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Skeletal Muscle Metabolism in Physiology and in Cancer Disease

Anna Giordano,[1,4] Menotti Calvani,[2] Orsolina Petillo,[4] Maria Carteni',[1] Maria Rosa A.B. Melone,[3] and Gianfranco Peluso[4]*

[1]Department of Experimental Medicine, II University of Naples, School of Medicine, Naples, Italy
[2]Scientific Department, Sigma-Tau S.A, Rome, Italy
[3]Institute of Neurological Sciences, Second University of Naples, Naples, Italy
[4]Department of Experimental Oncology, National Cancer Institute, Naples, Italy

Abstract Skeletal muscle is a tissue of high demand and it accounts for most of daily energy consumption. The classical concept of energy metabolism in skeletal muscle has been profoundly modified on the basis of studies showing the influence of additional factors (i.e., uncoupling proteins (UCPs) and peroxisome proliferator activated receptors (PPARs)) controlling parameters, such as substrate availability, cellular enzymes, carrier proteins, and proton leak, able to affect glycolysis, nutrient oxidation, and protein degradation. This extremely balanced system is greatly altered by cancer disease that can induce muscle cachexia with significant deleterious consequences and results in muscle wasting and weakness, delaying or preventing ambulation, and rehabilitation in catabolic patients. J. Cell. Biochem. 90: 170–186, 2003. © 2003 Wiley-Liss, Inc.

Key words: skeletal muscle; metabolism; cachexia; UCP; mitochondria; PPARs

SKELETAL MUSCLE FIBRES AND METABOLISM

Muscle tissue accounts for most of an individual's mass and daily energy consumption. Since skeletal muscle is a tissue of high-energy demand, muscle fuel economy is very important, not only for minimizing food requirements, but also for reducing unwanted heat production and for avoiding the catabolism of proteins important for muscle functions. Skeletal muscle consists mainly of slow twitch (type 1) and fast twitch (type 2a and 2b) fibers. The type 1 and type 2a fibers (red fibers) are mitochondria-rich and rely in their ATP supply mainly on oxidative phosphorylation, while type 2b fibers (white fibers) are mitochondria-poor and have a very effective glycolytic ATP production [Kunz, 2001]. The major functional difference between red and white muscle cells is that white fibers generate ATP by a short reaction pathway between substrates (e.g., glucose or fatty acids (FAs)) and the appearance of ATP, whereas in red muscle the pathway from substrate (again, glucose) to ATP is comprised of many more reaction steps (e.g., glycolysis plus plus) and is a correspondingly longer process. Consequently, fast-acting skeletal muscles are composed of dominantly glycolytic white fibers while slow-acting muscles such as those that maintain tone are generally red and oxidative [Lexell et al., 1984]. Moreover, the slow oxidative fibres have a slow contraction speed and a low myosin ATPase activity, and are specialized for steady, continuous activity, are highly resistant to fatigue, and have a good capillary supply for efficient gas exchange. They are rich in myoglobin, which gives them a red color, are built for aerobic metabolism and prefer to use fat as a source of energy.

For a prolonged exercise is, therefore, important the muscle fat supply. Indeed, the glucose in human blood is only sufficient for about 5 min of activity, then glycogen stored in the liver is broken down by hydrolysis into glucose and transported in the blood to muscle cells. After

*Correspondence to: Dr. Gianfranco Peluso, Institute of Protein Biochemistry (IBP), Via P. Castellino 111, 80131 Naples, Italy. E-mail: peluso@dafne.ibpe.na.cnr.it

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about 20 min of exercise triglyceride (TG) fats stored in adipose tissues are converted into FAs and glycerol. The FAs pass into the blood and can be oxidized to generate ATP in the muscle cells. After transport in the sarcoplasm by FAs binding protein, FABP, and before oxidation to CO_2 in tricarbossilic acids (TCA)-cycle, long-chain FAs must be activated to long-chain acyl-CoA, then translocated into mitochondria by the protein complex, carnitine palmitoyl transferase (CPT I), carnitin-acylcarnitine translocase (CACT), and carnitine palmitoyl tranferase (CPT II) [McGarry and Brown, 1997] (Fig. 1).

In the aerobic metabolism, the substrates metabolized into the mitochondria induce the production of NADH and $FADH_2$ that can be oxidized to NAD^+, FAD, and H^+ in the respiratory chain. In this way, substrate oxidation is coupled to the formation of ATP. According to chemiosmotic hypothesis [Mitchell, 1966], the protons are transported to the cytosolic side of the inner mitochondrial membrane by a series of reactions. This mechanism generates a proton gradient across the membrane, which leads protons to flow back across the inner mitochondrial membrane through a F_0F_1-complex. The energy thus generated is used by ATPase to convert ADP into ATP.

The coupling between substrate oxidation and ATP formation is not 100% efficient. The proton gradient can be reduced by so-called proton leaks, thereby dissipating energy as heat. It has been proposed that uncoupling proteins (UCPs) are involved in this mechanism. These proteins could either transport protons into the mitochondrial matrix [Klingenberg et al., 1999] or transport non-esterified FA anions out of the matrix in a process called FA cycling [Jezek et al., 1998]. Both these processes would reduce the proton gradient across the inner mitochondrial membrane (Fig. 2).

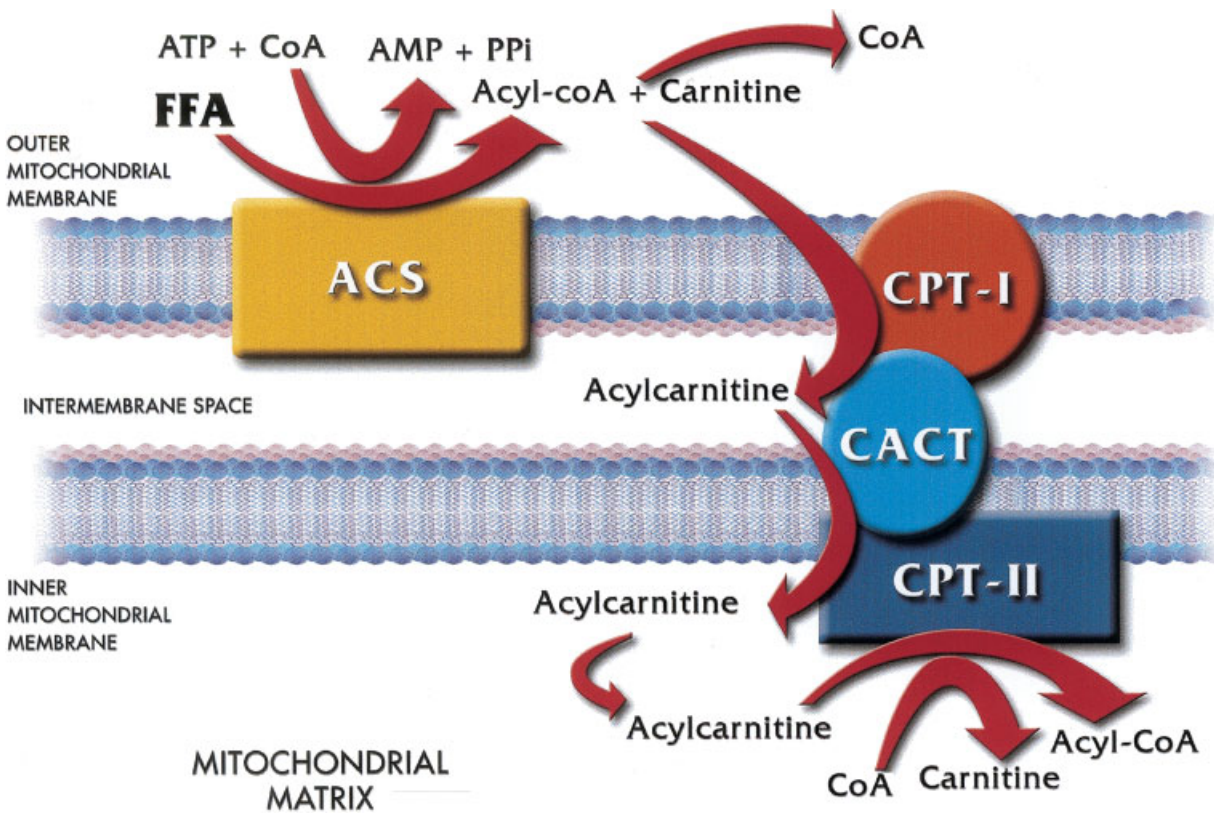

Fig. 1. Mitochondrial carnitine system. The enzyme carnitine palmitoyltransferase I (CPT-I) is located in the outer mitochondrial membrane and catalyzes the transfer of acyl groups from acyl-CoA to carnitine to synthesize acylcarnitine and to produce free CoA. The enzyme carnitine/acylcarnitine translocase (CACT) is located in the inner mitochondrial membrane and exchanges cytoplasmic acylcarnitine for mitochondrial free carnitine. The enzyme carnitine palmitoyltransferase II (CPT-II), is located on the matrix side of the inner mitochondrial membrane and reconverts acylcarnitine to acyl-CoA while free carnitine is produced.

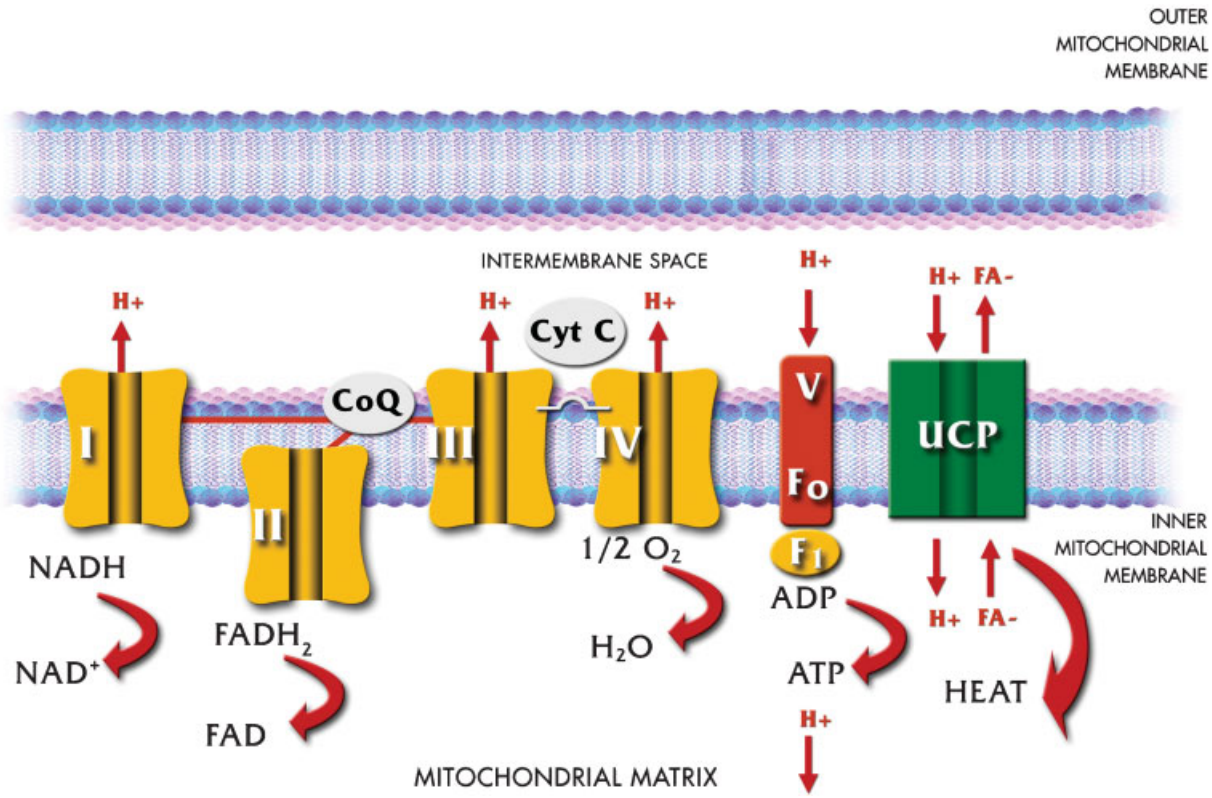

Fig. 2. Mitochondrial proton gradient and uncoupling protein(UCP) involvement in proton leaks. To synthesize ATP from ADP, substrates such as fat, carbohydrate, and proteins are metabolized, resulting in the production of NADH and $FADH_2$. NADH and $FADH_2$ can be oxidized to NAD^+, FAD, and H^+ in the respiratory chain. The protons are transported to the cytosolic side of the inner mitochondrial membrane a mechanism that generates a proton gradient across the membrane by four-enzyme complex: Complex I (NADH–ubiquinone reductase), Complex II (succinate–ubiquinone reductase), Complex III (ubiquinol–cytochrome reductase), Complex IV (cytochrome oxidize). The proton gradient causes protons to flow back across the inner mitochondrial membrane through a F_0F_1-complex (Complex V: ATPase). The energy thus generated is used by ATPase to transform ADP into ATP. The proton gradient can be reduced by so-called proton leaks, thereby dissipating energy as heat. UCPs mediate proton leakage and decrease the coupling of respiration to ADP phosphorylation, resulting in the generation of heat instead of ATP. UCPs also act as fatty acid (FA) anion transporters, transporting FA anions out of the mitochondrial matrix. CoQ, coenzyme Q; CytC, cytochrome c.

THE ROLE OF UCP IN SKELETAL MUSCLE METABOLISM

The UCPs 1, 2, and 3, are a family of mitochondrial membrane proteins that are involved in the control of energy metabolism.

UCP1, which is expressed in brown adipose tissue (BAT), plays an important role in adaptive thermogenesis, but it is also correlated with decreased metabolic efficiency and the prevention of the development of obesity [Himms-Hagen, 1984]. For example, fasting is associated with an increased metabolic efficiency and a decreased UCP1 expression and activity [Rothwell et al., 1984].

UCP2 is expressed in a wide variety of tissues, including white adipose tissue (WAT), skeletal muscle, and tissues of the immune system. Many findings suggest that UCP2 is related to energy expenditure and could, therefore, be involved in the development of obesity. Indeed, it has been shown in rodents that UCP2 mRNA expression is higher in obesity-resistant (A/J) mice than in obesity-prone (B6) mice [Fleury et al., 1997]. Furthermore, in humans, reduced UCP2 mRNA expression has been reported in the WAT [Oberkofler et al., 1998] and skeletal muscle [Nordfors et al., 1998] of obese subjects. Although many studies indicate that UCP2 is related to energy metabolism and obesity in rodents and humans, alternative functions have been demonstrated for this protein. For example, UCP2 could be involved in the regulation of radical production (reactive oxygen species,

ROS) by reducing the proton gradient and, therefore, it is beneficial for living cells, preventing them from damage to DNA, lipids, and proteins by ROS. Negre-Salvayre et al. have demonstrated that the administration of GDP, a molecule able to inhibit UCP2, to brown adipocytes increases both the electrical potential across the inner mitochondrial membrane and ROS production. The same effect of GDP on ROS production has been observed in liver mitochondria from non-parenchymal cells, but not in mitochondria from hepatocytes that have lacked UCP2 [Nègre-Salvayre et al., 1997].

UCP3 is expressed predominantly in skeletal muscle and has been associated with whole-body energy metabolism. Evidence for a relationship between UCP3 expression and energy metabolism comes from the finding that UCP3 mRNA expression is upregulated after thyroid hormone treatment, which is known to increase thermogenesis both in rodents [Gong et al., 1997] and in humans [Barbe et al., 2001]. Furthermore, UCP3 mRNA expression is upregulated after the consumption of a high-fat diet in BAT of rodents [Gong et al., 1999] and human [Schrauwen et al., 2001]. Both UCP3 mRNA level and UCP3 protein level are decreased after endurance training [Boss et al., 1998a; Schrauwen et al., 1999] and after weight reduction [Vidal-Puig et al., 1999; Schrauwen et al., 2000], conditions both characterized by a reduced resting metabolic rate and/or increased metabolic efficiency. In addition, other results indicate that UCP3 is able to uncouple oxidative phosphorylation in vivo, thereby dissipating energy as heat and it has been suggested that UCP3, as UCP2, may also be involved in reducing ROS production.

Generally speaking, excessive production of ROS occurs when the mitochondrial membrane potential is raised above a certain threshold [Skulachev, 1998]. Thus, uncoupling, lowering the mitochondrial membrane potential, prevents excessive ROS production. It has been demonstrated that after ablation of the *UCP3* gene, more ROS are produced [Vidal-Puig et al., 2000], while in transgenic mice overexpressing UCP3 a 12% decrease in membrane potential can be observed [Clapham et al., 2000]. This finding indicates that UCP3 is able to minimize the production of ROS, thus preventing excessive oxidative stress [Vidal-Puig et al., 2000].

Finally, a role for UCP3 in FAs β-oxidation has been proposed after the observed upregulation of UCP3 in case of high-fat feeding or fasting [Boss et al., 1998b]. In the latter situation, there is an increase in FA plasma levels, a condition mimicking the high-fat feeding. Thus, the increasing plasma FA are able to upregulate UCP3 mRNA expression probably because FA are ligands for peroxisome proliferator activated receptors (PPARs), factors upregulating many lipid-related genes as well as UCP [Emilsson et al., 2000]. Several studies have demonstrated that UCP3 is downregulated in conditions of increased ability to oxidize and upregulated in conditions in which FA delivery exceeds the capacity to oxidize fat, eventually leading to an increase in plasma FA levels, (fasting, acute exercise) [Boss et al., 1998b]. As mentioned before, UCP3 could act as FA anion transporters, exporting FA anions out of the mitochondrial matrix, preventing, therefore, the FA accumulation [Jezek et al., 1998].

PPARs AND MUSCLE METABOLISM

PPARs comprise an important subfamily of the nuclear hormone receptors that regulate systemic FA metabolism via ligand-dependent transcriptional activation of target genes [Kliewer et al., 2001]. PPARs exist in distinct subtypes known as PPAR-α, PPAR-δ (also known as PPAR-β and NUC1), and PPAR-γ, encoded by separate genes. Strong evidence indicates that their endogenous ligands consist of FAs and/or lipid metabolites and that they function to mediate adaptive metabolic responses to changes in systemic fuel availability.

PPARs exert diverse effects on fat and carbohydrate metabolism and are major targets for therapeutic agents in metabolic disease such as dyslipidemia and type 2 diabetes. They modulate gene expression in a manner similar to that of other nuclear receptors. Upon ligand activation, PPAR-α, PPAR-δ, and PPAR-γ, regulate transcription by dimerizing with the retinoid X receptor and binding to PPAR responsive elements (PPREs), within the regulatory regions of target genes. Known PPREs consist of a direct repeat (DR1 or DR2) of the hexanucleotide AGGTCA sequence separated by 1 or 2 nucleotides [Desvergne and Wahli, 1999]. PPARs form protein–protein interactions with a variety of nuclear proteins known as coactivators and corepressors, which mediate contact between the PPAR–RXR heterodimer, chromatin, and the basal transcriptional machinery

and which promote activation and repression of gene expression, respectively [Rosen and Spiegelman, 2001] (Fig. 3).

The expression levels of PPARs are modulated by stimuli as varied as circadian rhythm, fasting, stress, and cold. Furthermore, various factors, such as corticosteroids, insulin, phorbol esters, and different cytokines, affect PPAR expression. Additionally, PPAR and retinoid X receptor ligands also modulate PPAR mRNA levels [Lehmann et al., 1995; Pearson et al., 1996; Ferry et al., 2001].

PPAR-α is highly expressed in tissues such as liver, intestine, muscle, kidney, and heart, where it stimulates the β-oxidative degradation of FAs; PPAR-γ is predominantly expressed in adipose tissues, where it participates in biological pathways of intense interest, such as adipocyte differentiation, lipid storage, insulin sensitivity, and type 2 diabetes; PPAR-δ is expressed in a wide range of tissues, and recent findings indicate that it controls the adipogenesis and muscle metabolism.

Thus, the most part of studies have identified PPARs as important regulators of lipid and lipoprotein metabolism. PPARs control plasma levels of cholesterol and TGs, which constitute major risk factors for coronary heart disease [Barbier et al., 2002].

PPAR-α is expressed most abundantly in tissues that are characterized by high rates of FA oxidation (FAO), and mediates lipid-induced activation of *FAO* genes. It induces the expression of various enzymes involved in peroxisomal β-oxidation, and plays an essential role in maintaining lipid homeostasis also in skeletal muscle by modulating the expression of genes that regulate FA catabolism [Braissant et al., 1996]. Studies of PPAR-α knockout (KO) mice, have demonstrated that KO mice exhibit low rates of β-oxidation, decreased expression of *FAO* genes, failure of liver and heart to induce

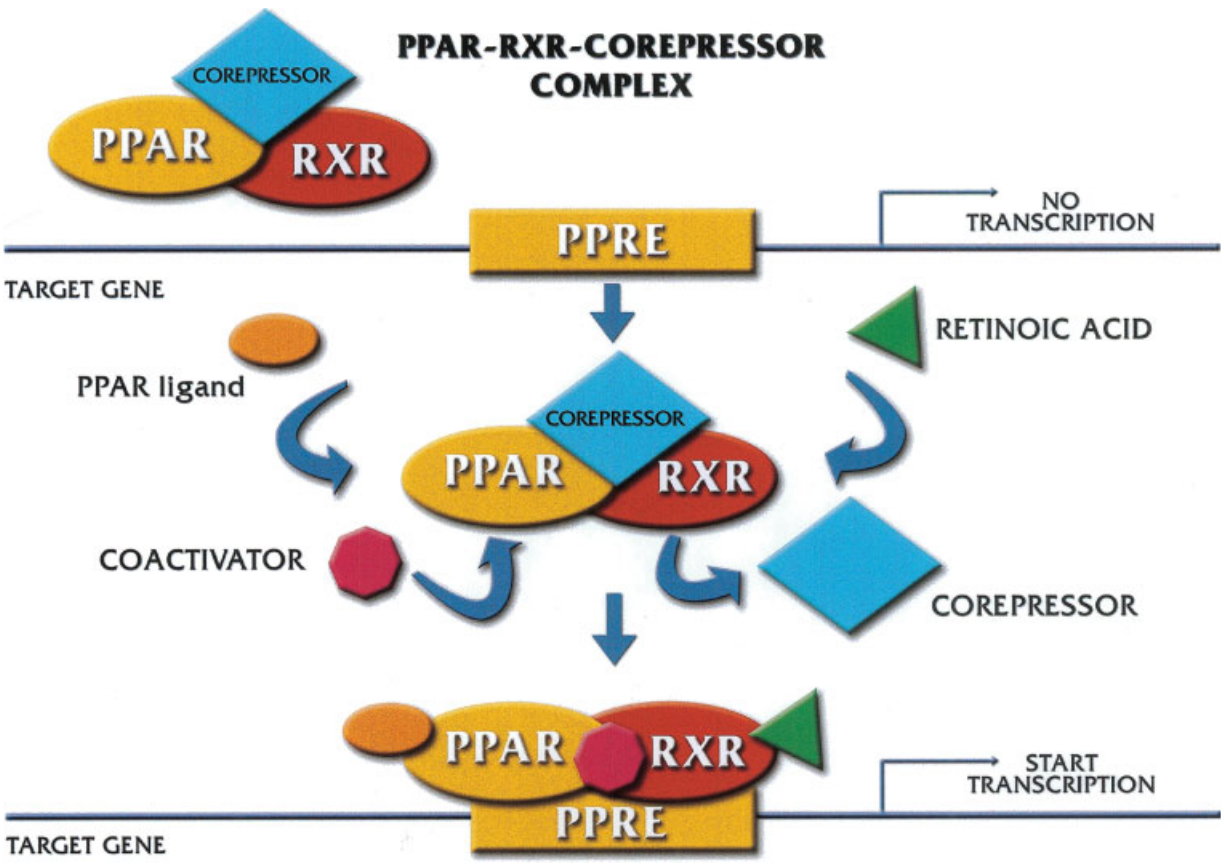

Fig. 3. Mechanism of action of peroxisome proliferator activated receptors (PPARs). Upon ligand activation, PPARs, regulate transcription by dimerizing with the retinoid X receptor and binding to PPAR response elements (PPREs), within the regulatory regions of target genes. PPARs form protein–protein interactions with a variety of nuclear proteins known as coactivators and corepressors, which mediate contact between the PPAR–RXR heterodimer, chromatin, and the basal transcriptional machinery and which promote activation and repression of gene expression, respectively.

β-oxidative pathways in response to physiological or pharmacological perturbations in lipid metabolism and abnormal accumulation of neutral lipids in both cardiac and hepatic tissues, compared with wild type (WT) littermates [Djouadi et al., 1998; Kersten et al., 1999; Leone et al., 1999].

Similar to other oxidative organs, mRNA expression of skeletal muscle genes involved in lipid catabolism is increased during physiological states such as exercise and starvation, which are associated with increased systemic delivery of free FAs (FFAs) [Pilegaard et al., 2000]. Furthermore, many of the same genes are also upregulated by in vivo administration of PPAR-α selective activators, suggesting that FA-induced activation of PPAR-α plays a critical role in mediating the adaptive response of muscle to starvation and exercise. PPAR-α protein expression is increased by exercise training and induced during myocyte differentiation, coincident with increased β-oxidative capacity [Muoio et al., 2002a].

In a recent study, to investigate the requirement for PPAR-α in regulating muscle FA metabolism and transcriptional activation of pyruvate-dehydrogenase kinase 4 (PDHK4) and UCP3, that are proposed to play key roles in mediating adaptive adjustments in muscle substrate selection, some authors evaluated metabolic and gene regulatory responses to 24 h of starvation and endurance exercise, as well as in vitro FAO, in muscles from PPAR-α KO mice and WT littermates [Muoio et al., 2002b]. They report that skeletal muscles from KO mice exhibited only minor changes in FA homeostasis and, moreover, that neither constitutive nor inducible mRNA expression of known *PPAR*-α target genes was negatively affected by its absence. They also found that skeletal muscle expressed high levels of PPAR-δ and that in both rodent and human skeletal muscle cells, activation of the δ-subtype increased FAO as well as mRNA levels of several classical *PPAR*-α target genes. These results indicate that both PPARs subtypes (α and δ) play important roles in mediating lipid-induced regulation of muscle β-oxidative pathways. They also demonstrated that in both human and rodent myocytes, the highly selective PPAR-δ agonist GW742 increased FAO about twofold and induced expression of several lipid regulatory genes, including PDHK4 and UCP3, responses that were similar to those elicited by the PPAR-α agonist GW647. These results show an overlap in the functions of PPARs-α and -δ as transcriptional regulators of FA homeostasis and suggest that in skeletal muscle high levels of the δ-subtype can compensate for deficiency of PPAR-α [Muoio et al., 2002b].

PPAR-γ is abundantly expressed in both WAT and BAT, mammary glands, muscles, and numerous other tissues. Thiazolidinediones (TZDs), a new class of anti-diabetic drugs known to bind and activate PPAR-γ, enhance insulin sensitivity in humans with insulin resistance. Although the expression of PPAR-γ has been reported in skeletal muscle, little is known about the potential effects of PPAR-γ on gene regulation in this tissue. Recent studies indicate that the expression of three genes important in lipid metabolism lipoprotein lipase (LPL), mCPT-1, and FABP correlates with the expression of PPARγ in skeletal muscle. This imply that also PPARγ may play a role in the regulation of lipid metabolism genes in skeletal muscle [Lapsys et al., 2000].

ROLE OF PPARs IN TG AND LDL METABOLISM

In vivo and in vitro data demonstrate that PPARs play a central role in the control of FA and lipoprotein metabolism. PPAR-α controls plasma lipid transport by acting on TG and FA metabolism and by modulating bile acid synthesis and catabolism in the liver.

In humans, PPARα activation with fibrates, the first efficient lipid lowering drugs, results in decreased plasma TG concentration and VLDL production by the liver [Staels et al., 1998]. Furthermore, PPAR-α stimulates HDL apolipoprotein expression. A strong positive regulation of FABP gene expression occurs upon dietary intake of long-chain FAs or direct ileal infusion of linoleic acid, which are PPAR-α ligands. Fibrate treatment induces FA transport protein 1 (FATP1), FA translocase (FAT) mRNA levels, and the intracellular FABP. Furthermore, PPAR-α induces FA uptake and catabolism in mitochondria via stimulation of muscle and liver carnitine palmitoyltransferase I and II and several mitochondrial FA catabolizing enzymes [Guerre-Millo et al., 2000]. When mitochondrial FA import is inhibited in PPAR-α null mice, massive cardiac and hepatic lipid accumulation and hypoglycemia are observed.

Fibrate-activated PPAR-α controls LPL activity by inducing its expression in the liver and by downregulating the hepatic expression of

apoC-III, an LPL activity and remnant catabolism inhibitor. These effects promote lipolysis and TG-rich lipoprotein catabolism, thus decreasing the plasma levels of TG [Schoonjans et al., 1996]. Direct support for these effects is provided by transgenic animal studies showing a correlation between liver apoC-III expression and plasma TG levels [Ito et al., 1990].

It has been demonstrated that PPAR-α controls circulating VLDL levels, since PPAR-α null/apoE-null mice display higer VLDL production on high-fat diet feeding than do control mice [Tordjman et al., 2001].

A link between PPAR-α and gluconeogenesis is revealed by treatment of WT and KO mice with etomoxir, an agent that blocks CPTI activity. This treatment induces a lethal hypoglycemia in PPAR-α KO mice while WT animals tolerate etomoxir administration and respond by a strong upregulation of *PPAR* target genes such as acyl-CoA synthetase (ACO), CYP4A1, CYP4A3, and medium-chain acyl-CoA dehydrogenase (MCAD). Additionally, a marked TG accumulation in liver and heart of the dead mice is revealed. PPAR-α null mice have low glycogen stores and, upon fasting, they exhibit a severe hypoglycemia and hypothermia [Kersten et al., 1999]. These manifestations are accompanied by an enhanced lipid accumulation in the liver and no increase in ketone body production, suggesting a dramatic impairment of FAs oxidation [Kersten et al., 1999; Leone et al., 1999].

Glitazones, that selectively activates PPAR-γ also affect the circulating levels of free FAs, cholesterol, and TG. In obese animal models, TZDs and non-TZD glitazones decrease the circulating levels of TG by inducing lipolysis (via activation of LPL expression levels in adipocytes) and clearance of TG-rich lipoproteins. The induction of LPL by PPAR-γ promotes FA delivery, while induction of FATP and acyl-CoA synthetase (ACS) results in increased FA uptake. These actions contribute to enhance TG storage in adipose tissue. PPAR-γ also modulates the expression of enzymes involved in the synthesis of FA and TG, such as malic enzyme [Castelein et al., 1994] (Fig. 4).

Several evidences indicates that PPAR-δ also regulates the expression of genes involved in FA metabolism, a novel PPAR-δ specific agonist

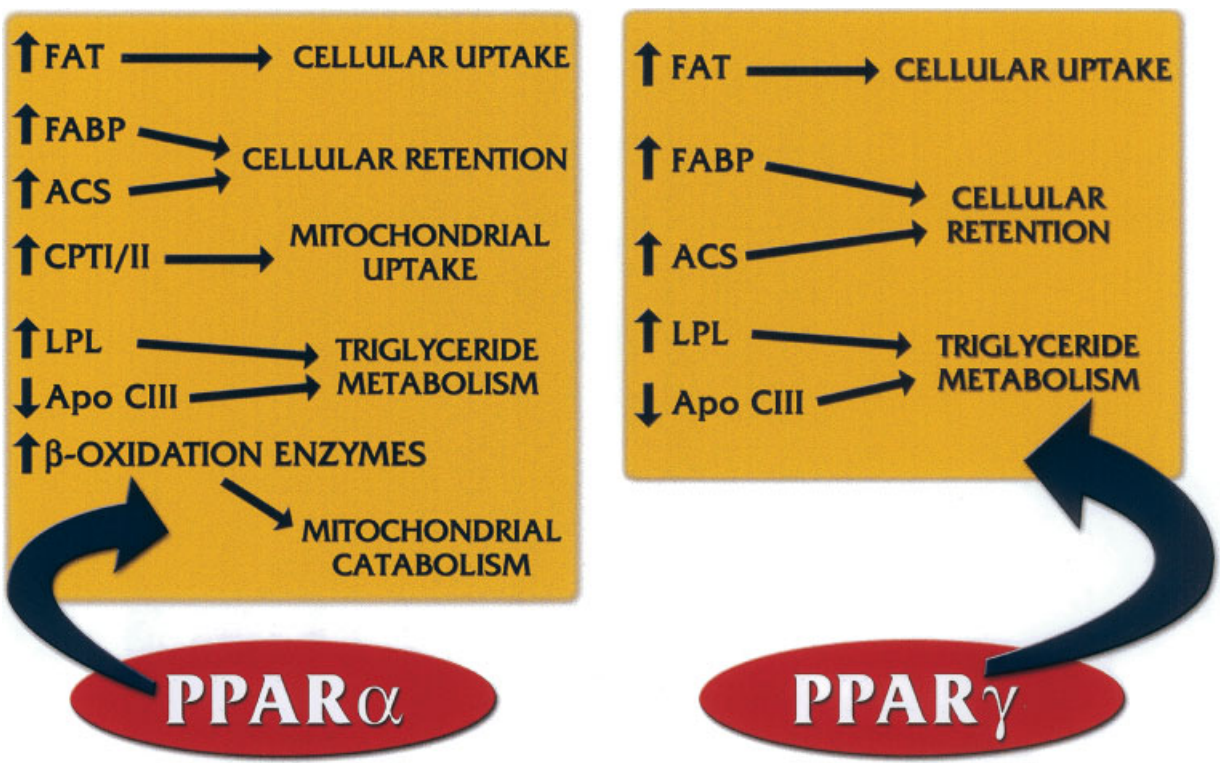

Fig. 4. Role of PPARs in the control of triglyceride (TG) and FA metabolism. PPARα regulates the expression of genes involved in FA cellular uptake, FA cellular retention, and FA mitochondrial uptake and catabolism. PPARγ promotes FA uptake and esterefication. PPARα and PPARγ induce the expression of LPL. FAT, fatty acid translocase; FABP, fatty acid binding proteins; LPL, lipoprotein lipase; ACS, acyl-CoA synthetase; CPT, carnitine palmitoyl transferase.

induces an increase in L-FABP expression in small intestine [Poirier et al., 2001]. PPAR-δ specific agonists also increase plasma HDL cholesterol concentrations in insulin-resistant mice and obese rhesus monkeys. The molecular mechanisms behind this increase remain to be clarified [Oliver et al., 2001].

PPARs influence the reverse cholesterol (RCT) pathway by regulating macrophage cholesterol efflux, HDL cholesterol transport in plasma, and bile acid synthesis. In humans, PPAR-α activation with fibrates increases the transcription of apoA-I and apoA-II, which are the major HDL apolipoproteins, an effect that contributes to the increase of HDL concentrations [Vu-Dac et al., 1994].

Taken together these finding demonstrate a possible role of PPARs in the skeletal muscle metabolism via an increase afflux of specific metabolites to the muscle bed.

METABOLIC CHANGES INDUCED BY CANCER

Many chronic or endstage diseases, such as cancer, infections, AIDS, congestive heart failure, rheumatoid arthritis, tuberculosis, cystic fibrosis, and Crohn's disease, are associated with a progressive weight loss and abnormalities of carbohydrate, lipid, and protein metabolism [Tisdale, 2002]. About half of all the cancer patients show a syndrome called cachexia in which the tumor induces metabolic changes in the host leading to loss of adipose tissue and skeletal muscle mass [Tisdale, 1997]. Patients with carcinomas of the pancreas and stomach have the highest incidence of cachexia (83–87%), while patients with breast cancer, acute non-lymphocytic leukemia, and sarcomas have the lowest incidence (31–40%) [Mitch and Price, 2001]. The body composition analysis of lung cancer patients who had lost 30% of their pre-illness stable weight showed an 85% decrease in total body fat and a 75% decrease in skeletal muscle protein mass [Fearon and Preston, 1990]. Patients with cancer present the loss of muscle that occur at a faster rate than any other known situation in human subjects. This condition reduces the quality of life and eventually results in death through the loss of respiratory muscle function. Cachexia commonly occurs when patients have lost more than 30% of their ideal body weight. The poor nutritional status also leads to a susceptibility to infection leading to death by sepsis. Patients with cachexia also show a decreased response to chemotherapy compared with weight stable patients [Mitch and Price, 2001]. Patients with weight loss live approximately half as long as those without, and those with greater weight loss live a shorter time than those with less or no weight loss. It has been estimated that almost one third of deaths in patients with cancer are related to muscle catabolism and weakness [Hasselgren and Fischer, 2001]. At the moment of diagnosis, 80% of patients with upper gastrointestinal cancers and 60% of patients with lung cancer have already experienced substantial weight loss [Inui, 2002].

LIPID AND GLUCOSE METABOLISM IN CANCER PATIENTS: A PREMISE FOR SKELETAL MUSCLE WASTING

Human cancer cachexia frequently involves an impairment of both lipid and cholesterol metabolism, with reduced adipose tissue mass and increased plasma levels of FFA and TG [Beutler, 1988; Kern and Norton, 1988], and with decreased high-density lipoprotein (HDL) cholesterol concentrations [Dessì et al., 1991a,b]. In cancer hosts, the activity of LPL, the enzyme responsible for the movement of FAs from blood into adipocytes, is decreased, and the decrement correlates with loss of body fat [Lanza-Jacoby et al., 1984; Carbo et al., 1994]. Cancer cachexia is often associated with a decreased insulin/glucagon ratio, and a decrease of insulin was proposed to be responsible for the progressive catabolism that is characteristic of cancer cachexia [Bartlett et al., 1994]. Hormonal changes promote the direction of energy and nutrients mainly to stressed body sites for the survival of the cells [Chrousos and Gold, 1992]. In cancer, such responses, by providing substrates more readily oxidized, i.e., glucose [Board et al., 1995], might help the growth and/or the survival of tumor. The tumor secretes a number of substances that serve to increase the supply of nutrients for its own growth and development. The main energy source for many solid tumors is glucose, which is converted into lactate rather than CO_2 because there is insufficient oxygen to allow the Krebs cycle and mitochondrial oxidative phosphorylation to operate. The lactate is transferred to the liver, where the carbon skeleton is used to synthesize glucose (Cory cycle). The

high rate of glucose import and glycolysis by tumor cells may also help to explain why the levels of glucose are normal despite the low insulinemia and the high gluconeogesis [Board et al., 1995]. In a recent study, the restoration of normal insulin levels in insulin-treated tumor-bearing rats, without lowering glucose levels, partially prevented most of the metabolic alterations consequent to tumor growth, including those in lipid and cholesterol metabolism as well as the dramatic reduction of food intake and the loss of body weight.

PROTEIN METABOLISM IN SKELETAL MUSCLE OF CANCER PATIENTS

Patients with cachexia experience abnormalities in protein metabolism. They show an increased whole body protein turnover [Norton et al., 1981], an increased rate of protein degradation and a decreased rate of protein synthesis in skeletal muscle, while hepatic protein synthesis is increased [Tisdale, 2001]. An increase in cathepsin-D activity is observed in biopsy specimens from the rectus abdominal muscle that correlates with protein degradation while there is no change in total body protein synthetic rate in the same subjects [Emery et al., 1984]. The maintenance of the total protein synthesis in these patients may be due to an increased hepatic production of acute phase proteins (APP). It has been suggested that the increased synthesis of hepatic APP is partly responsible for the catabolism of skeletal muscle proteins to provide the essential amino acids required for APP. The catabolism of muscle proteins may also have a positive effect on tumor growth. Glutamine is a direct source of nitrogen for the biosynthesis of purine and pyrimidine bases for tumors, and is an energy source. Alanine and glutamine represent more than 50% of the amino acids that are exported from skeletal muscle and both amino acids are efficient vehicles for the transport of nitrogen and carbon-skeletons between the various tissues in the body [Felig, 1975].

The skeletal muscle protein content depends on the relative rates of synthesis and degradation, which must be co-ordinately regulated to maintain the equilibrium. Pathological muscle depletion is characterized by a negative nitrogen balance, which results from disruption of this equilibrium due to reduced protein synthesis, increased breakdown, or both [Costelli et al., 2002]. The etiology of pathological muscle hypercatabolism is quite complex, involving nutritional, endocrine, metabolic, and immunological components. Although reduced protein synthesis and inhibited uptake of aminoacids contribute to the catabolic response in skeletal muscle, increased protein breakdown, in particular breakdown of the myofibrillar proteins actin and myosin, is the most important mechanism of muscle wasting [Tisdale, 2002].

The intracellular degradation of proteins depends on three proteolytic pathways. The lysosomal degradative system is mostly involved in the breakdown of endocytosed and membrane proteins [Dice, 1987]. It has been shown that the activity of lysosomal proteases is increased in the muscle and liver of tumor bearing rats [Tessitore et al., 1993]. The cytosolic compartment contains two major proteolytic pathways, the Ca^{2+} dependent system and the ATP-ubiquitin dependent system. Ca^{2+} dependent proteolysis in the muscle relies on the activity of three different enzymes, known as m-calpain, μ-calpain, and p94-calpain. It has been proposed that during cancer, the role played by the Ca^{2+}-dependent proteolysis is only marginal. The second proteolytic pathway is ATP-dependent and requires a peptide known as ubiquitin.

The steps involved in the breakdown of proteins by this mechanism include: the activation of ubiquitin by the ubiquitin activating enzyme (E1); the transfer of ubiquitin to the ubiquitin conjugating enzyme (E2); the interaction between the substrate protein and the ubiquitin ligase (E3); the interaction between E2 and E3 resulting in the multiubiquitination of the substrate protein; the degradation of the ubiquitinated protein by the 26S proteasome; and finally the deubiquitination resulting in the release and recycle of ubiquitin in the pathway [Hasselgren and Fischer, 2001] (Fig. 5). 26S proteasome is a large multisubunit proteolytic complex that consists of a central catalytic core (20S proteasome) and two terminal regulatory subcomplexes (19S complex). 20S proteasome contains proteolytic enzyme which are responsible of protein degradation, while 19S subcomplexes mediate the binding and the unfolding of substrate proteins before their transfer to 20S core. The 26S proteasome is associated with at least six ATPases that provide a continuous supply of energy for protein degradation. The proteasome releases short oligopeptides containing six to nine amino acid residues, which

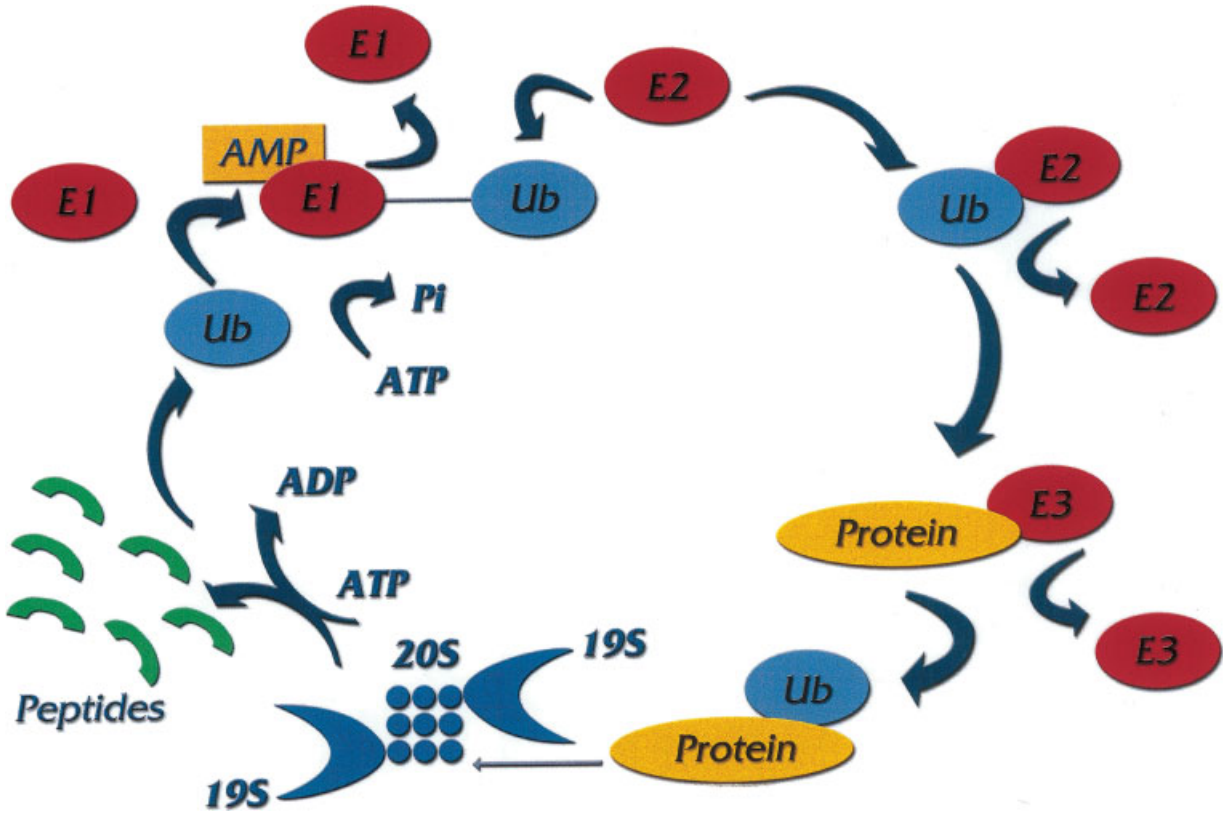

Fig. 5. Scheme of the ubiquitin-proteasome proteolytic pathway. The steps involved in the breakdown of proteins by this mechanism include: the activation of ubiquitin by the ubiquitin activating enzyme (E1); the transfer of ubiquitin to the ubiquitin conjugating enzyme (E2); the interaction between the substrate protein and the ubiquitin ligase (E3); the interaction between E2 and E3 resulting in the multiubiquitination of the substrate protein; the degradation of the ubiquitinated protein by the 26S proteasome; and finally the deubiquitination resulting in the release and recycle of ubiquitin in the pathway. The proteasome releases short oligopeptides containing six to nine amino acid residues, which are rapidly degraded into amino acids by cytosolic peptidase.

are rapidly degraded into amino acids by cytosolic peptidase [Tisdale, 1997]. All breakdown products enter the liver where they are used for the production of glucose. These breakdown products also stimulate the liver to produce APP at the expense of skeletal muscle proteins.

A number of reports have suggested that muscle protein hypercatabolism is mainly related to activation of the ATP-ubiquitin-dependent proteolysis. Indeed, the level of ubiquitin conjugates as well as the expression of genes pertaining to the ubiquitin proteasome degradative pathway are increased in conditions characterized by loss of muscle mass [Bossola et al., 2001]. In experimental cachexia models, in rats transplanted with the Yoshida ascites hepatoma [Baracos et al., 1995] and Yoshida sarcoma [Temparis et al., 1994] there is an increase in expression of ubiquitin, of the ubiquitin carrier protein E2, and of some proteasome subunits. In rats bearing the AH-130 Yoshida ascites hepatoma, muscle wasting is observed and has been associated with a 500% increase in polyubiquitin gene expression [Llovera et al., 1994]. These evidences indicate that ATP-ubiquitin-dependent proteolysis has an important role in muscle atrophy in cancer cachexia.

FACTORS INVOLVED IN THE INCREASING PROTEIN CATABOLISM IN SKELETAL MUSCLE BED

Several cytokines, such as tumor necrosis factor (TNF)-α, insulin-like growth factor (IGF)-1, IL-6, IL-1, interferon (IFN)-γ, and proteolysis-inducing factor (PIF) have been postulated to play a role in etiology of cachetic syndrome.

TNF-α

Direct evidence that TNF is involved in the mechanism of cachexia is that the administration of this cytokine to rats or humans induces

an enhanced protein degradation in skeletal muscle [Starnes et al., 1988; Llovera et al., 1993], while the administration of anti-TNF-antibodies decrease protein degradation rates in skeletal muscle, heart, and liver compared to animals receiving non-specific immunoglobulins. A recent report indicates that incubation of isolated rat muscle with TNF produces an increase in ubiquitin gene expression and an upregulation of the ubiquitin-dependent proteolytic pathway. TNF-α is known to be a potent activator of NF-κB. This molecule is activated through a process that involves its dissociation from a cytosolic, inhibitory protein IκB, permitting NF-κB to translocate to the nucleus, and influence the transcription of genes [Ladner et al., 2003]. Guttridge et al. [2000] report that activation of NF-κB by TNF-α in myocytes inhibits myoblast differentiation by suppressing production of MyoD mRNA at the post-transcriptional level. MyoD is a transcriptional factor that is essential for skeletal muscle differentiation and for repair of damaged tissue, and it may be particularly important for replenishing of wasted muscle. Indirect evidence that TNF may activate the proteasome system is provided from rats bearing the Yoshida sarcoma [Attaix et al., 1997]. Expression of an ATPase subunit of the proteasome is increased in wasting muscle from tumor-bearing rats and this condition is reverted when the animals were treated with pentoxifylline, which reduces the expression of TNF mRNA.

IGF-1

IGF-1 stimulates the rates of muscle protein synthesis, while reducing degradation rates [Umpleby and Russel-Jones, 1996]. Insulin administration to experimental animals and cancer patients reverses the muscle wasting pattern and the increase of protein degradation [Heslin et al., 1992]. Insulin has been shown to downregulate both E2 mRNA levels and proteasome activities [Duckworth et al., 1998] in cultures of L6 myotubes. In contrast, glucocorticoids exert a catabolic effect [Hasselgren, 1999]. Administration in rat skeletal muscle of glucocorticoid has been shown to increase the ubiquitin gene expression as well as that of the 14 kDa E2 and 20S proteasome subunit.

IL-6

Most of the studies linking IL-6 to cancer cachexia have come from mice bearing murine colon-26 adenocarcinoma. In this model, cachexia is associated with increasing serum levels of IL-6, and antibodies to the IL-6 receptor reduced the loss of gastrocnemius muscle but did not affect loss of body weight. Using C_2C_{12} myotubes in vitro Ebisui et al. [1995] have found that IL-6 shortened the half-life of long-lived proteins and increased the activity of the 26S proteasome and cathepsin-B and -L, suggesting the activation of proteolytic pathways. Administration of IL-6 in normal mice failed to cause weight loss, despite an increased APP production.

Another member of the IL-6 superfamily, ciliary neurotrophic factor (CNTF) when administered in normal mice does produce anorexia and lean tissue wasting with a 218% increase in carcass protein breakdown rate compared with freely fed controls.

IL-1

IL-1 has been found to have a role in protein catabolism in cancer cachexia. This cytokine stimulates muscle protein degradation in intact muscles [Baracos et al., 1983] but transfection of a cachexia-inducing tumor (colon-26) with the gene for the IL-1 receptor antagonist failed to inhibit the progression of cachexia [Costelli et al., 1995].

IFN-γ

Severe cachexia has been shown to develop rapidly in nude mice inoculated with cells constitutively producing mouse IFN-γ [Matthys et al., 1991] but there are no direct studies linking IFN to muscle protein catabolism.

PIF

A tumor product, PIF, is produced by human tumors and initiates muscle protein degradation directly through activation of the proteasome pathway as well as inhibiting protein synthesis. PIF is important during embryonic development of the liver and skeletal muscle, but it is not essential for normal cellular function [Watchorn et al., 1995]. Administration of the purified PIF to mice produces rapid weight loss, which is reversed by an antibody to PIF treatment [Cariuk et al., 1997]. Weight loss is associated with a significant decrease in the weight of the spleen and gastrocnemius muscles. PIF induces an accumulation of ubiquitin–protein conjugates in gastrocnemius muscles in weight-losing mice, and PIF-induced protein

catabolism in C_2C_{12} myoblasts is accompanied by an increased release of arachidonic acid [Smith et al., 1999]. The arachidonic acid is rapidly metabolized to prostaglandins (PG) E_2 and F_{2alpha} and to 5-, 12-, and 15-hydroxyeicosatetraenoic acids (HETES) that have been shown to directly increase protein degradation in muscle [Rodeman and Goldberg, 1982]. A metabolite of arachidonis acid, 15-HETE produces a significant increase in protein degradation in C_2C_{12} myoblasts, with a dose–response curve similar to that produced by PIF. Taken tighter, these data suggest that PIF may induce protein degradation as a result of an increased synthesis of 15-HETE. Protein degradation induced by PIF in C_2C_{12} myoblasts, together with arachidonate release and 15-HETE production is blocked by the polyunsaturated FA, eicosapentaenoic acid (EPA), which has been shown to attenuate the development of cachexia in pancreatic cancer patients [Downer et al., 1993].

SKELETAL MUSCLE ENERGY EXPENDITURE IN CANCER PATIENTS

As described before, weight loss is an important prognostic indicator for cancer patients, the survival of cancer patients being directly related to the total weight loss and also to the rate of weight loss. The acute wasting syndrome is typically associated with the development of significantly increased resting energy expenditure and negative nitrogen balance. Nitrogen is excreted primarily as urinary urea synthesized from amino acids released from skeletal muscle.

Resting energy expenditure can arise from decreased energy intake, increased energy expenditure, or both. Increased energy expenditure might be related to the upregulation of UCPs, particularly UCP3 in skeletal muscle. In mice bearing murine colon-26 adenocarcinoma with 24% of loss of their body weight, UCP1 mRNA levels are increased in BAT, while UCP2 and UCP3 mRNA levels are increased in skeletal muscle. An increased in UCP2 and UCP3 mRNA expression is also observed in skeletal muscle of rats bearing the Yoshida ascites hepatoma.

In humans, UCP3 mRNA level has been found to be more than five times higher in the skeletal muscle of patients with gastrointestinal adenocarcinoma and weight loss, while there are no changes in UCP3 mRNA expression in controls [Collins et al., 2002].

Tumor products or cytokines secreted during the anti-tumor response induce changes in UCP expression. A recent report indicates that a single injection of TNF-α to rats results in increased UCP2 and UCP3 mRNA levels in skeletal muscle and that administration of a tumor lipid mobilizing factor (LMF) results in increased UCP1, UCP2, and UCP3 expression in BAT and UCP2 in skeletal muscle [Busquets et al., 1998].

Several scientific reports indicate that both UCP2 and 3 protein levels in skeletal muscle are increased in patients with pancreatic cancer compared to controls. Similarly, UCP2 and 3 mRNA contents in skeletal muscle are also increased in other types of cancer, accompanied by reduced levels of ATP and phosphocreatine [Isaksson et al., 2002].

Consistent with the previous reports, mice bearing the MAC16 adenocarcinoma, a model for many human gastrointestinal and pancreatic cancer, display cachexia with both progressive weight loss from 13 days after tumor inoculation and a concomitant upregulation of UCP2 and 3 expression [Bing et al., 2000].

PPARs AND SKELETAL MUSCLE METABOLISM IN CACHEXIA

TNF-α or IFN-γ can also activate the PPAR-γ coactivator-1 (PGC-1) through the phosphorylation of p38MAPK (a mitogen activated protein kinase family member), which leads to stabilization and activation of PGC-1 protein. A PGC-1 related coactivator (PRC) has also been identified, with properties that overlap with those of PGC-1 [Andersson and Scarpulla, 2001]. When transcription factors/nuclear receptors (NRF-1 or PPAR) bind DNA, PGC-1 docks, and undergoes a conformational change that recruits other coactivator proteins thereby increasing transcriptional activity [Wu et al., 1999]. A growing body of evidence implicates PGC-1 in the control of mitochondrial biogenesis and certainly, increases in PGC-1 mRNA and protein levels accompany mitochondrial proliferation during adaptive thermogenesis [Lowell and Spiegelman, 2000] and muscle regeneration [Duguez et al., 2002]. It has been suggested that the overexpression of PGC-1 leads to mitochondrial proliferation in transgenic mouse heart [Lehman et al., 2000] and in adipocytes and myoblasts [Wu et al., 1999]. Because it appears that PGC-1 modulates transcription of

respiratory genes, attention was focused on the regulation of PGC-1 synthesis and activity [Herzig et al., 2000; Knutti et al., 2001]. Regulation of respiratory gene expression via p38MAPK-dependent pathways may be important in mediating mitochondrial proliferation induced by extrinsic pathways such as the response to cytokines [Herzig et al., 2000]. Although the role of membrane receptor signaling pathways in mediating mitochondrial proliferation in response to exercise is largely unknown, there is considerable evidence that intrinsic pathways, dependent on muscle metabolism, also influence respiratory gene expression.

Recently, some authors have found that adenovirus mediated expression of PGC-1 in cultured muscle cells results in a large increase in the GLUT4 isoform of the glucose transporter [Michael et al., 2001] and in mitochondrial proliferation and adaptative thermogenesis [Puigserver et al., 1998; Wu et al., 1999]. The effect of PGC-1 on mitochondrial biogenesis is probably explained at least in part by the finding that in addition to being a coactivator of PPARγ, PGC-1 coactives nuclear respiratory factors NRF-1 [Wu et al., 1999] and NRF2 that are key transcriptional activators of nuclear genes encoding a range of mitochondrial enzymes [Evans and Scarpulla, 1990; Virbasius and Scarpulla, 1994; Scarpulla, 1996]. Further evidence that PGC-1 promotes mitochondrial biogenesis was provided by the finding that PGC-1 activates PPARα [Vega et al., 2000] that has been shown to play a key role in the transcriptional control of the mitochondrial enzymes involved in β-oxidation of FAs [Gulick et al., 1994; Brandt et al., 1998].

Although the data are not conclusive for a direct involvement of PPARs on skeletal muscle wasting in case of cancer disease, there are all the premises for an important role played by these factors on modulating the metabolic response in muscle of neoplastic patients.

For example, troglitazone, a TZD that selectively activates PPAR-γ, has been postulated for the therapy of cachexia since it is able to reduce UCP2 and UCP3 mRNA levels in mouse myotubes, indicating that PPAR-γ ligands could decrease energy expenditure in cachexia.

CONCLUSIONS

Important progress has been made in the last 10 years in elucidating the role of various factors in skeletal muscle metabolism and the results of these studies are now being translated into treatment regiments for patients with cachexia. Cachexia is an important cause of mortality in cancer patients accounting directly for between 10 and 22% of all cancer deaths, as well as that from other causes such as infection.

Future research should, therefore, be directed towards an understanding of the mechanisms that are involved in loss of skeletal muscle protein and the mediators of this process.

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