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# NOVEL INVESTIGATIONAL DRUGS MIMICKING EXERCISE FOR THE TREATMENT OF CACHEXIA

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## **Abstract**

**Introduction:** Cachexia is a syndrome characterized by body weight loss, muscle wasting, and metabolic abnormalities, that frequently complicates the management of people affected by chronic diseases. No effective therapy is actually available, although several drugs are under clinical evaluation. Altered energy metabolism markedly contributes to the pathogenesis of cachexia; it can be improved by exercise, able to both induce anabolism and inhibit catabolism.

**Areas covered in this review:** This review will focus on exercise mimetics and on their potential inclusion in combined protocols to treat cachexia, with particular reference to the cancer-associated one.

**Expert opinion:** Despite exercise improves muscle phenotype, most patients retain sedentary habits quite difficult to disrupt. Moreover, they frequently present with chronic fatigue and co-morbidities that reduce exercise tolerance. For these reasons drugs mimicking exercise could be beneficial also in the absence of patient compliance to the practice of physical activity. Different exercise mimetics are now available and, yet some of them may exert serious side-effects, investigating their effectiveness on muscle phenotype will provide a new tool for the management of cachexia.

## 1. Introduction

Cachexia is a multi-organ syndrome that frequently occurs in chronic pathologies such as autoimmune or neurodegenerative diseases, sepsis, diabetes and cancer. The etiopathogenesis of cachexia is multifactorial, including reduced caloric intake, altered energy and protein metabolism, enhanced lipolysis, systemic inflammation, perturbations of the hormonal homeostasis. Among the different features that characterize the onset and progression of cachexia, body weight loss and inflammation are the most relevant <sup>(1)</sup>.

In cachexia, both skeletal muscle mass and function are markedly reduced. Muscle nitrogen balance is regulated by a complex network of factors, such as humoral mediators, nutrient and energy availability, or contractile activity, all of which concur to unbalance protein turnover rates, favoring degradation.  $\text{Ca}^{2+}$ -dependent, ATP-ubiquitin-dependent and autophagic lysosomal pathways have all been shown to contribute to muscle protein wasting <sup>(2)</sup>. Since the discovery and characterization of different muscle-specific ubiquitin ligases<sup>(3)</sup>, attempts have been made to antagonize muscle depletion by targeting these enzymes. The results, however, do not allow to validate ubiquitin ligases as therapeutic targets for muscle wasting. Not only, specific systemic inhibition of the different proteolytic pathways does not appear as an effective strategy to contrast the progression of cachexia. This has been shown for proteasome inhibitors such as bortezomib<sup>(4)(5)</sup>, while suppression of autophagy may even reveal detrimental<sup>(6)(7)(8)</sup>. The possibility that muscle-specific inhibition of proteolysis might prove effective remains an open question, however.

In various forms of cachexia muscle protein wasting is associated with an unbalance between pro- and anti-inflammatory cytokines, due to chronic inflammation. For example, circulating levels of Tumor Necrosis Factor (TNF) $\alpha$  and TNF soluble receptors are increased in patients affected by cancer, chronic renal or heart failure<sup>(9)(10)(11)</sup>, while mice lacking the anti-inflammatory IL-10 display muscle depletion and weakness<sup>(12)</sup>. Pro-inflammatory cytokines, in particular, play a pivotal role in modulating muscle protein turnover. Systemic inflammation is associated with enhanced rates of protein degradation, that can be paralleled or not by decreased protein synthesis. Cytokine-induced activation of the transcription factors Nuclear Factor (NF)- $\kappa$ B and Signal Transducer and Activator of Transcription (STAT)3 results in muscle atrophy<sup>(13)(14)</sup>. Several studies report that anabolism can also be affected by pro-inflammatory cytokines. As an example, TNF $\alpha$  down-regulates anabolic signaling pathways such as those activated by insulin and Insulin-like Growth Factor (IGF)-1<sup>(15)</sup>. Particularly relevant to muscle mass homeostasis

are the insulin receptor substrate (IRS)-1/PI3K/Akt axis<sup>(16)</sup> and the signaling pathways dependent on myostatin<sup>(15)(17)</sup> or on bone morphogenetic proteins<sup>(18)</sup>, all of which are subject to cytokine-mediated modulations.

## **2. Therapeutic approaches to cancer cachexia**

Cachexia develops in a large proportion of cancer patients, being estimated to account for about 20% of cancer-related deaths<sup>(1)</sup>. Its occurrence markedly complicates patient management, negatively impinging on the tolerance and response to antineoplastic treatments, and on the quality of life as well. Reverting overt cachexia, however, definitely is not an easy task, and the most likely scenario is to develop strategies aimed at delaying its onset. To achieve this goal, early stages of cachexia must be identified. Along this line, Fearon and collaborators<sup>(15)</sup> proposed a classification that defines three progressive stages (pre-cachexia, cachexia, and refractory cachexia), aimed to help clinicians to start the few available treatments as soon as possible.

Studies performed on experimental models have provided the basis for potential treatments of human cachexia, some of which are in phase I or II clinical trials. Provided the multifactorial nature of this syndrome, the rationale of such approaches ranges from nutritional support to the inhibition of specific molecular pathways.

The early institution of a personalized nutritional counseling is recommended to improve patient nutritional status, treatment tolerance and clinical outcome. Indeed, the maintenance of patient body weight by nutritional interventions allows the completion of chemotherapy, frequently interrupted because of severe side effects<sup>(19)</sup>. In this regard, several agents have been tested to treat anorexia and increase appetite, such as corticosteroids, progestins (megestrol acetate), cannabinoids, ghrelin and its analogues (anamorelin), branched-chain amino acids<sup>(20)</sup>.

Since systemic inflammation frequently occurs in cancer patients (see above), several anti-inflammatory drugs have been tested in experimental cachexia: steroids and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), anti-cytokine agents (pentoxifylline, thalidomide), anti-cytokine (IL-6, TNF) antibodies,  $\omega$ -3 polyunsaturated fatty acids. Most of these agents, however, have not been validated for the clinical use, although some of them (NSAIDs,  $\omega$ -3 polyunsaturated fatty acids) reached the clinical evaluation<sup>(20)</sup>.

The metabolic alterations underlying cancer cachexia point towards enhanced catabolism and reduced anabolism. Accordingly, anabolic and/or anticatabolic strategies have been adopted to modulate these processes and attempts have been made to modulate the Growth Hormone (GH)/insulin/IGF-1 axis, the androgen-dependent signaling (anabolic-androgenic steroids, selective androgen receptor modulators), or the availability of amino acids and their metabolites (branched-chain amino acids,  $\beta$ -hydroxy- $\beta$ -methylbutyrate, glutamine)<sup>(20)</sup>.

Finally, based on the mechanisms contributing to cancer-induced cachexia, some experimental drugs are currently under investigation, including anti-myostatin agents,  $\beta$ -adrenergic agonists, mitogen-activated protein kinase kinase (MEK), and extracellular signal regulated protein kinase (ERK) inhibitors, Interleukin (IL)-15. Although encouraging, in some cases at least, at the moment the results do not favor the possibility of their rapid translation into the clinical practice.

### **3. Novel aspects in the pathogenesis of cachexia: myogenesis and mitochondria**

In the last few years two aspects have added complexity to the pathogenesis of cachexia: 1) the reduced myogenic potential; 2) the altered mitochondrial structure and function.

#### **3.1 Impaired myogenesis**

Myogenesis allows the development of skeletal muscle during the embryonal life. In the adulthood it drives the repair of injured muscles and the maintenance of physiological muscle cell renewal. After damage, myofiber degeneration is compensated by the regeneration of new fibers afforded by proliferation and subsequent fusion of resident myogenic precursors, mainly satellite cells (SCs)<sup>(21)</sup>. Impaired myogenesis has been associated with pathological muscle wasting. The levels of Pax7, a marker of SC proliferation, and those of myogenin, an indicator of ongoing differentiation, respectively increase and decrease, respectively, in the muscle of mice bearing the colon 26 carcinoma (C26) as well as in cancer patients, suggesting that myogenic precursors accumulate in the skeletal muscle of tumor hosts<sup>(22)(23)(24)</sup>. This process appears to depend on the maintenance of elevated Pax7 expression, apparently related to activation of the

transcription factor NF- $\kappa$ B<sup>(23)</sup>. Impaired myogenesis is associated with enhanced signaling through the stress kinase ERK, which is known to maintain cells in an undifferentiated state<sup>(22)</sup>. Indeed, treatment of C26-bearing mice with PD98059, a MEK inhibitor able to block ERK activation, restores the physiological levels of both Pax7 and myogenin expression<sup>(22)</sup>. In agreement with these results, muscle gain has been observed in cholangiocarcinoma patients assuming selumetinib, another MEK inhibitor<sup>(25)</sup>. Finally, the administration of the multikinase inhibitor sorafenib has been shown to improve both muscle wasting and physical activity in mice hosting the C26 or the Lewis Lung Carcinoma (LLC). Such effects are associated with modulation of both STAT3 and ERK activity in the muscle, leading to reduced expression of Pax7 and atrogen-1<sup>(26)</sup>. Further investigations in this direction are warranted to clarify if ERK can be a suitable therapeutic target in cancer cachexia.

### 3.2 Impaired mitochondrial function

Since mitochondria are the main source of the energy required for contraction, alterations in their homeostasis markedly affect muscle function. While the concept of energy wasting is not new in the literature on cancer cachexia<sup>(1)</sup>, the underlying mechanisms became a hot topic just recently. Ultrastructural alterations have been reported in muscle mitochondria of animals bearing the C26<sup>(27)</sup>, the LLC (Pin, Busquets et al., under revision) or the Yoshida Ascited Hepatoma (AH)-130 tumors<sup>(28)</sup>. Mitochondrial uncoupling occurs and oxidative capacity decreases in the muscle of tumor hosts<sup>(29)(30)</sup>, consistently with the occurrence of a shift from oxidative to glycolytic fibers (Pin, Busquets et al., under revision). Humoral mediators contribute to mitochondrial impairment. As an example, TNF $\alpha$ -induced activation of NF- $\kappa$ B reduces muscle oxidative capacity and negatively regulates the expression of molecules relevant to mitochondrial biogenesis<sup>(29)</sup>. C2C12 myotubes cultured in the presence of LLC cell conditioned medium show alterations of the electronic flow in the electron transport chain<sup>(31)</sup> and finally, muscle wasting in the ApcMin/+ mice, an experimental model of IL-6 driven cancer cachexia, is associated with altered expression of proteins regulating mitochondrial biogenesis and fusion<sup>(32)</sup>.

A relation between mitochondria dynamics (balance between fission and fusion processes) and protein breakdown in the skeletal muscle has been previously

described<sup>(33)(34)</sup>. Mitochondrial dysfunction, leading to oxidative and energy stress, would lead to hyperactivation of muscle proteolytic systems through adenosine monophosphate-activated protein kinase (AMPK)- and Forkhead transcription factor (Fox)O-dependent pathways<sup>(33)</sup>, ultimately resulting in muscle wasting.

#### **4. Importance of exercise in the management of cachexia**

Most of the ongoing clinical trials are based on the use of nutritional and pharmacological interventions aimed at preventing the loss of body weight and muscle mass. However, muscle 'quality' is as important as muscle 'quantity'. Both aspects could be targeted by exercise training. Indeed, stimulating the increase of muscle mass and strength, it might improve cancer-induced wasting by both activating anabolic pathways and down-regulating the activity of pro-inflammatory cytokines<sup>(35)</sup>.

Two different exercise modalities can be chosen: 1) resistance exercise, that causes a mechanical strain on the muscle resulting in different adaptations, among which hypertrophy, that occurs primarily through the accretion of contractile proteins, increases the capacity to generate force; and 2) endurance exercise, that generally leads to metabolic adaptations such as increased number of mitochondria, fiber-type shift to an oxidative profile and increased capillary density, with little changes in the skeletal muscle mass<sup>(36)</sup>.

Endurance exercise increases the expression of the peroxisome proliferator-activated receptor (PPAR)- $\gamma$  coactivator-1 $\alpha$  (PGC-1 $\alpha$ ), a transcriptional co-regulator that binds to several transcription factors to promote mitochondrial biogenesis and oxidative metabolism<sup>(37)</sup>. Moreover, exercise also induces the expression of genes involved in mitophagy<sup>(38)</sup>, a process that accounts for the clearance of damaged mitochondria. Consistently, PGC-1 $\alpha$  has been recently shown to regulate mitophagy in the skeletal muscle<sup>(39)</sup>, while the reduced mitochondrial content reported in ApcMin/+ cachectic mice is associated with down-regulation of PGC-1 $\alpha$  and mitochondrial fusion proteins<sup>(32)</sup>. Finally, both resistance and endurance exercise appear to stimulate the proliferation of SCs, promoting myogenesis (see above)<sup>(40)(41)</sup>.

Taking into account that at least some of the metabolic changes occurring in the skeletal muscle of cancer patients (alterations of mitochondrial structure, reduced ATP



synthesis, shift from oxidative to glycolytic metabolism, impaired myogenesis)<sup>(1)(42)</sup> can be rescued by physical training, a combination of endurance and resistance exercise would probably be the right choice to improve cachexia.

## 5. Drugs mimicking exercise

At present three main classes of drugs mimicking the effects of aerobic/endurance exercise have been described: agonists of PPAR $\delta$  or of AMPK and molecules able to activate silent-information-regulator-two-protein (SIRT)1. In addition, hyperexpression of PGC-1 $\alpha$  could be an effective strategy, too.

PPAR $\delta$ , the most abundant PPAR in the skeletal muscle, is involved in the regulation of lipid metabolism, mitochondrial function and fiber-type determination. Its expression in the skeletal muscle is induced by exercise. As a consequence of its activation the energy source is switched from glucose to fatty acids and myofiber phenotype converted from fast (glycolytic) to slow (oxidative) twitch. Muscles in mice overexpressing PPAR $\delta$  are characterized by increased proportion of oxidative fibers, enhanced activity of enzymes involved in the oxidation/phosphorylation reactions, and increased mitochondrial biogenesis and uncoupling proteins<sup>(43)</sup>. Moreover, constitutive hyperactivation of PPAR $\delta$  results in increased exercise capacity and in protection against diet-induced obesity or type 2 diabetes<sup>(44)</sup>. By contrast, lack of PPAR $\delta$  leads to an opposite muscle phenotype, characterized by slow-to-fast myofiber conversion and down-regulation of gene products involved in fatty acid oxidation<sup>(45)</sup>.

Among the various PPAR $\delta$  agonists presently available, the most widely studied is GW501516, which has been shown to activate PPAR $\delta$  in an exercise-like manner, although not reproducing the increased mitochondrial gene expression and function or the switch of fiber type<sup>(46)(47)</sup>. Increased fat *versus* carbohydrate oxidation rate, up-regulation of mitochondrial biogenesis and increased expression of genes encoding contractile proteins characteristic of type I myofibers can be observed in mice after few days of synthetic PPAR $\delta$  agonist administration to mice<sup>(44)</sup>. Moreover, both angiogenesis and biogenesis of mitochondria are induced in mice 24 h after GW0742 administration, while a fiber type switch is evident after 48h<sup>(48)</sup>. Finally, myocyte cultures exposed to PPAR $\delta$  agonists show increased glucose and fatty acid metabolism, with enhanced expression and activation of AMPK and p38<sup>(48)</sup>.

The potential of small molecule PPAR $\delta$  agonists to treat various pathological conditions is highlighted by several studies. As an example, GW501516 improves experimental atherosclerosis in apoE<sup>-/-</sup> mice<sup>(49)</sup>, while its administration to obese mice reduces weight gain and increases both resting energy expenditure and peripheral insulin sensitivity<sup>(50)</sup>. Just few studies investigate PPAR $\delta$  agonist effects in humans. The results suggest that these drugs could reduce obesity, insulin resistance and hyperlipidemia. Indeed, treatment with GW501516 results in improved circulating apolipoproteins, LDL and HDL cholesterol and hepatosteatosis<sup>(51)</sup>. Finally, administration of MBX-8025 to moderately obese subjects improves circulating lipid and cholesterol profiles<sup>(52)</sup>. However, very few data are available to define if PPAR $\delta$  agonists can also affect muscle phenotype. GW501516 treatment improves muscle dystrophy by down-regulating inflammation-dependent pathways<sup>(53)</sup>. The same drug attenuates metabolic abnormalities and improves muscle phenotype in experimental models of cardiovascular disease<sup>(48)</sup>. A specific class of PPAR agonists, namely glitazones, known for their insulin sensitizing action in the skeletal muscle, appear promising to treat muscle atrophy, but their clinical use is controversial. Data obtained on a genetic model of diabetes, the *db/db* mice, show that rosiglitazone improves insulin resistance and Akt-dependent signaling pathway, while it inhibits both muscle proteasome and caspase 3 activities<sup>(54)</sup>. In addition, a transient muscle-sparing effect associated with normalization of PPAR $\delta$  expression has been reported in C26-bearing mice treated with rosiglitazone<sup>(55)(56)</sup>. Despite the above described positive effects, however, elderly obese patients practicing resistance training and assuming pioglitazone show both visceral fat and muscle depletion<sup>(57)</sup>, while contradictory results have been reported also for other PPAR $\delta$  agonists. Treatment of L6 myotubes with the GW0742 increases protein degradation and reduces myotube size, and rats administered the PPAR $\delta$  inhibitor GSK0660 are resistant to glucocorticoid or sepsis-induced muscle protein breakdown<sup>(58)</sup>. These findings are in line with previous data showing that PPAR $\delta$  expression is induced in the skeletal muscle of tumor-bearing rats<sup>(59)</sup>. A final consideration is that glitazones show several severe side effects, including cardiovascular and hepatic adverse events and increased risk of cancer development<sup>(60)</sup>. For these reasons, rosiglitazone has been withdrawn from the european market by EMA, while strict criteria for prescriptions and additional warning labeling are required by FDA<sup>(61)</sup>.

AMPK activation results in modulations of the metabolic balance aimed to spare energy, mainly achieved by stimulating catabolism and inhibiting anabolism. For example, AMPK activation induces the expression of muscle-specific ubiquitin ligases and

stimulates autophagy<sup>(62)(63)</sup>. Genetic AMPK inactivation in the muscle results in reduced mitochondrial mass, loss of type I fibers and reduced lipid metabolism. However, besides the pro-catabolic action of AMPK, the enhancement of its activity leads to increased oxidative metabolism and exercise efficiency<sup>(64)</sup>. In this regard, AMPK activation should be pursued in pathological states characterized by energy deficit, despite the concomitant occurrence of hypercatabolism.

The activation of AMPK can be achieved by drugs such as 5-aminoimidazole- 4-carboxyamide ribonucleoside (AICAR), metformin,  $\beta$ -guanidinopropionic acid ( $\beta$ -GPA), resveratrol, quercetin, and salicylate<sup>(48)</sup>. Increased fatty acid oxidation and oxygen consumption, IIb-to-IIx myosin isoform shift, enhanced aerobic capacity and running endurance have been reported after AICAR administration to experimental animals<sup>(46)</sup>. AICAR-induced AMPK activation improves muscle phenotype in dystrophic *mdx* mice by activating autophagy<sup>(53)</sup>. The same drug reverts angiotensin II-induced muscle wasting, down-regulating the induction of the muscle-specific ubiquitin ligases and improving PGC-1 $\alpha$  expression<sup>(65)</sup>. Chronic administration of  $\beta$ -GPA to experimental animals induces a fast-to-slow myofiber shift, increases PGC-1 $\alpha$  expression and reduces muscle content of Receptor-interacting protein (RIP)140, a repressor of the oxidative phenotype (reviewed in<sup>(48)</sup>).

As for metformin, few studies investigate its effects on muscle atrophy, both in humans and in experimental animals. Severely burned patients treated with metformin show reduced endogenous glucose production and increased glucose clearance and oxidation, with decreased glycemia. In parallel muscle wasting is improved, due to increased protein synthesis rather than to reduced breakdown<sup>(66)</sup>. Metformin appears to improve aging-related sarcopenia<sup>(67)</sup> and has been proposed to treat cancer-induced muscle wasting<sup>(68)</sup>.

AMPK can also be activated by resveratrol, since many effects exerted on the muscle by this drug are attenuated by AMPK inhibition. Consistently, the increased mitochondrial biogenesis and endurance performance induced by resveratrol in wild type mice cannot be observed when the drug is administered to AMPK $\alpha_1$  or  $\alpha_2$  deficient mice<sup>(69)</sup>. Similarly, muscle wasting and reduced AMPK activation in IL-10 deficient mice can be improved by grape seed extract<sup>(70)</sup>. Finally, resveratrol administration to obese men activates AMPK and increases PGC-1 $\alpha$  and SIRT1 protein levels, improving circulating lipid profile and inflammation<sup>(71)</sup>.

Mammalian sirtuins (SIRT1–7) are a class of deacetylases involved in several processes such as metabolic regulation, cell death, autophagy, DNA repair and circadian rhythm. In addition, SIRT1s appear crucial in lifespan regulation in lower eukaryotes, while not in healthy mice<sup>(72)</sup>. SIRT1 deregulation is involved in aging as well as in chronic pathologies such as obesity and cancer<sup>(73)</sup>.

SIRT1 is the most extensively studied. It is involved in the modulation of various epigenetic mechanisms and also behaves as a critical regulator of energy homeostasis. SIRT1 positively modulates several processes such as cell survival, autophagic protein degradation, insulin production, regulation of mitochondrial mass, lipid metabolism and glucose homeostasis<sup>(73)</sup>. Consistent with its role as energy regulator, SIRT1 expression is induced in response to caloric restriction<sup>(73)</sup>, and can be activated in the skeletal muscle by AMPK, linking a critical energy sensor to a regulator of aerobic capacity<sup>(74)</sup>.

Mice genetically deficient of SIRT1 show inflammation and metabolic defects<sup>(75)</sup>, while SIRT1 hyperexpression improves high fat diet-induced metabolic alterations<sup>(76)</sup>. Recent data show that muscle-specific SIRT1 overexpression causes a fast-to-slow fiber type transition. Such modification is paralleled by reduced gastrocnemius weight, which is consistent with the lower cross sectional area of oxidative compared to glycolytic fibers. However, transgenic animals are protected, partially at least, from fasting or denervation-induced atrogene hyperexpression<sup>(77)</sup>. Finally, muscle phenotype in *mdx*/SIRT1 double transgenic mice is improved<sup>(78)</sup>.

Both natural and synthetic SIRT1 activators are available. Among the former, polyphenols, such as resveratrol, are the most studied. Resveratrol administration to mice increases increased muscle mitochondrial biogenesis and oxidative metabolism, resulting in enhanced exercise capacity and in protection against obesity and type 2 diabetes<sup>(79)</sup>. Resveratrol improves the lifespan of mice maintained on high fat diet<sup>(80)</sup> and is also beneficial in obese humans (see above). Part of the effects exerted by resveratrol derive from SIRT1-dependent modulations of PGC-1 $\alpha$  acetylation state, and are recapitulated by selective SIRT1 activators<sup>(81)</sup>.

Synthetic selective SIRT1 activators such as SRT2104 appear well tolerated in healthy adults and in elderly people<sup>(82)(83)</sup>. SRT2104 administration to healthy volunteers improves plasma lipid profile and insulin sensitivity<sup>(82)(84)</sup>. At present no studies address the effectiveness of synthetic SIRT1 activators on muscle mass and function in humans. Such

aspect has been recently investigated in an experimental study where short-term treatment with SRT2104 has been shown to attenuate muscle mass depletion induced by inactivity or fasting<sup>(85)</sup>. The protection exerted by SRT2104 on inactivity-induced muscle atrophy is associated with increased PGC-1 $\alpha$  levels<sup>(85)</sup>, consistent with previous observations showing that PGC-1 $\alpha$  overexpression is able to protect against muscle atrophy induced by denervation or fasting<sup>(86)</sup>.

PGC-1 $\alpha$  is currently considered one of the major regulators of exercise-induced phenotypic adaptation in the skeletal muscle. It modulates mitochondrial biogenesis and its hyperexpression leads to increased mitochondrial mass and enhanced oxidative metabolism<sup>(87)</sup>.

Both transcriptional and post-translational mechanisms contribute to regulate PGC-1 $\alpha$  activity. As an example, endurance exercise leads to PGC-1 $\alpha$  deacetylation, up-regulating the expression of several target genes. Exercise-induced activation of PGC-1 $\alpha$  may also be influenced by different signaling pathways such as those dependent on Mitogen Activated Protein Kinases (MAPKs), oxidative stress, AMPK, SIRT1<sup>(87)</sup>.

In the skeletal muscle PGC-1 $\alpha$  participates in fiber-type specification, promoting a fast-to-slow transition. When specifically overexpressed in the muscle, it leads to a markedly oxidative phenotype characterized by high respiratory capacity and increased resistance to fatigue that result in improved exercise performance, without affecting muscle mass. Such phenotype closely resembles the one induced by aerobic training. By contrast, lack of PGC-1 $\alpha$  results in decreased exercise performance and oxidative metabolism, paralleled by a slow-to-fast fiber type transition<sup>(87)</sup>. Evidence has been provided of a link between PGC-1 $\alpha$  and the TNF-like weak inducer of apoptosis (TWEAK)-Fibroblast growth factor inducible (Fn)14 signaling pathway<sup>(88)</sup>, known to be involved in muscle atrophy<sup>(89)</sup>. Indeed, muscle-specific PGC-1 $\alpha$  overexpression has been shown to inhibit TWEAK-induced expression of atrogenes and to prevent the inducible expression of TWEAK receptor Fn14 in denervated muscle<sup>(88)</sup>. Finally, recent results obtained in our laboratory show that PGC-1 $\alpha$  overexpression improves muscle wasting in tumor-bearing mice (Pin, Busquets et al., under revision). These results are consistent with those reported by Ruas and coworkers<sup>(90)</sup>, showing that transgenic mice overexpressing the PGC-1 $\alpha$ 4 isoform, characterized by muscle hypertrophy, are more resistant than wild-type animals to hindlimb-associated muscle atrophy as well as to cancer-induced cachexia.

## 6. Conclusion

In the last decades several mechanisms underlying muscle wasting in cachexia have been unraveled, however further research is required, with particular reference to the need to confirm in humans data arising from animal models. The reduction of physical activity that generally occurs in cancer patients likely significantly contributes to the pathogenesis of muscle wasting in cachexia. For this reason, exercise training is regarded as a promising intervention that can attenuate cancer-induced muscle wasting. However, since exercise capability can be impaired in cancer patients, drugs able to mimick exercise effects would be welcome. In this regard, PPAR $\delta$  or AMPK agonists as well as SIRT1 activators have been proposed as potentially useful drugs (Table 1). The results actually available are encouraging but still at the pre-clinical level. Further studies are thus warranted to extend the experimental observations and to translate them to the clinical practice.

## 7. Expert opinion

Cancer cachexia originates from a complex network of profound metabolic alterations that reflects the action of inflammatory mediators, of energy deficit and of hypercatabolic stimuli. At the molecular level at least, such perturbations may occur at very early disease stages, well before any evidence of general wasting.

Tumor eradication obviously is the only radical approach to deal with cachexia. However, not only this goal is totally beyond reach, but cachexia compromises its feasibility. Therefore, reliable therapeutic strategies aimed at interfering with the onset and/or progression of cachexia need to be pursued and timely implemented in patients. On the basis of results obtained in experimental studies, several drugs are now under evaluation in clinical trials to test their effectiveness in preventing/delaying cachexia in cancer patients. The rationale underlying such trials is quite variable. In general, however, it is important to realize that monotherapies are hardly adequate to deal with cachexia and this view seems to gain a growing consensus. Rather, the most suitable approach should likely be a multidirectional one, selectively tailored, whenever possible, on a panel of the manifold and variable (e.g., inflammatory cytokines) pathogenic factors involved.

In the last years the knowledge about the mechanisms underlying cachexia has expanded considerably, also taking into consideration impaired myogenesis and the occurrence of energy deficit, paving the way to new potential therapeutic targets. Few attempts to improve cachexia by impinging, directly or indirectly, on myogenesis have been reported so far (see above). While the results appear encouraging, further studies are needed to clarify this issue.

The occurrence of energy dysmetabolism in cachexia is long recognized, however the research on the underlying mechanisms and the definition of useful therapeutic strategies is at the very beginning. In the last years, alterations in mitochondrial structure and function have been reported in the skeletal muscle of cancer hosts. Being mitochondria the main source of energy used by the muscle for contractile activity, their dysfunction would result in oxidative and energy stress, ultimately contributing to muscle wasting. Exercise has proven to be very effective to improve muscle phenotype by impinging on both anabolic and catabolic (pro-inflammatory) pathways.

Despite the potentially favorable perspectives reported above, to disrupt the sedentary habits shared by most patients is not an easy task. In addition, chronic fatigue and co-morbidities frequently occurring in cancer patients, such as anemia and cardiac dysfunctions, are limiting factors for practicing physical activity, and can eventually lead to exercise intolerance. Consistently, muscle wasting is not prevented by exercise in mice bearing the C26 tumor (Pin, Busquets et al., under revision), in which cachexia is associated with anemia and cardiac alterations<sup>(91)</sup>, while excessive endurance exercise is associated with increased mitochondrial fission in the absence of mitophagy induction<sup>(92)</sup>. For these reasons, the availability of drugs that mimic the effects of exercise action would be desirable in order to maintain the beneficial effects of exercise avoiding the negative aspects represented by potential muscle injury and by the need of a 'permissive' energy metabolism, frequently lacking in cancer patients, especially in advanced disease stages (Figure 1).

The strength of the research on drugs mimicking exercise is to clarify if they can effectively become part of a tailored therapeutic protocol aimed at preventing/delaying cachexia. This would allow the maintenance of cancer patients in the pre-cachexia stage as long as possible, markedly improving the possibility to complete the scheduled antineoplastic treatment, achieving the most possible effectiveness. However, since in most cases the data available on drugs mimicking exercise are at the pre-clinical level,

studies aimed at validating the results also at the clinical level are warranted. This is not an easy task, however, since such studies would be more complicated, in terms of both invasiveness of the approach and ethical issues, than prospective clinical investigations based on anthropometric and/or biological fluid-derived data.

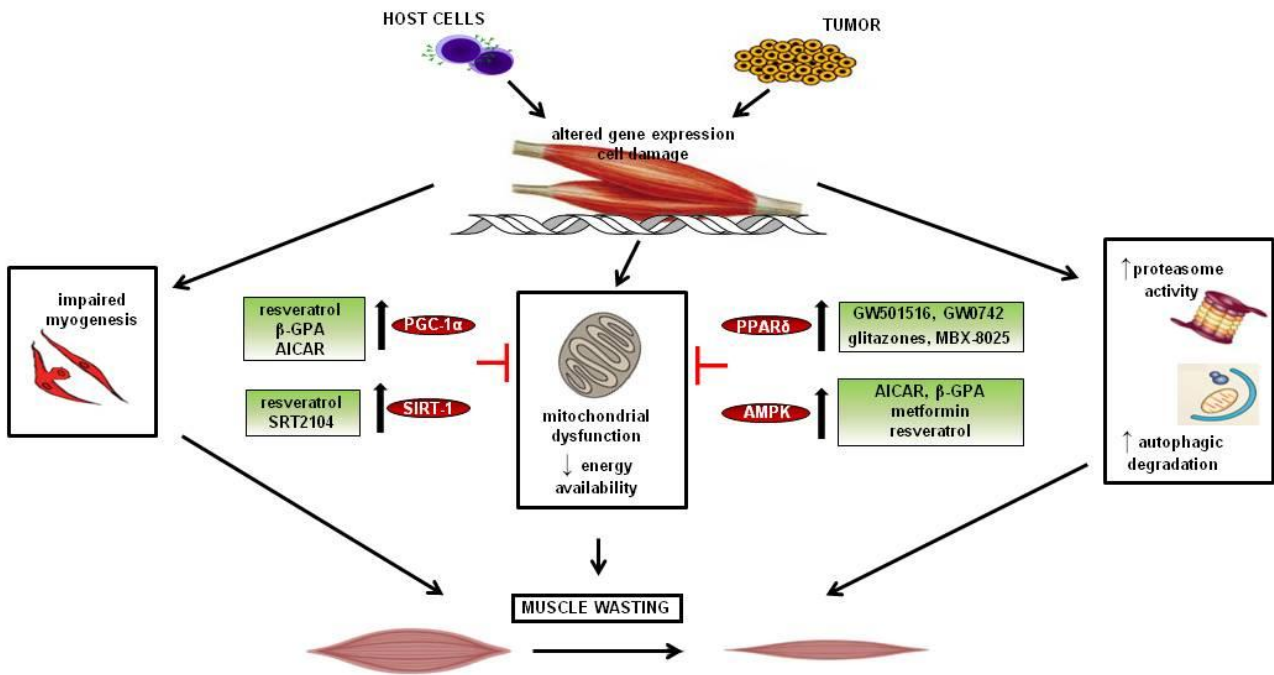
Finally, a note of care must be posed, since many exercise mimetics, in particular those able to induce PPAR $\delta$  (glitazones, metformin), may produce dangerous side-effects such as worsening the cardiovascular function or increasing the oncogenic risk. In this regard, studies should concentrate on those drugs able to improve energy metabolism, acting on mitochondrial turnover and oxidative metabolism, being well tolerated at the same time.



**Table 1. Drug mimicking exercise: the state of the art**

	<b>CURRENT STATUS</b>	<b>REFERENCES</b>
<b>PPAR<math>\delta</math> AGONISTS</b>		
GW501516	pre-clinical	44, 46-48, 53
GW0742	pre-clinical	48
glitazones	pre-clinical	54-56
<b>AMPK ACTIVATORS</b>		
AICAR	pre-clinical	46, 53, 65
$\beta$ -GPA	pre-clinical	48
metformin	clinical	66-68
resveratrol	pre-clinical	69, 70
<b>SIRT1 ACTIVATORS</b>		
resveratrol	pre-clinical	79, 81
SRT2104	pre-clinical	85, 86

Drugs currently under investigation for their use as exercise mimetics are listed. References reporting effects on target tissues different from the skeletal muscle are not included.



**Figure 1. Different classes of molecules are able to mimic the effects of aerobic/endurance exercise.** Both host cells and the tumor secrete humoral factors and produce metabolic changes which modify skeletal muscle gene expression and cause cell damage. These alterations lead to the hyperactivation of protein degradation, affect myogenesis and impair mitochondrial function, ultimately leading to muscle wasting. Exercise mimetics (green squares) act on exercise-responsive factors (AMPK, PPAR $\delta$ , PGC-1 $\alpha$ , SIRT1), improving the energy deficit by restoring mitochondrial biogenesis, oxidative capacity and ATP synthesis rates.

## Disclosure

The authors declare that there is no conflict of interest.

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Papers of special note have been highlighted as either of interest (●) or of considerable interest (●●) to readers.

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