

## Polyunsaturated fatty acid regulation of gene transcription: a mechanism to improve energy balance and insulin resistance

Steven D. Clarke

*Graduate Program of Nutritional Sciences, The University of Texas at Austin,  
115 Gearing Building, Austin, Texas 78712, USA*

This review addresses the hypothesis that polyunsaturated fatty acids (PUFA), particularly those of the *n*-3 family, play essential roles in the maintenance of energy balance and glucose metabolism. The data discussed indicate that dietary PUFA function as fuel partitioners in that they direct glucose toward glycogen storage, and direct fatty acids away from triglyceride synthesis and assimilation and toward fatty acid oxidation. In addition, the *n*-3 family of PUFA appear to have the unique ability to enhance thermogenesis and thereby reduce the efficiency of body fat deposition. PUFA exert their effects on lipid metabolism and thermogenesis by up-regulating the transcription of the mitochondrial uncoupling protein-3, and inducing genes encoding proteins involved in fatty acid oxidation (e.g. carnitine palmitoyltransferase and acyl-CoA oxidase) while simultaneously down-regulating the transcription of genes encoding proteins involved in lipid synthesis (e.g. fatty acid synthase). The potential transcriptional mechanism and the transcription factors affected by PUFA are discussed. Moreover, the data are interpreted in the context of the role that PUFA may play as dietary factors in the development of obesity and insulin resistance. Collectively the results of these studies suggest that the metabolic functions governed by PUFA should be considered as part of the criteria utilized in defining the dietary needs for *n*-6 and *n*-3 PUFA, and in establishing the optimum dietary ratio for *n*-6 : *n*-3 fatty acids.

### **Polyunsaturated fatty acids: Gene transcription: Insulin resistance**

#### **Introduction**

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 which 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 proteins of energy metabolism, cell differentiation and cell growth. Nutrients govern the tissue content of different proteins by functioning as regulators of gene transcription, nuclear RNA processing, mRNA stability and mRNA degradation. One dietary constituent

which has a strong influence on cell differentiation, growth and metabolism is fat. The fatty acid component of dietary lipid not only influences hormonal signalling events by modifying membrane lipid composition, but fatty acids have a very strong direct influence on the molecular events governing gene expression. It is the regulation of gene expression by dietary fats which we believe has the greatest impact on the development of obesity and insulin resistance.

#### **Type of dietary fat influences energy balance and insulin resistance**

Dietary fat is deposited into adipose tissue with greater energetic efficiency than is the dietary energy derived from carbohydrate. This is one reason why energy-dense diets appear to be positively associated with the development of obesity (Boden & Chen, 1995). However, the development of obesity is not required for dietary fat to adversely affect glucose metabolism (Anderson *et al.* 1973). The impairment in glucose metabolism associated with the ingestion of fat appears to be the cumulative product of:

- (1) impaired translocation of glucose transporter-4 activity (Van Epps-Fung *et al.* 1997; Zierath *et al.* 1997)
- (2) suppressed expression of glucose transporter-4 (Kahn & Pedersen, 1993)
- (3) inhibited expression of hepatic glycolytic and lipogenic enzymes (Clarke & Jump, 1993; Jump & Clarke, 1999)
- (4) impaired insulin signalling (Kolterman *et al.* 1979).

In addition, the chronic ingestion of a high-fat diet may enhance insulin release (Prentki & Corkey, 1996) which could in turn lead to the development of hyperinsulinaemia, and subsequently a decrease in insulin receptor number (Olefsky & Saekow, 1978).

Unfortunately, many of the nutritional studies designed to examine the impact of dietary fat on body fat accumulation and glucose metabolism have failed to consider the possibility that not all fats exert the same effects. Over 30 years ago, Allmann & Gibson (1969) 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 while the addition of 16:0 had no effect. Since these first observations, numerous studies have demonstrated that the ingestion of fats rich in  $n$ -6, and particularly  $n$ -3 PUFA, suppress hepatic lipogenesis (Jump & Clarke, 1999); reduce hepatic triglyceride output (Nestel *et al.* 1984); enhance ketogenesis (Wong *et al.* 1984); and induce fatty acid oxidation in both liver and skeletal muscle (Thomassen *et al.* 1982; Power & Newsholme, 1997). These changes in fuel metabolism are accompanied by a decrease in body fat deposition (Hill *et al.* 1993; Takada *et al.* 1994; Couet *et al.* 1997). Dose–response studies comparing vegetable oils rich in 18:2 $n$ -6 with fish oils rich in 20- and 22-carbon  $n$ -3 PUFA clearly demonstrate that the 20- and 22-carbon  $n$ -3 oils are significantly more effective than the vegetable oils (Clarke & Jump, 1993; Baillie *et al.* 1999). Although part of the difference in potency between fish oils and vegetable oils may be due to the fact that the 18:2 $n$ -6 must undergo  $\Delta$ -6 desaturation in order to be metabolically active (Clarke & Jump, 1993), recent studies comparing 18:2 $n$ -6 with 18:3 $n$ -3 indicate that the 18:3 $n$ -3 continues to be more potent than 18:2 $n$ -6.

Although the re-partitioning of fatty acids away from triglyceride synthesis and toward oxidation may explain the hypotriglyceridaemic action of dietary PUFA, it can not explain why fat deposition is less efficient in animals fed diets that are rich in long-chain PUFA. Such an explanation requires the induction of biochemical processes that enhance heat production and are inducible by dietary PUFA. In this regard, peroxisomal fatty acid oxidation and the uncoupling of mitochondrial oxidative phosphorylation appear to fill this requirement (Reddy & Mannaerts, 1994; Vidal-Puig *et al.* 1997; Schrauwen *et al.* 1999; Weigle *et al.* 1999). Peroxisomal fatty acid oxidation is thermogenic because the first step in the pathway utilizes molecular oxygen as the electron acceptor rather than FAD. The result is that each acetyl-CoA produced by peroxisomal fatty acid oxidation yields two ATP in contrast to the three ATP produced by mitochondrial fatty acid oxidation. With respect to mitochondrial oxidative phosphorylation, at least three mitochondrial uncoupling proteins (UCP) have been cloned (Boss *et al.* 1998; Schrauwen *et al.* 1999), and two,

UCP-2 and UCP-3, appear to be inducible by dietary fat (Fleury *et al.* 1997; Baillie *et al.* 1999).

Peroxisomal fatty acid oxidation is generally considered the key pathway by which very long chain fatty acids (e.g. 22- and 24-carbon) are shortened prior to their oxidation in the mitochondria (Reddy & Mannaerts, 1994). However, peroxisomal fatty acid oxidation may also be a significant site of fatty acid oxidation for 16- and 18-carbon fatty acids. In fact, studies with isolated rat hepatocytes (Kondrup & Lazarow, 1985) have estimated that as much as 25% of hepatic fatty acid oxidation may occur in the peroxisome. Peroxisomal fatty acid oxidation also occurs in skeletal muscle (Baillie *et al.* 1999). Moreover, the enzymes of peroxisomal fatty acid oxidation in both liver and muscle are induced two- to threefold by dietary fish oil rich in 20- and 22-carbon  $n$ -3 PUFA and by peroxisome proliferator activated receptor- $\alpha$  (PPAR $\alpha$ )-specific ligands (Baillie *et al.* 1999; C. M. Nelson & S. D. Clarke, unpublished results). Although the amount of fatty acid oxidation that takes place in the skeletal muscle is unknown, a twofold increase in the peroxisomal oxidative capacity of skeletal muscle, combined with the large size of the skeletal muscle pool, suggests that the peroxisome could be a significant site of fatty acid oxidation and diet-induced thermogenesis. The idea that peroxisomes may play an important role in energy balance was recently underscored by the finding that PPAR $\alpha$ -knock-out mice, which completely lack PPAR $\alpha$ , developed adult-onset obesity and experienced increased triglyceride deposition in the liver (Costet *et al.* 1998). Unfortunately, the potential contribution of peroxisomal fatty acid oxidation to human energy balance remains to be established. Nevertheless, this appears to be an important pathway for thermogenesis in rodents and pigs (Takada *et al.* 1994; Costet *et al.* 1998; Baillie *et al.* 1999).

Until recently the role of UCPs in human thermogenesis was unclear because the only UCP identified was largely expressed in brown adipose tissue, and humans possess very limited amounts of brown fat (Boss *et al.* 1998). However, recently UCP-2 and UCP-3 were cloned (Fleury *et al.* 1997; Solanes *et al.* 1997), and both proteins appear to possess the ability to uncouple mitochondrial oxidative phosphorylation (Jaburek *et al.* 1999). UCP-2 is expressed in a wide array of tissues, but UCP-3 expression is largely restricted to skeletal muscle (Vidal-Puig *et al.* 1997). We have found that feeding rats on fish oil not only induced peroxisomal fatty acid oxidation in skeletal muscle, but also increased the abundance of UCP-3 mRNA nearly twofold (Baillie *et al.* 1999). More importantly, the induction of UCP-3 expression was associated with a 25% reduction in body fat deposition (Baillie *et al.* 1999). These data support the idea that PUFA, notably the  $n$ -3 family, are less efficiently utilized for body fat deposition because they induce the thermogenic pathways of peroxisomal fatty acid oxidation and mitochondrial uncoupling.

Interestingly, the enhancement of fatty acid oxidation and thermogenesis by dietary PUFA is also associated with an improvement in glucose uptake and insulin sensitivity (Storlien *et al.* 1987; Field *et al.* 1990). As an example, Storlien *et al.* (1987) found that the 50% reduction in whole-body glucose disposal and oxidation

observed in rats fed 40% of their calories as safflower oil was completely prevented by replacing 20% of the fat calories with tuna oil. Similarly, Field *et al.* (1990) reported that adipocytes were more insulin sensitive and transported more glucose when the polyunsaturated to saturated fatty acid ratio of the diet was increased from 0.25 to 1.0. An improvement in glucose metabolism in the face of accelerated fatty acid oxidation would appear to be in conflict with the idea that higher rates of fatty acid oxidation actually impair glucose utilization (Saloranta *et al.* 1993). An explanation for this paradox may lie with the role that dietary fatty acids play in determining membrane fluidity (Podolin *et al.* 1998). Dietary regimes that reduce membrane fluidity (e.g. low 18:2 $n$ -6 to 16:0 ratio or high sucrose intakes) also impair insulin receptor signalling and reduce glucose transporter activity (Field *et al.* 1990; Podolin *et al.* 1998). On the other hand, enhancing skeletal muscle membrane fluidity by simply providing 5–10% of the dietary energy as fish oils, and consequently enriching the membranes with 20:5 and 22:6 $n$ -3, accelerates glucose uptake and maintains normal glucose metabolism even at high levels of fat intake (Storlien *et al.* 1987). Recent data have revealed that the degree of insulin resistance in humans is negatively correlated to the amount of 22:6 $n$ -3 within skeletal muscle phospholipid (Baur *et al.* 1998). The improvement in glucose uptake that occurs when membranes are enriched with very long chain PUFA appears to be due to the fact that glucose transporter-4 resides in the plasma membrane for a prolonged period of time (Gasperikova *et al.* 1997). This increase in residence time associated with higher membrane fluidity is associated with an enlargement of the intracellular pool of glucose-6-phosphate (Podolin *et al.* 1998). Enlargement of the glucose-6-phosphate pool would potentially explain the increase in skeletal muscle glycogen synthesis that is observed in rats fed on fish oil (Storlien *et al.* 1987). More importantly, the ability of fish oil to enhance the rate of glycogen storage would allow the skeletal muscle to increase its uptake of glucose, even under conditions where fatty acid oxidation is accelerated. A second event elicited by dietary fish oil is a reduction in the intramuscular content of triglyceride (Storlien *et al.* 1991). A decrease in lipid droplet size and number has been found to be associated with an improved insulin sensitivity (Storlien *et al.* 1991). Presumably the decrease in lipid droplet number and size is due to the fact that dietary fish oils induced skeletal muscle fatty acid oxidation (Power & Newsholme, 1997; Baillie *et al.* 1999), but the mechanism coupling lipid droplet size with insulin sensitivity is unknown. One possibility is that large lipid droplets are the outcome of slower rates of fatty acid oxidation. A reduction in fatty acid oxidation may be related to an elevation in the intramuscular content of malonyl-CoA (Saha *et al.* 1995). High levels of malonyl-CoA inhibit the activity of carnitine palmitoyltransferase and consequently slow the entry of fatty acids into the mitochondria (Wilson *et al.* 1990; Saha *et al.* 1995). The resulting decrease in fatty acid oxidation would presumably enhance the flux of fatty acyl-CoA into the triglyceride synthetic pathway and thereby increase triglyceride accumulation. Finally, weight loss itself is often accompanied by an improvement in

insulin sensitivity. Thus the induction of thermogenesis resulting from  $n$ -3 PUFA ingestion may improve insulin sensitivity and glucose metabolism by decreasing the efficiency of fat deposition.

Collectively, these data indicate that the consumption of  $n$ -3 PUFA, particularly the 20- and 22-carbon fatty acids, could play a preventive role in the development of insulin resistance. In support of this conclusion, Podolin *et al.* (1998) recently reported that as little as 6% fish oil in the diet of rats prevented the development of insulin resistance. However, it should be noted that some human studies suggest that fish oil ingestion by patients who have already developed non-insulin-dependent diabetes may actually exacerbate the insulin resistance (Malasanos & Stacpoole, 1991), but this conclusion is not consistently observed in all studies. Overall, dietary  $n$ -3 PUFA appear to play an essential dietary role in the prevention of insulin resistance, and may be important to total energy balance.

In summary, the Randle hypothesis (fatty acid oxidation impairs glucose utilization) may be true when discussing the relationship between the oxidation of saturated and monounsaturated fatty acids and the utilization of glucose, and it may have applications within individuals who have developed the metabolic syndrome. However, a large amount of data now indicate that  $n$ -6 and particularly  $n$ -3 PUFA uniquely regulate glucose and lipid metabolism and play a useful role as a nutritional component for prevention of insulin resistance. Moreover, the unique ability of PUFA to govern fuel partitioning should be taken into consideration when discussing the interactions between dietary fats and carbohydrates.

#### **Polyunsaturated fatty acid regulation of fuel partitioning: a transcriptional explanation**

The PUFA-dependent repartitioning of metabolic fuels away from storage and toward oxidation reflects the fact that PUFA coordinately suppress the transcription of lipogenic genes, while they simultaneously induce the transcription of genes encoding proteins of lipid oxidation and thermogenesis (Clarke *et al.* 1997; Baillie *et al.* 1999; Jump & Clarke, 1999). Specific examples of lipogenic genes that are controlled by PUFA include hepatic glucokinase (Jump *et al.* 1994); pyruvate kinase (Liimatta *et al.* 1994); pyruvate dehydrogenase (Da Silva *et al.* 1993); acetyl-CoA carboxylase (Katsurada *et al.* 1990); fatty acid synthase (Clarke *et al.* 1990; Xu *et al.* 1999); and adipocyte fatty acid synthase (Mater *et al.* 1998). Fatty acids of the  $n$ -3 family may also interfere with the terminal steps of fat cell differentiation (Mater *et al.* 1998). The PUFA induction of genes encoding proteins involved in lipid oxidation and thermogenesis include carnitine palmitoyltransferase (Mascaro *et al.* 1998); mitochondrial HMG-CoA synthase (Rodriguez *et al.* 1994); peroxisomal acyl-CoA oxidase (Baillie *et al.* 1999; Jump & Clarke, 1999); fatty acid binding proteins (Kletzien *et al.* 1992; Kahn & Sorof, 1994); fatty acid transporter and fatty acyl-CoA synthetase (Martin *et al.* 1997); and UCP-3 (Baillie *et al.* 1999).

The changes in metabolic pathways affected by dietary PUFA have traditionally been thought to reflect alterations in hormone receptor signalling caused by fatty acid changes

in membrane phospholipids, or to eicosanoid synthesis and subsequent signalling via eicosanoid receptors. As the mechanisms by which PUFA regulate the genes encoding proteins of lipid synthesis and oxidation begin to unravel, we found that the PUFA regulation of gene transcription occurred within a matter of minutes (Clarke *et al.* 1990; Jump *et al.* 1994; Xu *et al.* 1999). Such a time frame was too rapid to be explained simply by changes in membrane composition and altered hormone release or signalling. However, the rapidity with which PUFA modified gene transcription was most consistent with a ligand-mediated event, i.e. a PUFA-binding transcription factor. The concept of a fatty acid activated transcription factor gained significant support when the PPAR was cloned in 1990 (Issemann & Green, 1990). The amino acid sequence predicted by the open reading frame for PPAR indicated that it possessed structural features that were characteristic of steroid receptors, i.e. a ligand-binding domain and a zinc finger DNA-binding domain (Issemann & Green, 1990). Ligand activation assays revealed that fatty acids, fibrates and plasticizers were possible ligands for PPAR, and that ligand binding enhanced the interaction of PPAR with its DNA recognition sequence (Kliwer *et al.* 1997). Characterization of the PPAR response element that resided in the 5'-flanking region of the peroxisomal acyl-CoA oxidase gene revealed that the response element consisted of a hexameric repeat (AGGTCA) and that PPAR binding to the repeat sequence was greatly enhanced when PPAR formed a heterodimer partnership with RXR $\alpha$  (Varanasi *et al.* 1996). Interestingly, the direct repeat-binding motif was identical to the repeat sequence recognized by the thyroid hormone receptor and the vitamin D<sub>3</sub> receptor, and by a wide collection of orphan receptors (Clarke *et al.* 1999). Since the initial identification of the liver PPAR (now designated PPAR $\alpha$ ), a number of PPAR isoforms have been identified including PPAR $\gamma$ 1 and PPAR $\gamma$ 2, the ubiquitous PPAR $\delta$  (NUC-1/FAAR), and two related lipid-activated transcription factors, LXR and FXR (Clarke *et al.* 1999). The relative abundance and function of each PPAR varies with type of tissue. For example, PPAR $\alpha$  is found in many tissues but has its highest abundance in liver. On the other hand, PPAR $\gamma$  has been found in adipocytes, macrophages and colonocytes, and appears to play a pivotal role in cell differentiation (Brun *et al.* 1996; DuBois *et al.* 1998; Tontonoz *et al.* 1998).

Functional PPAR response elements have been found to exist in several genes including carnitine palmitoyltransferase, acyl-CoA oxidase, fatty acyl-CoA synthetase, mitochondrial HMG-CoA synthase, lipoprotein lipase and fatty acid-binding protein (Rodriguez *et al.* 1994; Varanasi *et al.* 1996; Martin *et al.* 1997; Mascaro *et al.* 1998). In addition, a functional PPAR response element appears to reside in the 5'-flanking region of the UCP-1 gene (Foellmi-Adams *et al.* 1996), and PPAR $\alpha$  activators induce the expression of UCP-3 in skeletal muscle (C. M. Nelson and S. D. Clarke, unpublished results). Although a number of genes contain recognition sequences for PPAR, the PPAR isoform which binds to the direct-repeat response element depends on the type of ligand that interacts with the ligand domain of the PPAR. In this respect, a wide array of lipophilic factors including PUFA, fibrates, prostaglandins and leukotrienes,

oxidized fatty acids and the anti-diabetic drugs called thiazolidenediones will bind to a variety of PPARs, but the specificity of activation depends on the binding affinity and activation constant that each ligand has for the respective PPAR (Issemann & Green, 1990; Forman *et al.* 1995; Kliwer *et al.* 1997; Tontonoz *et al.* 1998). In the liver, fibrates (e.g. clofibrate, gemfibrozil) have a high affinity for PPAR $\alpha$ . Fibrate activation of PPAR $\alpha$  leads to an enhanced binding of PPAR $\alpha$  to its AGGTCAAGGTCA recognition sequence localized in the 5'-flanking region of the carnitine palmitoyltransferase, and peroxisomal acyl-CoA oxidase, mitochondrial HMG-CoA synthase, fatty acyl-CoA synthetase, and the fatty acid transporter (Rodriguez *et al.* 1994; Varanasi *et al.* 1996; Martin *et al.* 1997; Mascaro *et al.* 1998). The outcome is an increased rate of gene transcription leading to a rise in protein abundance, and finally an enhanced rate of hepatic fatty acid oxidation. The physiological consequence is a decrease in circulating blood triglycerides. PUFA activation of PPAR $\alpha$  may also be responsible for the induction of peroxisomal enzymes and UCP-3 in skeletal muscle, but this remains to be definitively established.

PUFA are not only ligands for PPAR $\alpha$ , but are also strong activators of PPAR $\gamma$  and PPAR $\delta$ , both of which play roles in the terminal steps of fat cell differentiation (Brun *et al.* 1996). Ligand activation of PPAR $\gamma$  greatly accelerates the rate of conversion of pre-adipocytes to mature fat cells (Brun *et al.* 1996). In particular, PPAR $\gamma$  activation leads to an induction in the expression of lipoprotein lipase, A-fatty acid-binding protein, and glucose transporter-4 (Kletzien *et al.* 1992; Forman *et al.* 1995; Brun *et al.* 1996). However, unlike PPAR $\alpha$ , PPAR $\gamma$  appears to be more influenced by *n*-6 fatty acids than *n*-3 fatty acids (Forman *et al.* 1995; Mater *et al.* 1998). This may be due to the fact that the natural ligand for PPAR $\gamma$  is a prostanoid product of 20:4*n*-6 metabolism (Forman *et al.* 1995). In this regard, Mater *et al.* (1998) have observed that 20:5 *n*-3 actually inhibited fat cell differentiation, which suggests that 20:5*n*-3 may have given rise to a biologically less potent PPAR $\gamma$  ligand. These findings are consistent with the reports that diets with a high ratio of *n*-6 to saturated fatty acids result in greater rates of adipocyte glucose uptake (Field *et al.* 1990), and that diets rich in 20:5 *n*-3 are associated with reduced adipose stores (Hill *et al.* 1993; Baillie *et al.* 1999).

The discovery of PPAR, combined with the knowledge that PUFA were effective ligands for all PPAR isoforms, brought forth the attractive hypothesis that PPAR was the 'master switch' transcription factor which was targeted by PUFA to coordinately up-regulate genes encoding proteins of lipid oxidation while down-regulating the genes encoding proteins of lipid synthesis. However, numerous reports now indicate that the PUFA suppression of lipogenic genes does not directly involve PPARs, but rather reflects changes in the expression and nuclear localization of the transcription factor, sterol-regulatory element-binding protein-1 (SREBP-1) (Worgall *et al.* 1998; Xu *et al.* 1999). SREBP-1 exists as a 125 kDa membrane-anchored precursor protein which, upon proteolysis, releases a 68 kDa peptide that translocates to the nucleus (Brown & Goldstein, 1999). Binding of SREBP-1 to its response element within



the 5'-flanking region of lipogenic genes induces gene transcription (Shimomura *et al.* 1998). As might be expected, over-expression of SREBP-1 in the liver is associated with high rates of *de novo* fatty acid biosynthesis, and a several-fold induction in the transcription of lipogenic genes (Shimomura *et al.* 1998). Recently we reported that the ingestion of safflower oil or fish oil reduced the membrane content of precursor SREBP-1 by 60 and 75 %, respectively, and that this in turn led to a 65 and 90 % reduction in the nuclear content of 68 kDa SREBP-1 (Xu *et al.* 1999). The decrease in SREBP-1 was accompanied by a comparable decrease in the transcription of hepatic fatty acid synthase and a reduction in fatty acid synthase promoter activity (Xu *et al.* 1999). Unlike PUFA, the ingestion of saturated or mono-unsaturated fats had no effect on the nuclear content of SREBP-1 or on lipogenic gene expression. Nuclear run-on assays revealed that PUFA suppressed SREBP-1 expression by decreasing the stability of SREBP-1 mRNA (Xu *et al.* 1999). The PUFA suppression of hepatic lipogenic gene expression was paralleled by a significant decrease in the hepatic production of malonyl-CoA (Wilson *et al.* 1990). This decrease in malonyl-CoA not only reduced the rate of fatty acid biosynthesis, but it also may have facilitated fatty acid oxidation by releasing the malonyl-CoA inhibition of carnitine palmitoyltransferase (Saha *et al.* 1995). It remains to be determined if PUFA reduce SREBP-1 levels in skeletal muscle and thereby reduce the expression of acetyl-CoA carboxylase II and the production of malonyl-CoA, but such a mechanism would be consistent with the observations that PUFA, particularly the *n*-3 family, enhance fatty acid oxidation in skeletal muscle (Power & Newsholme, 1997).

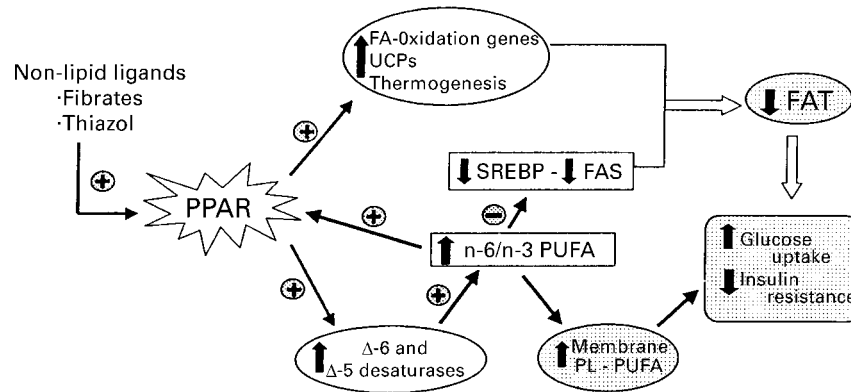
Although the PUFA regulation of SREBP-1 appears to be a key player in the PUFA suppression of lipogenic genes, not all glycolytic and lipogenic genes that are suppressed by dietary PUFA contain recognition sites for SREBP-1. While it is possible that SREBP-1 regulates the expression of a secondary transcription factor, which in turn governs the lipogenic gene, an alternative explanation is that a second PUFA-regulated transcription factor may exist in the nucleus of liver cells. One nuclear protein which may fulfil this role is hepatic nuclear factor-4 (HNF-4). Like PPAR, HNF-4 is also a member of the steroid receptor superfamily, but unlike PPAR, which bind free fatty acids, HNF-4 will bind only fatty acyl-CoA esters (Hertz *et al.* 1998). HNF-4 appears to enhance the promoter activity of select genes (Hertz *et al.* 1998). This enhancer activity is suppressed when fatty acyl-CoA esters, particularly PUFA esters, bind to the ligand domain of the HNF-4 (Hertz *et al.* 1998). Interestingly, an HNF-4 response element appears to reside in the first intron of the fatty acid synthase gene, which is the reported site of a glucose response sequence (Jump & Clarke, 1999). In addition, an HNF-4 recognition sequence is also a component of the PUFA-response region of the pyruvate kinase gene (Liimatta *et al.* 1994). The presence of the HNF-4 response element within the fatty acid synthase and pyruvate kinase genes suggests that it may play a role in the PUFA regulation of these genes, but to date there are no data to support this hypothesis.

### Do PPAR activators improve the metabolic syndrome by enhancing the $\Delta$ -6 and $\Delta$ -5 desaturase pathway?

The reduction of blood triglycerides and the improvement in glucose metabolism elicited by the ingestion of oils rich in 20- and 22-carbon PUFA, particularly those of the *n*-3 family, is reminiscent of what is observed when humans and animals are treated with the non-lipid activators of PPAR (fibrates, thiazolidinediones) (Kletzien *et al.* 1992; Nolan *et al.* 1994; Staels *et al.* 1998). Given these similarities, we hypothesized that non-lipid activators of PPAR may in part improve the metabolic syndrome by enhancing the production of 20- and 22-carbon PUFA. In support of this concept, we recently found that feeding rats the PPAR $\alpha$ -specific activator WY 14,643 induced hepatic  $\Delta$ -6 and  $\Delta$ -5 desaturase gene transcription several-fold (H. P. Cho and S. D. Clarke, unpublished results). This induction of  $\Delta$ -6 and  $\Delta$ -5 desaturase gene transcription was paralleled by an increase in the hepatic abundance of  $\Delta$ -6 and -5 desaturase mRNA (H. P. Cho and S. D. Clarke, unpublished results) and a rise in the enzymatic activity of the two enzymes (Kawashima *et al.* 1990). Moreover, the ingestion of WY 14,643 significantly increased the amount of 20:5*n*-3 and 22:6*n*-3 found in skeletal muscle phospholipid (C. M. Nelson and S. D. Clarke, unpublished results). As might be predicted from previous dietary studies with fish oils, the enhanced activity of the  $\Delta$ -6 and  $\Delta$ -5 desaturase pathway elicited by the ingestion of a PPAR $\alpha$  ligand was accompanied by an increase in the expression of skeletal muscle UCP-3, and a decrease in the hepatic expression of SREBP-1 and fatty acid synthase (Clarke, 1999). It is not known if prolonged consumption of a non-lipid PPAR $\alpha$  agonist will also decrease body fat deposition. However Matsui *et al.* (1997) recently reported that bezafibrate feeding improved insulin sensitivity in rats by increasing the skeletal muscle content of 20-carbon PUFA. Moreover, Costet *et al.* (1998) discovered that as PPAR $\alpha$ -knock-out mice grow older they develop the classic symptoms of the metabolic syndrome (body fat gain, insulin resistance). It remains to be determined if the  $\Delta$ -6 and  $\Delta$ -5 desaturase pathway can be enhanced by ligands for other PPARs such as PPAR $\gamma$  and PPAR $\delta$ . Nevertheless, the data suggest that agents which induce the  $\Delta$ -6 and  $\Delta$ -5 desaturase pathway appear to improve insulin sensitivity. Furthermore, an induction of skeletal muscle  $\Delta$ -6 and  $\Delta$ -5 desaturase (Cho *et al.* 1999) by PPAR $\gamma$  and PPAR $\delta$  ligands may potentially enrich the membrane phospholipids with 20:5 and 22:6*n*-3, and thereby explain how these ligands improve skeletal muscle glucose uptake (Nolan *et al.* 1994).

### Conclusions

In this review we present a collection of studies which argue that *n*-6 and *n*-3 PUFA may play a beneficial role in the prevention of obesity and insulin resistance. In this respect, oils which are rich in *n*-3 PUFA (e.g. fish oils) appear to be more efficacious than vegetable oils rich in *n*-6 PUFA. More specifically *n*-3 PUFA increase thermogenesis, decrease body fat deposition, and improve glucose clearance. The mechanism by which PUFA elicit these metabolic outcomes appears to be largely exerted at the genomic level, i.e.



**Fig. 1.** Schematic mechanism of PUFA regulation of fuel partitioning. Thiazol, thiazolidinediones; PPAR, peroxisome proliferator activated receptor; UCPs, mitochondrial uncoupling proteins; SREBP, sterol response element-binding protein; FAS, fatty acid synthase; PUFA, polyunsaturated fatty acids; PL, phospholipids; +, activation or induction; -, suppression.

regulation of gene transcription. The complexity of the interrelationships between dietary PUFA and PPAR activators, and the possible influences exerted by PUFA on energy balance and glucose metabolism, are depicted in Fig. 1. In essence we are proposing that dietary PUFA, particularly those derived from fish oils, improve glucose uptake and insulin sensitivity by enriching the plasma membranes with the long-chain PUFA and thereby lengthening the amount of time the glucose transporter-4 is associated with the membrane. In addition, the PUFA inhibit lipid synthesis and decrease malonyl-CoA production by suppressing the expression of lipogenic genes. Concomitantly, the dietary PUFA induce the genes of lipid oxidation and induce thermogenesis by increasing the expression of UCP-3. Finally, we propose that all of these effects of PUFA can be mimicked by non-fatty-acid activators of PPARs such as fibrates and thiazolidinediones, which function to induce  $\Delta$ -6 and  $\Delta$ -5 desaturase gene expression and thereby accelerate the synthesis of 20- and 22-carbon PUFA. The data emerging from these studies strongly suggest that *n*-3 polyenoic fatty acids may be essential dietary fatty acids for the maintenance of energy balance and the prevention of insulin resistance.

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