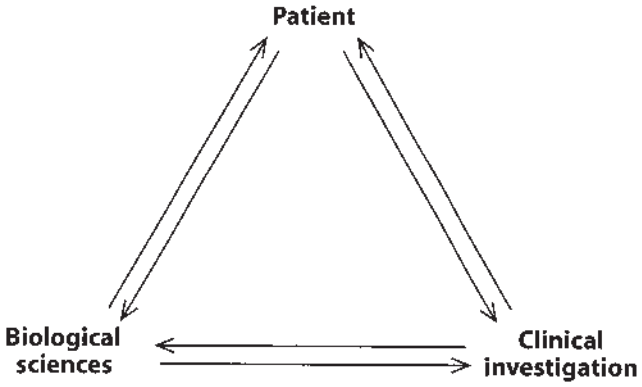

FIGURE 1. The Herman triangle depicts a plan for the clinical investigation of a patient with a metabolic or nutritional disease in which attention is paid to the basic science underlying the problem being investigated.

and was working in the laboratory of Sir Hans Krebs at Oxford University, where I was engaged in the study of cardiac metabolism in the isolated perfused rat heart. It so happened that Krebs gave many of the lectures to the first-year honors students in biochemistry, and I went to most of those lectures. During one of the lectures, devoted to gluconeogenesis, Krebs discussed the control points in the conversion of lactate to glycogen. At one point, he mentioned the fact that glucocorticoids had been shown to stimulate gluconeogenesis in rats (13), which I knew from my own work (7, 8), and digressing slightly from the main theme of the lecture, he mentioned the paper by Jacob and Monod (12) and wondered out loud whether glucocorticoids could increase the concentration of given enzymes at control points in gluconeogenesis. It suddenly struck me that there was at that time no explanation for the action of the fat-soluble vitamins, including vitamin D, which is a secosteroid. After the lecture I went up to Krebs, and commenting on his suggestion that corticoids could be inducers of gluconeogenic enzymes, I asked him if he had any ideas about the fat-soluble vitamins, particularly vitamin D. He said that he had no idea about

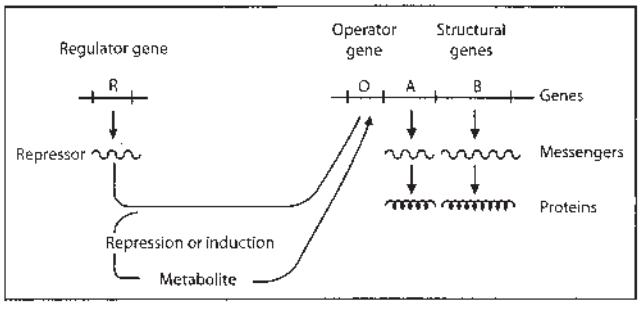

FIGURE 2. The model proposed by Jacob and Monod (12) for the genetic regulation of protein synthesis in *Escherichia coli*. This system controls the induction or repression of β-galactosidase (A) and 2 other structural genes, those for galactoside permease and galactoside acetylase. In this model, a regulator gene elaborates a messenger RNA that inhibits the operator. The inducer, lactose or a thiogalactoside (labeled metabolite), overcomes the inhibition of the operator gene and permits the expression of the structural genes. Adapted from reference 12.

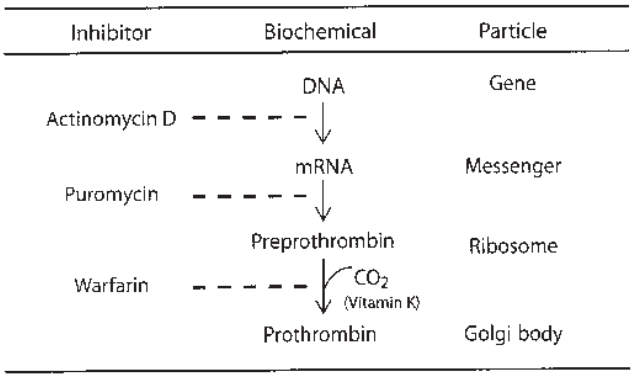

FIGURE 3. The pathway of prothrombin synthesis in birds and other animals. The pathway involves the transmission of biochemical information from gene to messenger RNA (mRNA) to preprothrombin, which is an acarboxyprothrombin and is inactive in the coagulation scheme. Posttranscriptionally, preprothrombin is converted to prothrombin by the vitamin K–dependent enzyme γ-glutamylcarboxylase. The inhibitors of this process, actinomycin D, puromycin, and warfarin, act at the specified steps.

the fat-soluble vitamins but urged me to look into that hypothesis. It is of interest that Krebs did not follow up on his own suggestion about the corticoids, but I decided to begin some studies on the fat-soluble vitamins as soon as I got back to Pittsburgh. From that time forward, I began to think about genetics as an essential variable in the solution of nutritional problems. Two examples of the use of genetic concepts to explain a nutritional phenomenon are presented in this article.

VITAMIN K AS A MODEL SYSTEM

After my return to Pittsburgh, the first question that faced me was the selection of a fat-soluble vitamin to investigate. I had no experience with vitamin D, and no one knew at that time what the gene products of vitamins A, D, and E were. I did remember, however, the work of my former mentor at St Louis University, Edward Doisy, who had successfully isolated phylloquinone from alfalfa (14) and determined its structure (15). Furthermore, at that time it was known that phylloquinone controls the activity of several coagulation proteins that could be putative gene products. In addition, these proteins were easy to measure in plasma.

I decided to order some chicks and feed them a vitamin K–deficient diet. After 2 wk their prothrombin times were extended from the usual 20–30 s to >240 s. These vitamin K–deficient chicks responded to an oral dose of menadione (2 μg/100 g body wt) in 6 h with a return of their prothrombin times from >240 to <160 s. When actinomycin D, an inhibitor of DNA transcription, was given 4 h before administration of vitamin K, there was no response to vitamin K. By labeling these chicks with [8-^{14}C]adenine and hydrolyzing the liver RNA, we showed a progressive decrease in the specific activity of the hydrolytic products of RNA, namely, 2′-AMP and 3′-AMP, which indicated that the actinomycin D had blocked messenger RNA formation. Later, we also showed that puromycin, an inhibitor of protein synthesis, also blocked vitamin K action in chicks (16). As shown in **Figure 3**, these results were consistent with our hypothesis and led to the following global conclusion (17):

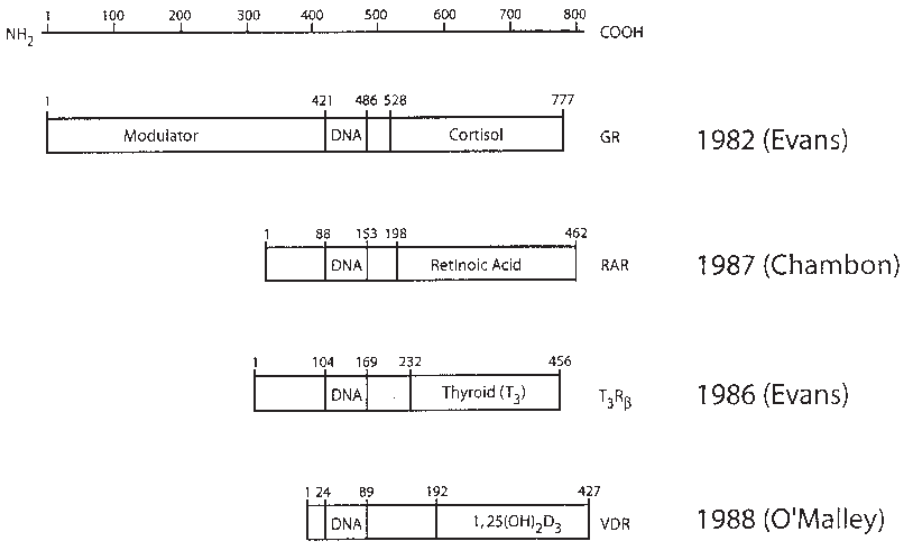

FIGURE 4. Detailed structures of 4 receptors from the steroid hormone–thyroid hormone receptor superfamily. The lengths of primary amino acid sequences in specific domains are shown. The structures are aligned to show that the DNA binding core of 66 amino acids is the same in these receptors. The amino-terminal domain of ≈250 amino acids is the ligand-binding domain. The amino-terminal portion is highly variable in size but appears to provide maximum biological activity in each case. The year of discovery of each of these transcription factors is shown along with the name of the director of each laboratory involved (26, 27, 29–31). GR, glucocorticoid receptor; RAR, retinoic acid receptor; T_3, triiodothyronine; T_3R_β, triiodothyronine receptor β; $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; VDR, vitamin D hormone receptor.

> The work suggests the possibility that all of the fat-soluble vitamins operate to control the synthesis of specific proteins and enzymes in the highly differentiated mammal. This speculation is strengthened by the parallel similarity of the other fat-soluble vitamin deficiency diseases to genetically conditioned disorders. Evidence already exists that some of the chemically related steroid hormones act at the genetic level.

Shortly after the publication of our report, Johnson's group (18) and Suttie (19) reported that drugs inhibiting DNA or protein synthesis in vitamin K–deficient rats did not completely block the action of vitamin K in forming prothrombin in that species. Later, it turned out that the precursor of prothrombin accumulates in the liver of vitamin K–deficient rats, which, unlike chickens, do not secrete the precursor into the plasma (20). As a result, the concentration of preprothrombin in the liver of chicks was insufficient to provide a response to vitamin K that was visible in a clotting assay. This fact complicated the interpretation of our experiments with actinomycin D in chickens and led to the wrong conclusion. In other words, chicks required ongoing protein synthesis to respond to vitamin K, whereas rats initially did not.

In 1974 workers in 3 laboratories (21–23) showed that prothrombin and the other vitamin K–dependent proteins contain 9–13 molecules of γ-carboxyglutamate near their N termini and that these residues are responsible for the biological activity of the vitamin K–dependent proteins. Vitamin K deficiency arrested the synthesis of normal vitamin K–dependent proteins and permitted descarboxyglutamate variants to accumulate. The enzyme responsible for the carboxylation of glutamyl residues in vitamin K–dependent proteins, γ-glutamylcarboxylase, was isolated in 1991 by Wu et al (24), and its complementary DNA was cloned, sequenced, and expressed in cells in vitro by the same group in the same year (25).

It is ironic that the fat-soluble vitamin that I selected to test for a genetic function was the only 1 of the 4 that is not genetotrophic (26–28). In the early 1980s, a family of receptors for glucocorticoids, mineralocorticoids, progesterone, estrogen, androgen, thyroid hormone, and metabolic products of vitamin A (retinoic acid) and vitamin D (1,25-dihydroxycholecalciferol) was discovered and named the steroid hormone–thyroid hormone superfamily (29). These receptors were also transcription factors, and each caused the expression of several genes that mediated the specific actions of each ligand. The general structure of these receptors is shown in **Figure 4** (26, 27, 29–31), and their gene products are shown in **Table 1** (32–35). With regard to the glucocorticoids that Krebs speculated about in 1961, they have been shown to

TABLE 1
Ligands and gene products of the human steroid hormone–thyroid hormone receptor superfamily[1]

Ligand	Gene products
Cortisol	PEP carboxykinase, FDP-ase, G-6-P-ase, and glycogen synthetase. Cortisol inhibits collagenase and cytokines NFκB, IL-1, IL-2, and interferon
Retinoic acid	Retinoid-binding proteins (CRBP-1, CRABP II), keratins, laminin, alcohol dehydrogenase, and transglutaminase
Triiodothyronine	Myelin basic protein, embryonic maturation factors, myosin heavy chain, and fatty acid synthetase
Vitamin D hormone	Calbindin, osteocalcin, 25-OH-D-24-hydroxylase, and differentiation factors for keratinocytes and promyelocytes

[1] From Orth and Kovacs (32), Ross (33), Brent (34), Holick (35), and Tsai et al (36). PEP, phosphoenolpyruvate; FDP-ase, fructose 1,6-bisphosphatase; G-6-P-ase, glucose 6-phosphatase; NFκB, nuclear transcription factor κB; IL, interleukin; CRBP-1, cellular retinol-binding protein 1; CRABP II, cellular retinoic acid–binding protein II.

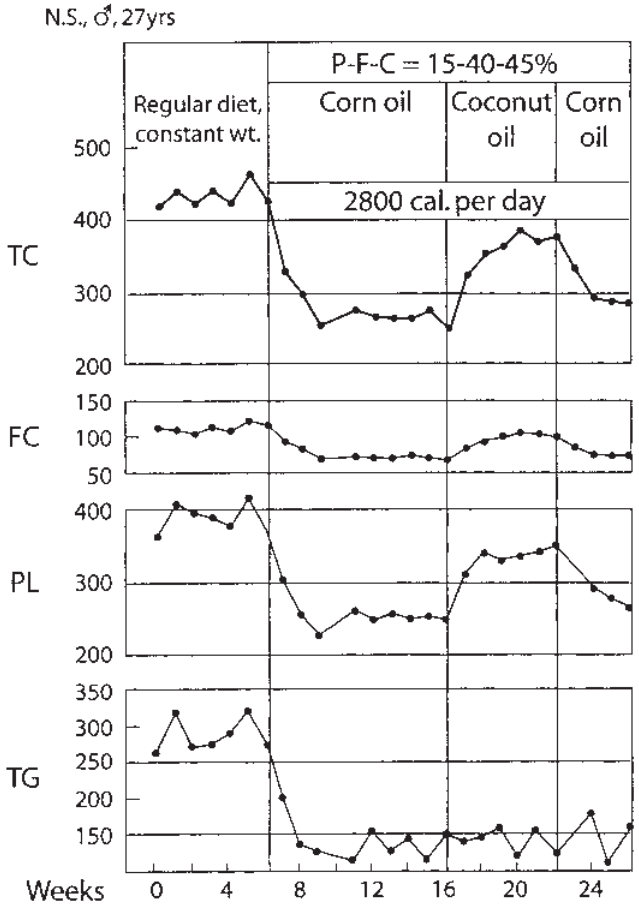

FIGURE 5. Twenty-six–week study by Ahrens (40) of serum lipids in a 27-y-old man (N.S.) with hypercholesteremia. TC, total cholesterol; FC, free cholesterol; PL, phospholipids; TG, triacylglycerol; P-F-C, dietary protein, fat, and carbohydrate intakes as percentages of total energy; wt., weight; cal., calories. All concentrations are in mg/100 mL serum. Reprinted with permission from reference 40.

enhance all the potentially limiting reactions in gluconeogenesis. By the 1990s, this superfamily had increased in number from 13 to >50 factors (36) and had come to include the peroxisome proliferator–activated receptors (α, β, and γ).

POLYUNSATURATED FATTY ACIDS EXERT GENETIC CONTROL ON FATTY ACID STORAGE AND OXIDATION

The second problem that led me from clinical investigation to genetics was the mechanism of action of linoleic acid in lowering serum cholesterol concentrations in humans. In 1952 Kinsell et al (37) and Groen et al (38) were the first to report that the substitution of vegetable oil for animal fat in the diet of humans would markedly lower their serum cholesterol. By 1957 the hypolipemic action of linoleic acid in humans was confirmed in many laboratories around the world, and a formula, which featured a strong role for linoleic acid, was devised by Keys et al (39) to predict the quantitative effect of dietary fat on serum cholesterol.

Using formula diets, Ahrens (40) confirmed the hypolipemic action of linoleic acid in patients with hyperlipemia (**Figure 5**)

TABLE 2
Effect of unsaturated dietary fats on the absorption, excretion, synthesis, and plasma concentration of cholesterol in 11 hypercholesterolemic patients[1]

Variable	Control diet	PUFA diet
Cholesterol absorption (%)	41 ± 14 [5]	39 ± 9 [5]
Plasma cholesterol (mg/dL)	369 ± 102 [11]	283 ± 108[2] [11]
Fecal steroids (mg/d)	812 ± 411 [11]	871 ± 463 [11]
Isotopic cholesterol $t_{1/2}$ (d)	8.5 ± 3.0 [4]	8.0 ± 3.2 [4]

[1] $\bar{x}$ ± SD. *n* in brackets. From Grundy and Ahrens (41). PUFA, polyunsaturated fatty acid; $t_{1/2}$, half-life.
[2] Significantly different from control diet, $P < 0.05$.

and then carried out a series of experiments to determine the mechanism of action of linoleic acid (41). He studied cholesterol absorption, cholesterol turnover measured with isotopic cholesterol, and the excretion of the sterol products of cholesterol metabolism in the stool. Although his hyperlipemic subjects had marked changes in serum lipids when they were changed from a diet containing saturated fats to one containing polyunsaturated fats, there were no changes in cholesterol absorption or metabolism, as shown in **Table 2**. Because of these negative data, Ahrens postulated that the effect of dietary linoleic acid must be to change the distribution of cholesterol or cholesterol-containing lipids between plasma and other tissues even though this effect had not been observed in animals (42).

At about this time, Nestel and Steinberg (43) observed that palmitic acid was preferentially incorporated into triacylglycerol in the perfused rat liver, whereas linoleic acid was channeled mainly into oxidative pathways. Bjorntorp (44) then showed that rat liver mitochondria oxidized linoleic acid to carbon dioxide >10 times as fast as they oxidized palmitic acid. Because of these studies, it occurred to us that the hypocholesterolemic action of linoleic acid could be due to changes in its fatty acid metabolism rather than to changes in cholesterol metabolism. We undertook studies to test this hypothesis in humans (45, 46).

We recruited 2 normolipemic and 2 hyperlipemic volunteers from our clinic for these studies. The normolipemic subjects had a normal lipoprotein profile, whereas the 2 hyperlipemic subjects had high VLDL concentrations and plasma triacylglycerol concentrations >1000 mg/dL. The hyperlipemic subjects were diagnosed as having familial hypertriglyceridemia. The 4 subjects were fed diets sequentially for 4 wk each, beginning with a diet containing 4% of energy from linoleic acid and then switching to a diet containing 18% of energy from linoleic acid. These diets were composed of 16% of energy from protein, 48% from carbohydrate, and 36% from fat. The high–linoleic acid diet was obtained by substituting corn oil for the animal fat in the control diet. At the end of 3 wk of each diet, each subject was given a tracer dose of 100 μCi [1-^{14}C]linoleic acid (specific activity of 30 mCi/mmol) by mouth, and the radioactivity was measured in plasma lipids and respiratory carbon dioxide for the next 48 h. The original plasma cholesterol and triacylglycerol concentrations in the 4 subjects and the effect of the high–linoleic acid diet on their plasma lipid concentrations are shown in **Figure 6**. The increase in dietary linoleic acid lowered plasma triacylglycerol concentrations 30% in the normolipemic subjects and 50% in the hyperlipemic subjects. Plasma cholesterol

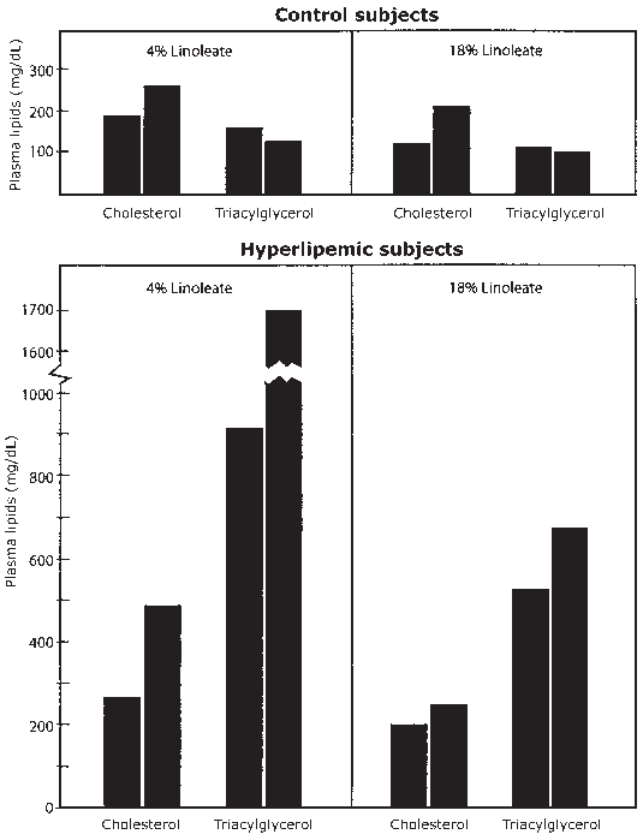

FIGURE 6. Effect of dietary linoleic acid on serum lipid concentrations in 2 control (normolipemic) and 2 hyperlipemic subjects. The subjects consumed diets containing either 4% of energy from linoleic acid or 18% of energy from linoleic acid. There was a significant ($P < 0.05$) difference in mean cholesterol and triacylglycerol concentrations between the 2 diets (45).

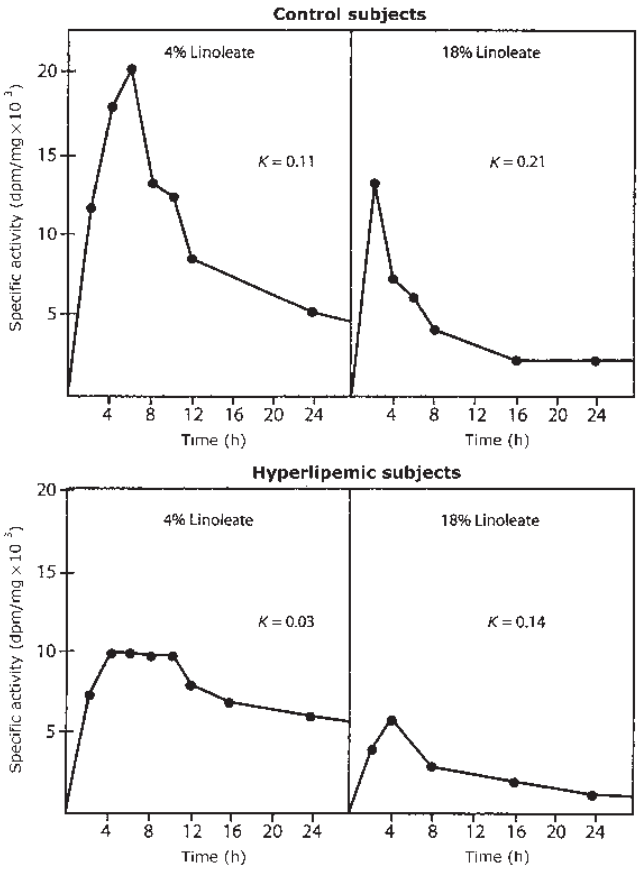

FIGURE 7. Mean turnover of linoleic acid in plasma triacylglycerol, expressed as the specific activity of radioactive linoleic acid plotted against time, in 2 control (normolipemic) and 2 hyperlipemic subjects. Fractional turnover constants for triacylglycerol linoleic acid are shown in each graph. dpm, disintegrations per minute. There was a significant ($P < 0.05$) difference in mean linoleic acid turnover between the 2 diets (46).

concentrations fell ≈25% in the normolipemic subjects and 50% in the hyperlipemic subjects.

As noted in **Figure 7**, the fractional turnover constant for linoleic acid in triacylglycerol in the normolipemic subjects increased from 0.11 to 0.21/h. In the hyperlipemic subjects, the fractional turnover constant went from 0.03 to 0.14/h. Moreover, as shown in **Figure 8**, when the high–linoleic acid diet was consumed, the conversion of the labeled linoleic acid to carbon dioxide increased 2.9 times in the normolipemic subjects and 4.6 times in the hyperlipemic subjects. From these data we concluded that "high linoleate feeding may contribute to the control of hyperlipemia by diverting more fatty acids toward oxidative pathways, thus leaving less for hepatic biosynthesis of low density lipoproteins" (46).

To understand the genetic implications of this work, one must flash forward to the 1990s and review the work of Clarke and coworkers (47, 48), who discovered that PUFAs down-regulate genes that control fatty acid synthesis and storage and up-regulate genes that promote fatty acid oxidation. Animal studies have shown that the ingestion of fats that are rich in PUFAs, particularly n−3 PUFAs, suppresses hepatic lipogenesis (48), reduces hepatic triacylglycerol output (49), induces ketogenesis (50), and increases fatty acid oxidation in both liver and muscle (51). PUFAs appear to interact with transcription factors like sterol regulatory element–binding protein 1 and peroxisome proliferator–activated receptor α, the latter being a member of the steroid hormone–thyroid hormone superfamily of transcription factors. A summary of these actions of PUFAs is shown in **Figure 9**. Some of the enzymes involved in partitioning fatty acids between storage and oxidation are shown. These enzymes include fatty acid synthetase and acetyl-CoA carboxylase, which are inhibited by PUFAs, and acyl-CoA oxidase, a peroxisomal enzyme, and carnitine palmitoyl transferase, a mitochondrial enzyme, both of which are enhanced by PUFAs. It is clear that the effects of linoleic acid that we observed in our study in 1967 (46) are well accounted for by these new findings (47, 52). Regarding the relative effectiveness of n−6 and n−3 PUFAs, both animal and clinical studies suggest that n−3 PUFAs are more powerful.

Clarke (53) has also suggested that the genetic regulatory effects of PUFAs may ameliorate the metabolic syndrome, which was first described by Reaven et al (54) as consisting of abdominal obesity, hypertension, hypertriglyceridemia, hyperglycemia, insulin resistance, and a low plasma HDL concentration, and may precede the onset of clinical type 2 diabetes in overweight persons. It is of interest that genetic knockout mice devoid of the peroxisome proliferator–activated receptor α gene express many characteristics of the metabolic syndrome (55–58). According to findings

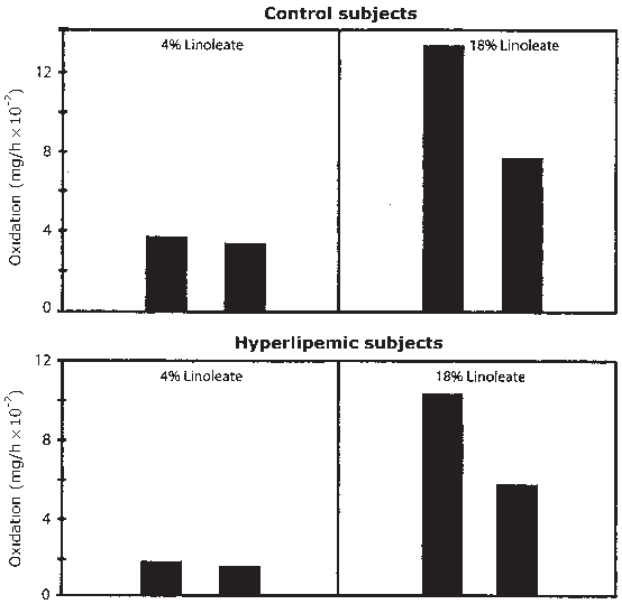

FIGURE 8. The rate of oxidation of triacylglycerol linoleic acid to carbon dioxide in 2 control (normolipemic) and 2 hyperlipemic subjects. Expired air was collected from subjects who consumed diets containing either 4% or 18% of energy from linoleic acid, and the $^{14}CO_2$ concentration was measured. The rates were calculated from the $^{14}CO_2$ in the expired air and the triacylglycerol specific activity. There was a significant ($P < 0.05$) difference in mean rates of oxidation between the 2 diets (46).

from the third National Health and Nutrition Examination Survey (NHANES III), the unadjusted prevalence of the metabolic syndrome among US adults is 22%, ranging from 7% in persons aged 20–29 y to 43% in persons aged 60–69 y (57). These numbers indicate that 47 million persons in the United States have this disorder. On the basis of the data presented above, clinical studies of the effects of PUFA supplementation in persons with the metabolic syndrome should be given high priority.

PROMISE FOR POLYUNSATURATED FATTY ACIDS IN THE TREATMENT OF OBESITY

Ravussin and coworkers (58–60) have studied the metabolic aspects of obesity in Pima Indians and whites for many years.

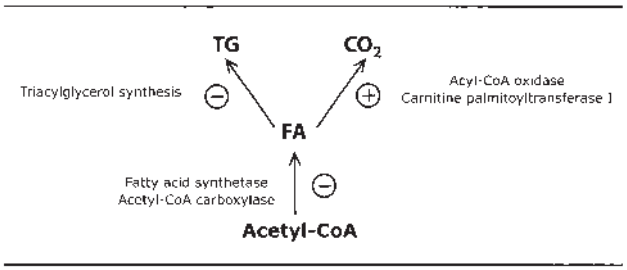

FIGURE 9. Genetic effects of polyunsaturated fatty acids on fat metabolism. Fatty acid synthesis is impeded by down-regulation of fatty acid synthetase and acetyl-CoA carboxylase. In addition, the oxidation of fatty acid is accelerated by the up-regulation of acyl-CoA oxidase and carnitine palmitoyl transferase I. These effects reduce triacylglycerol synthesis by the classic pathway (47). TG, triacylglycerol; FA, fatty acid.

TABLE 3
Effect of a fish supplement on the energy metabolism of 6 adults over 3 wk[1]

Variable	Control	Fish oil
Energy intake (MJ/d)	10.4 ± 0.7	10.3 ± 1.2
Energy expenditure (MJ/d)	7.17 ± 0.65	7.43 ± 0.58[2]
Fat oxidation (mg·kg^{-1}·min^{-1})	0.87 ± 0.13	1.06 ± 0.17[2]
Respiratory quotient	0.834 ± 0.019	0.815 ± 0.020[2]
Change in fat mass (kg)	−0.30 ± 0.34	−0.88 ± 0.15[2]

[1] $\bar{x}$ ± SD. From Couet et al (65).
[2] Significantly different from control, $P < 0.05$.

Ravussin's numerous studies have led him to believe that the 3 main risk factors for obesity are family history, a low resting energy exchange, and a high respiratory quotient, which indicates a low rate of fat oxidation. These findings mesh nicely with those of Clarke (47) and may indicate that the lack of n−3 PUFAs in the American diet (61) contributes to the epidemic of obesity in the United States. Studies have shown that fish oil reduces body weight and body fat mass and increases energy expenditure in animals (62–64). Futhermore, Couet et al (65) studied 6 healthy persons (5 men and 1 woman) who consumed a constant diet with or without a fish-oil supplement (6 g/d) for 3 wk. The washout between the control and supplemented periods was 12 wk. The diet contained 16% of energy from protein, 51% from carbohydrate, and 32% from fat. All meals were ingested in the metabolic unit. Energy intake, energy expenditure, fat oxidation, respiratory quotient, and change in fat mass were measured. With consumption of the fish-oil supplement, there was no change in energy intake, a 4% increase in energy expenditure, a 5% increase in fat oxidation, and a 290% increase in body fat loss, as shown in **Table 3**.

These data indicate that human subjects who have a constant energy intake and are given 6 g fish oil/d for 3 wk increase their fat oxidation, overall energy exchange, and loss of body fat. Although the number of subjects studied was small, the data are in accord with those from animal studies. It is hoped that other clinical investigators will test this hypothesis.

In conclusion, I have presented 2 examples of my own work in which I explored the possibility that nutrient-gene interactions hold the key to specific nutritional problems. I believe that although obesity has many causes, it is also affected by genes that partition fat between storage and oxidation. It is possible that the genes discussed in the present article in relation to linoleic acid metabolism may respond not only to PUFAs but also to other unidentified endogenous ligands that may play a role in controlling body weight and energy exchange. I hope that these new findings will modify the current arguments about the macronutrient composition of diets that are most suitable for weight reduction (66). Furthermore, it is my opinion that the double helix will dominate much of clinical investigation devoted to nutritional problems in the future.

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