Fatty Acid Regulation of Gene Expression: A Genomic Mechanism to Improve the Metabolic Syndrome

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The development of obesity and associated insulin resistance involves a multitude of gene products, including proteins involved in lipid synthesis and oxidation, thermogenesis, and cell differentiation. The genes encoding these proteins are in essence the blueprints that we have inherited from our parents. However, what determines the way in which blueprints are interpreted is largely dictated by a collection of environmental factors. The nutrients we consume are among the most influential of these environmental factors.

During the early stages of evolutionary development, nutrients functioned as primitive hormonal signals that allowed the early organisms to turn on pathways of synthesis or storage during periods of nutrient deprivation or excess. As single-cell organisms evolved into complex life forms, nutrients continued to be environmental factors that interacted with hormonal signals to govern the expression of genes encoding the proteins involved in energy metabolism, cell differentiation, and cell growth. Nutrients govern the tissue content and activity of different proteins by functioning as regulators of gene transcription, nuclear RNA processing, messenger RNA (mRNA) degradation, and mRNA translation, as well as functioning as posttranslational modifiers of proteins.

One dietary constituent that has a strong influence on cell differentiation, growth, and metabolism is fat. The fatty acid component of dietary lipid not only influences hormonal signaling events by modifying membrane lipid composition, but fatty acids also have a very strong direct influence on the molecular events that govern gene expression. In this chapter we discuss the influence of n-9, n-6, and n-3 fatty acids on gene expression in the liver and skeletal muscle, and the impact this has on intra- and interorgan partitioning of metabolic fuels (Fig. 1).
FIG. 1. Polyunsaturated fatty acids (PUFA) as hepatic fatty acid repartitioning agents. This schematic describes the mechanisms by which dietary polyunsaturated fatty acids redirect fatty acids away from triglyceride storage and toward fatty acid oxidation. The data supporting the schematic have been derived from studies with the liver. AOX, peroxisomal acyl-CoA oxidase; ACC, acetyl-CoA carboxylase gene; CPT, liver type carnitine palmitoyltransferase; ER, endoplasmic reticulum; FA, fatty acids; FAS, fatty acid synthase gene; PL, phospholipid; TG, triglycerides; VLDL, very low density lipoproteins.

POLYUNSATURATED FATTY ACIDS AS FUEL REPARTITIONING AGENTS

In 1967 Nichaman et al. (1) reported that raising the linoleate content of the diet from 4% of energy to 18% reduced plasma triglyceride concentrations in normal and hyperlipemic subjects by 30% and 52%, respectively. Shortly after this, Allmann and Gibson (2) discovered that the induction of hepatic lipogenesis associated with carbohydrate feeding could be inhibited by the inclusion of 18:2(n-6) in the diet but not by the addition of 16:0. Since the initial reports by these investigators (1,2), numerous studies have shown that the ingestion of fats rich in n-6 and n-3 fatty acids suppress hepatic de novo fatty acid biosynthesis, triglyceride production, and triglyceride secretion, while they enhance hepatic and skeletal muscle fatty acid oxidation (3-16). These metabolic effects cannot be mimicked by dietary monounsaturated and saturated fatty acids or by polyunsaturated fatty acids not derived from the n-6 and n-3 fatty acid families (for example, 5,13-22:2) (15). Moreover, in order for 18:2(n-6) and 18:3(n-3) to influence gene expression and metabolism, they must be metabolized to highly unsaturated fatty acid (HUFA) products of the Δ-6 desaturase pathway (16).

In addition to regulating lipid metabolism, HUFA may improve insulin sensitivity and increase nonoxidative glucose metabolism (17,18), but this promising action appears to be highly dependent upon the amount of HUFA consumed. For example, we have recently discovered that rats fed a diet containing 40% fish oil energy showed a pattern of muscle gene expression that suggested a severe impairment of glucose metabolism (a 20-fold increase in the expression of pyruvate dehydrogenase kinase 4). Interestingly, feeding rats a 40% olive oil diet [rich in 18:1(n-9)] resulted in a muscle gene expression pattern suggesting minimal interference with skeletal muscle
glycolysis, an appreciable increase in glucose conversion to glycogen, and significantly reduced DNA damage (Nelson CM, Heird W, Clarke SD, unpublished data).

A key component of the HUFA repartitioning mechanism involves an HUFA-dependent reduction in the hepatic activity of several glycolytic and lipogenic enzymes, including glucokinase, pyruvate kinase, acetyl-CoA carboxylase, fatty acid synthase, citrate lyase, malic enzyme, glucose-6-phosphate dehydrogenase, pyruvate dehydrogenase, and diacylglycerol acyl transferase (2-15,19). Expression of these enzymes is very sensitive to the suppressive effects of HUFA. Diets that contain as little as 2% to 4% of their dietary energy as HUFAs are sufficient to reduce hepatic fatty acid synthase activity by 25% to 40%, but maximum inhibition requires a diet that derives 10% of its energy from HUFA (15).

Paralleling the HUFA-mediated decrease in hepatic lipogenesis is an increase in the rate of hepatic fatty acid oxidation and ketogenesis (12-14). HUFAs enhance fatty acid oxidation by coordinately increasing the expression of mitochondrial L-type carnitine palmitoyltransferase and peroxisomal acyl-CoA oxidase, by decreasing the synthesis of malonyl-CoA, and by decreasing the sensitivity of carnitine palmitoyltransferase to malonyl-CoA inhibition (20). Carnitine palmitoyltransferase regulates the entry of fatty acids into the mitochondria, and acyl-CoA oxidase catalyzes the rate-limiting step in peroxisomal fatty acid oxidation. The contribution of hepatic peroxisomal fatty acid oxidation to overall fatty acid oxidation is unclear but has been reported to be as high as 20%. Dietary HUFAs readily induce the expression of both liver-type carnitine palmitoyltransferase and acyl-CoA oxidase. We have found that a diet providing as little as 7% of energy as HUFA will significantly increase the hepatic abundance of both transcripts [(21) and Nelson CM, Heird W, Clarke SD, unpublished data].

In addition to the HUFA-dependent up-regulation in hepatic capacity for fatty acid oxidation, metabolite regulation of fatty acid entry into the mitochondria is an important determinant of fatty acid oxidation (22-24). In this respect, malonyl-CoA has an important negative influence on carnitine palmitoyltransferase activity (22-24). The concentration of malonyl-CoA is highly dependent upon the rate of substrate flux through the fatty acid biosynthetic pathway (22-24). Hepatic concentrations of malonyl-CoA increase 10- to 20-fold when fasted rats are fed a high-carbohydrate meal. Several years ago we showed that if 18:2(n-6) was supplemented to the carbohydrate meal, malonyl-CoA synthesis was inhibited (20). This is one of the earliest metabolic changes detectable with HUFA feeding. Thus the fatty acid biosynthetic pathway (and its inhibition by dietary HUFA) is not only important for governing the rate of conversion of dietary carbohydrate to fatty acids and triglycerides, but is also a key determinant of the rate of fatty acid flux through the pathway of β oxidation. This latter function may be most important in humans, where dietary HUFAs are known to enhance fatty acid oxidation and lower hepatic triglyceride output but where net fatty acid synthesis is reportedly very low (25,26).

In addition to suppressing the production of malonyl-CoA, HUFAs also reduce the sensitivity of carnitine palmitoyltransferase to malonyl-CoA inhibition (27). In this respect, 20- and 22-carbon (n-3) HUFA appear to be more potent than 18:2(n-6) (27).
Thus, dietary HUFAs regulate hepatic lipid metabolism at three different levels: gene expression and enzyme abundance, malonyl-CoA synthesis, and membrane fluidity (27,28).

Malonyl-CoA is also a key regulator of fatty acid oxidation in skeletal muscle (23,24). Like the liver, the concentration of malonyl-CoA in soleus and gastrocnemius is highly dependent upon nutritional state. For example, feeding fasted rats a high-carbohydrate diet increased skeletal muscle malonyl-CoA levels by 50% to 250%, and, as in the hepatic system, the rate of fatty acid oxidation decreased as the level of skeletal muscle malonyl-CoA increased (23). However, unlike the liver, the production of malonyl-CoA in gastrocnemius muscle was not dependent upon the amount of muscle-type acetyl-CoA carboxylase protein found in the muscle (23).

The expression of muscle-type acetyl-CoA carboxylase is also unaffected by the type or amount of dietary HUFA (Nelson CM, Heird W, Clarke SD, unpublished data). Moreover, unlike the situation in the liver, muscle-type carnitine palmityltransferase and peroxisomal acyl-CoA oxidase gene expression is very resistant to up-regulation by dietary HUFA. In fact, achievement of a modest 50% to 100% increase in muscle-type carnitine palmityltransferase activity requires a diet containing less than 35% of HUFA energy (12-14), and even under these conditions peroxisomal acyl-CoA oxidase expression in gastrocnemius muscle remains virtually unchanged (Nelson CM, Heird W, Clarke SD, unpublished data).

A close examination of published reports reveals that investigators have only used high HUFA diets or cells in culture to investigate the influence of fatty acid composition on muscle fatty acid oxidation (12-14,21,29,30). Thus, although HUFAs may increase mitochondrial membrane fluidity and reduce the malonyl-CoA sensitivity of muscle-type carnitine palmityltransferase, the impact of physiologic intakes of HUFA on skeletal muscle fatty acid oxidation remains an open question. If increases in skeletal muscle fatty acid oxidation are to be seriously considered in formulating dietary recommendations for improving glucose sensitivity and for lowering blood triglycerides (17,18,31), the influence of HUFA on skeletal muscle metabolism must be examined at lower, more meaningful dietary levels.

A GENOMIC MECHANISM FOR THE POLYUNSATURATED FATTY ACID REGULATION OF LIPID METABOLISM

The Role of PPAR in PUFA Fatty Acid Oxidation and Synthesis

HUFA inhibition of gene expression occurs too quickly (< 90 minutes) to be explained simply by modification of hormone receptor signaling by HUFA (7,30–33). Rather, the changes are more consistent with the idea that HUFAs regulate the activity or abundance of a nuclear transcription factor directly. The 1990 discovery of a novel lipid activated transcription factor, peroxisome proliferator activated receptor α (PPARα), provided the first evidence that the nucleus did in fact contain transcription factors that were dependent on fatty acid ligands (34). PPARα is a member of the steroid receptor superfamily and like other steroid receptors, it possesses a DNA binding domain and a ligand binding domain (34–38). Interaction of PPARα with its
DNA recognition site is markedly enhanced by ligands such as the hypotriglyceridemic fibrate drugs, conjugated linoleic acid, and HUFA (34–38). In general, PPARα activation leads to the induction of several genes encoding proteins involved in lipid transport, oxidation, and thermogenesis (Fig. 2) (29,39–42).

The importance of PPARα in overall glucose and fatty acid homeostasis was shown by the observation that PPARα −/− mice lack the ability to increase rates of fatty acid oxidation during periods of food deprivation, and they develop characteristics of adult onset diabetes including fatty liver, raised blood triglycerides, and hyperglycemia (43). The fact that PPARα is essential for lipid oxidation was further underscored when hyperglycemia was found to suppress PPARα expression, induce PPARγ expression, increase β cell and cardiomyocyte lipids, and accelerate cell death (44,45).

That PPARα may play a pivotal role in both fatty acid and glucose metabolism quickly led to the idea that PPARα was a “master switch” transcription factor that was targeted by HUFAs to coordinate the suppression of genes encoding proteins of lipid synthesis and to induce genes encoding proteins of fatty acid oxidation and thermogenesis. More important, PPARα regulation by HUFA—particularly (n-3) HUFA and possibly conjugated linoleic acid—offered an explanation for the reported benefits of these fatty acids in protecting individuals from developing the detrimental characteristics of non-insulin-dependent diabetes (18). This attractive hypothesis was strengthened by reports that potent pharmacologic activators of PPARα modestly reduced lipogenic gene transcription (33). However, PPARα does not appear to interact with HUFA response sequences of lipogenic genes (10). Moreover, HUFA

FIG. 2. Nuclear mechanism for polyunsaturated fatty acids (PUFA) regulation of gene expression. FA, fatty acids; NF-Y, nuclear factor Y; PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator-activated receptor response element; Sp1, stimulatory protein 1; SREBP-1, sterol regulatory element binding protein-1; TG, triglycerides.
continue to suppress the transcription of hepatic lipogenic genes in PPARα−/−mice (46). Thus the HUFA inhibition of lipogenic gene expression is independent of PPARα and may simply reflect the increase in HUFA synthesis resulting from the PPARα-dependent induction of the Δ-6 desaturase pathway (Fig. 2) (16,47).

HUFA Regulation of Transcription Factors in the Insulin Response Element and Lipogenic Gene Transcription

Dietary HUFAs inhibit hepatic lipogenesis by suppressing the transcription of various hepatic enzymes involved in glucose metabolism and fatty acid biosynthesis, including glucokinase, pyruvate kinase, acetyl-CoA carboxylase, fatty acid synthase, stearoyl-CoA desaturase, and the Δ-6 and Δ-5 desaturases (2–11,33). HUFA response sequences have been well characterized in only three genes: fatty acid synthase, S14, and L-type pyruvate kinase. The rat fatty acid synthase gene contains two independent HUFA regulatory sequences that are located between −118 and −43 and between −7250 and −7035 (Teran-Garcia M, Clarke SD, unpublished data). Approximately 65% and 35%, respectively, of the HUFA control of the fatty acid synthase gene can be attributed to the proximal and distal elements. Interestingly, the proximal HUFA response region of the fatty acid synthase gene has characteristics that are very similar to the HUFA response region of the S14 gene (−220 to −80), while the distal HUFA response region of the fatty acid synthase has similarities to the L-type pyruvate kinase HUFA response region (−160 to −97) (10).

The proximal HUFA response region of the fatty acid synthase gene imparts insulin responsiveness to the gene and contains DNA binding sites for sterol regulatory element binding protein 1 (SREBP-1), USF (upstream stimulatory factor), Sp1 (specific transcription factor 1), and nuclear factor Y (NF-Y) (48–50). The nuclear abundance of USF is unaffected by dietary HUFA (33). In contrast, HUFAs rapidly reduce the nuclear content of hepatic SREBP-1, and this reduction is associated with a decreased rate of fatty acid synthase and S14 gene transcription (33). SREBP-1 exists in two forms, 1α and 1c. SREBP-1α is the dominant form in cell lines and is a regulator of genes encoding proteins involved in both lipogenesis and cholesterologenesis. SREBP-1c constitutes 90% of the SREBP-1 found in vivo and is a determinant of lipogenic gene transcription (54,55).

SREBP-1 is synthesized as a 125-kDa precursor protein that is anchored in the endoplasmic reticulum membrane (48,53). Proteolytic release of the 68-kDa mature SREBP-1 occurs in the Golgi system, and movement of SREBP-1 from the endoplasmic reticulum to the Golgi apparatus requires the trafficking protein, SREBP cleavage activating protein (SCAP) (53,56). Once released, mature SREBP-1 translocates to the nucleus and binds both to the classical sterol response element and to a palindrome CATG sequence. In the case of fatty acid synthase, SREBP-1 interacts with a CATG palindrome that also functions as an insulin response element (48).
Overexpression of mature SREBP-1a in the liver is associated with high rates of fatty acid biosynthesis and the development of a fatty liver (57). In contrast, the ablation of the SREBP-1 gene results in low expression of lipogenic genes (58). These observations led us to hypothesize that HUFAs inhibit lipogenic gene transcription by impairing the proteolytic release of SREBP-1c or by suppressing SREBP-1c gene expression, or both. Diets rich in 18:2 (n-6) or 20:5 and 22:6 (n-3) were found to reduce the hepatic nuclear and precursor content of mature SREBP-1 65% and 90%, and 60% and 75%, respectively (33). The decrease in SREBP-1c was accompanied by a comparable decrease in the transcription rate of hepatic fatty acid synthase (33).

Unlike HUFAs, saturated and monounsaturated fats have no effect on the nuclear content or precursor content of SREBP-1 or on lipogenic gene expression (33). The HUFA-dependent reduction in hepatic content of SREBP-1 may explain how HUFAs inhibit the transcription of several genes encoding proteins that are involved in hepatic glucose metabolism and fatty acid biosynthesis, including glucokinase, acetyl-CoA carboxylase, stearoyl-CoA desaturase, and the Δ-6 and Δ-5 desaturases (59). Interestingly, the inhibition of lipogenic gene expression that reportedly occurs in adipose tissue with the ingestion of fish oil does not involve an SREBP-1–dependent mechanism (51).

HUFAs reduce the nuclear content of SREBP-1 by a two-phase mechanism. The first phase is a rapid (< 60 minutes) inhibition of the proteolytic release process (54,56,60). The second phase involves an adaptive reduction (taking about 48 hours) in the hepatic content of SREBP-1 mRNA, which is subsequently followed by a reduction in the amount of precursor SREBP-1 protein (33). The mechanism whereby HUFAs acutely inhibit the proteolytic processes is unknown. However, nuclear run-on assays suggest that HUFAs reduce the hepatic content of SREBP-1 mRNA by posttranscriptional mechanisms (33). Using rat liver cells in primary culture, we have determined that HUFAs reduce the half-life of SREBP-1c mRNA from 11 hours to less than 5 hours (54). The mechanism by which HUFAs control the half-life of SREBP-1 is unknown, but it may require the synthesis of a rapidly turning-over HUFA-dependent protein (54).

Recently, we found that feeding rats the PPARα-specific activator WY 14,643 induced hepatic Δ-6 and Δ-5 desaturase gene expression severalfold. The induction in Δ-6 and Δ-5 desaturase gene transcription was paralleled by an increase in the hepatic abundance of the respective transcripts and by a rise in enzymatic activity (Tang Z, Cho H, Clarke SD, unpublished data). This apparent increase in the rate of conversion of 18-carbon HUFA to 20- and 22-carbon products was paralleled by a 50% reduction in the expression of SREBP-1 and its target gene, fatty acid synthase (33). Thus it appears that nonlipid ligand activators of PPARα reduce hepatic lipogenic gene transcription by inducing the activity of the Δ-6 and Δ-5 desaturase pathway. This suggests that PPARα activators (for example fibrates) lower hepatic triglyceride secretion and hence blood triglycerides in two ways: first, by stimulating the hepatic oxidation of fatty acids and thereby diverting fatty acids away from triglyceride synthesis; and second, by inducing the Δ-6 desaturase pathway and the production of 20- and 22-carbon fatty acids, which in turn lower the SREBP-1, thereby decreasing lipogenic
gene expression and the synthesis of malonyl-CoA for fatty acid biosynthesis and carnitine palmitoyltransferase regulation (Fig. 3). It remains to be determined whether the Δ-6 and Δ-5 desaturase pathway is enhanced by ligands for other PPARs such as PPARγ and PPARδ. However, it is interesting that enrichment of skeletal muscle phospholipids with 20- and 22-carbon HUFAs improves glucose uptake (17,18), and that PPARγ enhances skeletal muscle insulin sensitivity (61).

SREBP-1c by itself possesses weak trans activating power, but the binding of SREBP-1c to its recognition sequence enhances the upstream DNA binding of NF-Y and Sp1 and this in turn amplifies the trans activating activities of the three transcription factors (48). NF-Y is a heterotrimeric nuclear protein that reportedly plays a role in regulating chromatin structure by way of its interaction with histone acetyltransferases. The binding sites for NF-Y are essential for fatty acid synthase (49) and S14 promoter activity (62). Mutations within the Y box region of −104 to −99 of the S14 gene eliminate promoter activity by preventing NF-Y from interacting with upstream T3 (−2800 to −2500) and carbohydrate response (−1600 to −1400) regions (62). Similarly mutating the Y box motif of the rat fatty acid synthase gene eliminated 80% of the promoter activity (Teran-Garcia M, Clarke SD, unpublished data). In contrast, eliminating the SREBP-1 site (−67 to −53) reduced fatty acid synthase promoter activity by only 40%. More important, only 25% of the HUFA inhibition of fatty acid synthase promoter activity was lost with the SREBP-1 site mutation, whereas 55% was lost with the NF-Y site mutation. Apparently, HUFAs regulate fatty acid synthase gene transcription by governing the nuclear content of SREBP-1 and by interfering with the enhancer activity of NF-Y.
HUFA Regulation of the Carbohydrate Response Element and Lipogenic Gene Transcription

The insulin response region and its associated transcription factors (SREBP-1, NF-Y, and Sp1) are not the only nuclear factors regulated by HUFA. Transfection-reporter analyses indicate that HUFAs exert a negative influence on the carbohydrate response element of the L-type pyruvate kinase (10,63) and fatty acid synthase genes (Teran-Garcia M, Clarke SD, unpublished data). The nature of the transcription factors and the mechanism by which HUFAs regulate them are not well defined. One hepatic protein that may be an HUFA target is hepatic nuclear factor 4 (HFN-4) (64). HFN-4 is a member of the steroid receptor superfamily. It enhances the glucose/insulin induction of L-type pyruvate kinase transcription by binding as a homo-dimer to a direct repeat-1 motif (63). Like PPARα, HFN-4 has a ligand binding domain that interacts with acyl-CoA esters, but unlike PPARα, fatty acyl-CoA binding to HFN-4 decreases its DNA binding activity (63). This suggests that HUFA may exert part of its negative influence on gene transcription by reducing HNF-4 DNA binding activity. Linker scanner mutations through the carbohydrate response region of the L-type pyruvate kinase promoter (that is, −183 to −97) did in fact reveal that the HNF-4 recognition elements were essential for HUFA suppression of the promoter (63). Recently we found that sequences between −7242 and −7150 of the fatty acid synthase gene were required for glucose to induce fatty acid synthase gene transcription (65,66). Subsequent studies have shown that −7242 to −7150 contains DNA recognition sites for HNF-4 and a novel carbohydrate response factor (66). Moreover, deleting this sequence eliminated 30% to 40% of the total HUFA suppression of the fatty acid synthase promoter (Teran-Garcia M, Clarke SD, unpublished data). Thus, HUFA suppression of lipogenic gene expression may involve two mechanisms: interference with the insulin regulation of transcription factors interaction with the insulin response element; and interference with the glucose signal governing the transcription factors affecting the carbohydrate response element.

HUFA Regulation of mRNA Processing

One of the first transcripts shown to be reduced by dietary HUFA was glucose-6-phosphate dehydrogenase mRNA. Interestingly, nuclear run-on assays revealed that this did not reflect an inhibition of gene transcription (67,68). Recently, Salati and coworkers have shown that HUFA govern the hepatic abundance of glucose-6-phosphate dehydrogenase mRNA by inhibiting the processing events involved in mRNA maturation (67,68). HUFA regulation of transcript processing may be unique for glucose-6-phosphate dehydrogenase, because transcription studies indicate that HUFAs exert their inhibitory influence primarily by suppressing the rate of lipogenic gene transcription. Nevertheless, these results show that the dietary HUFA influence lipid synthesis and oxidation at several different regulatory steps including transcription, mRNA processing, mRNA decay, and post-translational modifications.
ARE Ω-3 FATTY ACIDS BETTER FUEL REPARTITIONERS THAN Ω-6?

To answer the relative potency question requires first, a knowledge of the structural requirements for a HUFA to possess inhibitory activity, and second, an appreciation that much of the work evaluating the effectiveness of ω-6 and ω-3 fatty acids has compared vegetable oils with marine oils. Such a comparison not only mixes the effects of n-6 with n-3 fatty acids, but also in essence compares Δ-6 desaturase substrates [for example, 18:2(n-6)] with Δ-6 desaturase products [for example, 20:5(n-3)].

To begin with, an inhibitory HUFA must consist of 18 carbons and contain at least two conjugated double bonds in the 9,12 position. The loss of one of the double bonds by hydroxylation renders the fatty acid inactive, but one of the double bonds may be in a trans configuration (15). A fatty acid may contain additional double bonds and retain inhibitory potency (for example, columbinic acid, 5t,9c,12c-18:3) (15). Most important, in order for an HUFA to influence fuel repartitioning it must be a product of the Δ-6 desaturase pathway (16). These fatty acid structural requirements appear also to be instrumental in the HUFA regulation of skeletal muscle metabolism, but unequivocal data establishing these requirements remain to be acquired.

Δ-6 Desaturase has a two- to three-fold greater affinity for 18:3(n-3) than for 18:2(n-6). Consequently, 18:3(n-3) is more effective than 18:2(n-6) as a regulator of lipid synthesis and oxidation. However, only a small amount of 18:2(n-6) and 18:3(n-3) undergoes Δ-6 desaturation (<1%) (69). Thus it is not surprising that dietary oils rich in fatty acid products of Δ-6 desaturase are approximately three-fold more effective as suppressors of hepatic lipogenesis and inducers of fatty acid oxidation than the 18-carbon HUFA substrates (15). Moreover, when n-6 and n-3 HUFAs are compared on an equal carbon basis, n-6 and n-3 HUFAs appear to be equipotent regulators of lipid metabolism. For example, when 18:3(n-6) and 20:4(n-6) were compared with fish oil, those fatty acids were equipotent inhibitors of hepatic fatty acid biosynthesis (15). Thus, the greater effectiveness of fish oil may have less to do with its n-3 fatty acid content than with the fact that fish oils are rich in 20- and 22-carbon HUFA, which bypass the regulated and required steps of desaturation and elongation. However, that said, one cannot overlook the fact that 20:4(n-6) may increase the production of eicosanoids and thereby offset the health benefits of HUFA (for example, by enhancement of inflammatory responses). On the other hand, 20:5(n-3) would give rise to eicosanoids with low bioactivity, and in this way, would provide benefits for both lipid metabolism and eicosanoid target diseases.

Finally, although n-6 and n-3 fatty acid products of Δ-6 desaturase are modest inducers of fatty acid oxidation, both fatty acid groups are relatively weak ligand activators of PPARα. In contrast, eicosanoid products of n-6 and n-3 HUFAs have two- to three-fold greater affinity for PPARα and hence are more effective activators of PPAR-responsive genes (70). This raises the possibility that the bioactive n-6 or n-3 fatty acid that governs lipid metabolism may be a prostaglandin, leukotriene, or hydroxylated fatty acid. While this question remains unresolved, numerous studies using a wide array of eicosanoid synthesis inhibitors have failed to detect eicosanoid involvement in the regulation of hepatic lipid and glucose metabolism (5,30).
Prostaglandins have been implicated in the inhibition of lipogenic gene expression in 3T3-L1 adipocytes (71), but their involvement in the in vivo regulation of lipogenesis remains to be established. In general, there are few data to support the argument that dietary HUFAs exert their influence on hepatic lipogenesis by enhancing eicosanoid production. In fact, the signaling mechanism whereby HUFAs govern lipogenesis and lipogenic gene expression remains a mystery.

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REFERENCES


**DISCUSSION**

*Dr. Kon:* What about the antioxidant effects of ω-3 fatty acids? You spoke mainly about oxidation, but there are data on the antioxidant effects of fish oil supplements, in woman particularly.

*Dr. Clarke:* I removed a data set because I needed to shorten my presentation. We’ve finished the DNA arrays and measured 10,000 gene products. Interestingly, a high olive oil and high fish oil diet decreased DNA repair enzymes, high olive oil having the greatest effect. Therefore, the implication is that there’s a decrease in apoptosis, perhaps due to the antioxidant components of olive oil or perhaps due to the 18-1 itself. We also saw some changes in skeletal muscle inflammatory responses, but they didn’t go the way you’d expect them to—that is, the olive oil had some effects but fish oil did not, and it didn’t enhance inflammation. So it doesn’t look as though fish oil is going to cause any additional oxidative stress in skeletal muscle. We saw more positive effects with olive oil than we did with fish oil. Peroxisomal proliferation occurs, which means there will be increased hydrogen peroxide production as well, but that’s about as much as we’ve done in terms of looking at antioxidant gene expression.

*Dr. Scott:* I have a philosophical question. If you go along with the idea that the genetic blueprint was determined thousands of years ago when men were hunter-gatherers and presumably living off vegetables and animal foodstuffs, where did all this fish come from?

*Dr. Clarke:* Fish oil happens to be an effective tool. Arachidonic acid or GLA, its precursor, will have the same inhibitory effects as fish oil fatty acids. Anything that’s a product of the Δ6 desaturase that’s derived from n-6, n-3 family will inhibit lipogenic gene expression and decrease triglyceride synthesis. Now, why did that evolve? Probably because too much oleic acid increases the essential fatty acid requirement. When we used oleic acid in our hepatocyte preparation, we always got increased fatty acid synthase expression. So, my guess is that this evolved as a regulatory mechanism so that the liver would not accumulate excessive amount of triglyceride or synthesize excessive 18-0 and 18-1, while being able to conserve its precursors for essential fatty acids.

*Dr. Böhles:* In classical biochemistry, the shorter the fatty acid and the less unsaturated, the greater the degree of self-oxidation. From your research, would you say that PUFAs are only signal-providing substances or are they also oxidized themselves?

*Dr. Clarke:* They are oxidized, but depending on length. Their rate of oxidation depends greatly upon the number of double bonds in the carbon link. The first four steps in 20-carbon polyunsaturated oxidation take place in the peroxisome, which is probably why we get peroxisomal proliferation. After that, the mitochondria take over. Therefore, something like DHA is oxidized very slowly. Linoleate is readily oxidized, but probably not more rapidly than palmitate or oleate.
Dr. Wanders: What always strikes me about these nuclear hormone receptors is that the most powerful ligands are the free fatty acids, but in the cell I would be very surprised if the ligands were present as free acids. Could you comment?

Dr. Clarke: I agree with you that there is very little free acid in the cell. My guess is that the ligands for these fatty acid binding receptors are not truly fatty acids. The assays are set up to make it look as though fatty acids are the activators, but any lipophilic compound will bind and activate. You can't get the type of separation of function between saturates, monounsaturates, and polyunsaturates if your investigation is solely based upon binding constants of lipid to proteins—they are just not large enough to give you adequate levels of difference in biologic responses. In order to achieve that level of selectivity, you must arrange for some sort of metabolic transformation of the fatty acid, probably to an oxide or a hydroxide. In that way, you can get orders of magnitude difference in binding activity. I'm sure that it's not directly the result of the fatty acid.

Dr. Bachmann: How are the PUFAs transported across the plasma membrane, and how are they exchanged in plasma?

Dr. Clarke: I think there is good evidence that CD36 is a fatty acid transport protein in certain cell types, and there has been a lot of work showing impaired fatty acid utilization in knockout mice (1). So there is involvement of a transporter for fatty acids. The question is whether that transporter has selectivity for polyunsaturates versus saturates. With knockout mice and using genetic manipulations such as overexpression, we should be able to determine eventually whether one fatty acid or another is preferred.

Dr. Bier: What was called the nuclear hormone super receptor family was discovered because when individual examples of the class were identified people started looking for other receptors and other members of the class, using molecular characteristics and concentrations. Thus, they completely missed a whole family of receptors because the concentration differences and characteristics were different. Now that we've dropped the "hormone" out of the name of the class, we're discovering a new family of orphan receptors or potential ligands. These studies may provide us with information about the dietary ligands that regulate these receptor families.

Dr. Clarke: In my opinion, nutrients were the first hormones. The cell had to respond to its nutrient environment and be able to survive, and hormones represented a more sophisticated system to ensure this. I think the next hot target will be the LXR receptor. This is an orphan receptor that apparently binds oxysterols and may also bind polyunsaturates. Thus, it is another member of the steroid receptor superfamily and has some very interesting characteristics. There are several members of this family of orphan receptors that are being found to bind metabolites of lipids.

Dr. Daniel: I would like to comment on the fatty acid transport proteins. As far as I know, six genes have now been isolated. These proteins are expressed at different levels in different tissues but all the functional data I'm aware of show that they have a very high preference for saturated fatty acids and show only weak interaction with polyunsaturated fatty acids.

Dr. Clarke: That could well be. Although I'm not a lipid biochemist, I think that there are many problems with fatty acid transport studies, and I don't have a lot of confidence that we can do them correctly. There was a time when it was thought that fatty acids inhibited virtually every enzyme in glycolysis and fatty acid biosynthesis, but it turned out that this was a denaturing effect and had no biological significance at all. Therefore, I'm a skeptic when it comes to those kinds of studies.

Dr. Daniel: You said that PUFAs could inhibit fatty acid synthase, or reduce its expression, and so diminish triglyceride synthesis in liver, while at the same time improving β oxidation.
What is the mechanism behind that? Just reducing synthesis of triglycerides does not necessarily mean that the fatty acids go to β oxidation, at least not in humans.

Dr. Clarke: In the rat, the mechanism is that we inhibit the lipogenic side, but at the same time we stimulate the oxidative side and lower malonyl-CoA concentrations, which allows the fatty acid to enter the mitochondria more readily. So you have polyunsaturates working in both ways: down-regulating synthesis and up-regulating oxidation. That happens in the liver and probably in the β cell; it also definitely happens in the heart. Whether or not it happens in skeletal muscle seems to be an open question. All the studies I’m aware of in skeletal muscle that have claimed that polyunsaturates stimulate fatty acid oxidation in the muscle have used diets that are very high in fat—40% polyunsaturated or 40% fish oil, which are not meaningful levels. When you look at lower levels, you don’t see the kind of gene expression control that one would predict from the high-level studies. I think the increased oxidation of fatty acid in skeletal muscle—if it occurs at all—will most probably occur through a metabolite control mechanism rather than through changes in enzyme amount and gene expression.

Dr. Borum: Is the metabolic syndrome-like phenotype that is seen in HIV patients treated with some antiretroviral agents associated with the story you have recounted? In addition, could you utilize any of this information for the benefit of such patients?

Dr. Clarke: My familiarity with this is not great. My understanding of this condition is that it resembles a lipid dystrophy, so the problem is a different one in that there is an inability to get glucose into the fat cells. Here we are talking about a high correlation between lipid accumulation and cell death, and then the development of insulin resistance, so I think the scenarios are different. In adipose tissue, you don’t see this polyunsaturate control of the fat.

Dr. Böhles: If you take the situation where malonyl-CoA influences CPT-1 (carnitine palmitoyltransferase 1), have you any idea how polyunsaturated fatty acids could influence the KI with respect to infinity of fatty acids for CPT?

Dr. Clarke: There have been good data in the last year or so showing that making the mitochondrial membranes more unsaturated increases the Vmax for CPT-1 (2). Therefore, that is a case where you do have changes in kinetic behavior that accelerate fatty acid oxidation by CPT-1 transport. We have no data yet with respect to KIs for polyunsaturates in lowering malonyl-CoA. Several years ago, we did show the decrease in malonyl-CoA in the liver, but we didn’t look at KIs. The evolving malonyl-CoA decarboxylase story opens another door in determining whether polyunsaturates accelerate decarboxylation of malonyl-CoA, and I think that is going to be important. The polyunsaturates we’re talking about here must be products of the Δ6-desaturase. If we knock out the Δ6-desaturase, we do not see any of this gene expression control on the lipogenic side.

Dr. Bachmann: In relation to PUFAs, are the differences between the two chain lengths, 20 and 22?

Dr. Clarke: Probably not in the studies we’re looking at in terms of lipogenic control and fat synthesis. In terms of fatty acid oxidation, I would say that DHA is probably going to be more potent. There aren’t many dose–response studies available, however.

Dr. Bachmann: You also mentioned the monounsaturated fatty acids, and you said at the end of your presentation that you should use four parts of olive oil, one part of fish oil, and one part of vegetable oil. Can you comment on that?

Dr. Clarke: That’s based on our micro arrays. These have turned up at least six DNA repair enzymes that are down-regulated with olive oil. This is a significant change and indicates that the olive oil is protecting the muscle DNA against damage. The antioxidant components of olive oil may be responsible, but we don’t know that yet. My recommendation may be somewhat provocative, but we can accomplish the triglyceride effects—the lowering of blood and
tissue triglycerides, in the liver particularly—with about 10% of dietary energy from fish oil type fatty acids. In respect of the safflower oil, we need certain amounts of essential fatty acids for growth, but the effects on gene expression don’t saturate until you reach about 10% of energy. So for both the n-3 and the n-6 fatty acids about 10% of dietary energy is needed. Then based upon the marked changes in reducing DNA repair enzymes, I would provide the balance as olive oil. That’s where those suggestions came from.

REFERENCES
