Chapter

Leucine and Its Importance for Cell Signalling Pathways in Cancer Cachexia-Induced Muscle Wasting

Andre Gustavo Oliveira, Bread Cruz,
Sarah Christine Pereira de Oliveira, Lais Rosa Viana,
Natalia Angelo Da Silva Miyaguti,
Luiz Alberto Ferreira Ramos, Rafael Rossi Valentim
and Maria Cristina Cintra Gomes-Marcondes

Abstract

The anabolic effects of a supplemented diet with branched-chain amino acids, especially leucine, on skeletal muscle wasting and as a co-adjuvant in cancer treatment have been well-studied. Leucine is a precursor of protein synthesis and acts as a nutritional signal, affecting multiple metabolic processes (e.g., satiety, thermogenesis, energy efficiency, and body composition). Previous studies related to nutritional therapy have mainly focused on myopenia, which is the loss of skeletal muscle mass in some pathologies, including cancer. Leucine plays a role in the maintenance and even increase of lean body mass in healthy individuals as well as the prevention of disease states that culminate in myopenia. Herein, we review the available data addressing the mechanisms by which leucine acts as a cellular signal, thereby stimulating muscle protein synthesis, leading to the inhibition of muscle catabolism, especially in an experimental model of cancer cachexia. We also show differences found in the metabolomic and proteomic analyses, including the use of leucine in maternal diets as a preventative for muscle wasting as supported by our experimental data.

Keywords: leucine, cell signalling, protein metabolism, protein synthesis, protein degradation, muscle wasting, experimental cachexia models

1. Introduction

Cancer remains an enigmatic pathology for some patient types and can also cause deleterious effects, e.g., in some cases ending in a cachexia state. Cancer cachexia is a complex syndrome that results from anorexia associated with glucose intolerance, depletion of body fat, and severe wasting of lean mass, which corresponds to the more significant proportion of metabolically active tissue—the muscle tissue. In particular, the loss of skeletal mass, which is referred to as myopenia in the pathological process, is clinically relevant as this process is directly related to the loss of muscle function in cancer patients. In every type of cachexia, the pathogenesis of muscle loss is complex and multifactorial. Due to the high energy expenditure produced by neoplastic cells, the patient presents inefficiency of energy production, as well as so-called futile energy processes, which is a major cause of muscle wasting. These energy expenses include high glucose production via gluconeogenesis from lactate or gluconeogenic precursors where there is excessive consumption associated with reduced the production of ATP. Then, the cancer patient loses weight involuntarily with severe loss of muscle mass due to increased protein degradation, which produces gluconeogenic amino acids. Moreover, this process also includes lipolysis, resulting in glycerol as a glucose precursor, inducing a spoliation cycle. All these points lead the cancer patient into fatigue and asthenia, thereby leading to a worse prognosis. Recently, the number of studies on new cancer treatment therapies has increased; most of these studies focus on the patient's responses to conventional treatments and improvement of survival and quality of life.

A novel therapeutic approach to cancer involves preserving, restoring, or even an epigenetic influence to maintain an adequate nutritional status for cancer patients, thereby slowing the onset of muscle mass wasting. In this context, nutritional supplementation has been identified as a potentially useful intervention. During protein synthesis, branched-chain amino acids (BCAAs), mainly leucine, act as precursors of the carbon skeleton and nitrogen. Also, leucine can primarily be oxidised in the muscle for energy supply and contribute nitrogen for the synthesis of other amino acids. Leucine also plays an essential role in cell signalling, stimulates protein synthesis, and modulates catabolism, mainly in skeletal muscle. In an experimental cachexia model that includes a leucine-rich diet (data in print), we report an improvement in functional muscle tests (verified by CatWalk test) and the influence of maternal leucine supplementation on the offspring's adulthood responses in the improving of the muscle tissue response. Since recent works have indicated that the most important goal during cancer progression is the maintenance of lean body mass and considering the key role of leucine in modulating skeletal muscle protein synthesis and degradation, our research group has been evaluating the effects of a leucine-rich diet in an experimental model of cancer cachexia. Herein, we summarise the findings of our group as well as others that show that a leucine-rich diet can ameliorate the prognosis, reduce the risk of death, and help to maintain the quality of life in cancer patients.

2. Cancer cachexia

Cachexia is a condition characterised by reduced food intake; involuntary and progressive weight loss; and intense catabolism of carbohydrates, lipids, and proteins [1], thereby resulting in intense deterioration of host tissues, severe weight loss, and adipose tissue and muscle mass wasting [2]. Weight loss and malnutrition are the most common characteristics observed in advanced cancer patients [3]. Cachexia is responsible for almost 30% of all cancer-related deaths and associated with significantly decreased physical activity and psychological burden [3]. Cachexia is also related to other pathophysiological changes, such as systemic inflammation, insulin resistance, and oxidative stress [4]. Several pro-inflammatory cytokines (e.g., IL-6 and TNF-α) and pro-cachectic factors (e.g., factor inducing proteolysis [PIF] and lipid mobilisation factor [LMF]), which are considered mediators of muscle wasting, act during the cachectic process [5, 6]. Considering the high prevalence (50–80%) of cachexia in advanced cancer, the investigation of the molecular process of cancer cachexia is important when considering the most efficient targets of treatment. The use of nutritional interventions to minimise the side effects of cancer is a novel and promising approach [7]. As such, supplementation with BCAA, especially leucine, has been shown to improve skeletal muscle mass because leucine plays an important role in skeletal muscle metabolism, regulates protein synthesis by stimulating the mTOR pathway, and inhibits the ubiquitin-proteasome pathway [8, 9].

3. Muscle protein synthesis and leucine

Muscle mass is a controlled balance of protein turnover by the cellular processes of protein synthesis and breakdown. In some pathological conditions, such as cancer cachexia, protein synthesis could also be compromised, which results in skeletal muscle atrophy and weakness [10, 11]. The regulation of protein turnover in skeletal muscle is a complex process, usually involving interactions between gene transcription, translation, and protein degradation. Stimulation and signalling processes are initiated by principal agents of these activities, such as anabolic hormones (e.g., insulin), growth factors, glucose, and amino acids. One such signalling pathway is triggered by insulin, which initiates protein synthesis after binding to its receptor, thereby activating several downstream components. The activated insulin receptor triggers the tyrosine phosphorylation of the insulin receptor substrate (IRS) 1 and 2, followed by activation of the phosphoinositol 3 kinase (PI3-kinase). Then, PI3-kinase activates phosphoinositide kinases-dependent 1 and 2 (PDK1/2) to phosphorylate the protein kinase Akt/PKB. The activated PKB phosphorylates tuberous sclerosis 2 (TSC2) inactivates the tuberous sclerosis complex 1 and 2 (TSC1/TSC2), which are no longer able to perform GTPase activity at Rheb (brain-enriched ras-homologue), allowing Rheb to release and activate the mechanistic target of rapamycin (mTOR), the key component of this machinery [12]. Also, the Akt substrate, PRAS40, when phosphorylated by PKB, loses its inhibitory effect over mTOR. In fact, mTOR acts as a sensor and integrator of diverse inputs, such as nutrients, growth factors, and energy status. mTOR, which consists of the mTORC1 and mTORC2 complexes, is a master regulator of protein synthesis and is essential for the maintenance of muscle mass and function [13]. Upon encountering anabolic factors, such as amino acids, mTORC1 is activated and signals to ribosomal protein S6 protein kinase 1 (S6K1) and eukaryotic initiation factor 4E binding protein 1 (4EBP1)—these are the best-known downstream effectors of mTOR signalling and control the protein synthesis pathway. Activated p70S6K subsequently leads to phosphorylation of the downstream target S6K1, which results in the translation of messenger RNA (mRNA) encoding for ribosomes and transcription factors [14]. In parallel, mTOR phosphorylates the dissociation of the 4E-BP1/eIF4E complex, releasing the eukaryotic initiation factor 4E (eIF4E), which subsequently binds to eIF4G, thus forming the eIF4F translation initiation complex, and allows the recruitment of the 40S ribosomal subunit to initiate protein translation [15].

Although the impairment of muscle protein synthesis in cachexia is not an obligate feature, many studies are working in strategies to improve the muscle mass and also the patient's muscle function which could imply in better prognosis and quality of life in those patients. Thereby, leucine together with valine and isoleucine, or even alone, can stimulate protein synthesis and act as cell signalling molecules in skeletal muscle by activating the mTOR pathway [16, 17]. Multiple studies have shown that leucine alone stimulates protein synthesis, mediating the translational control of protein synthesis in skeletal muscle independently of other BCAAs [18, 19]. For example, some studies have shown that the oral administration or infusion of leucine in adult humans or animals elevates muscular protein synthesis [20]. Moreover, leucine appears to have a much more potent anabolic effect (i.e., stimulating the mTOR pathway) than anabolic hormones, such as insulin. The administration of leucine after fasting or amino acid starvation stimulates protein synthesis and promotes the phosphorylation and activation of S6K1 via the rapamycin-sensitive mTOR in skeletal muscle [21, 22]. On the other hand, several studies have emphasised the specific contribution of cell membrane transport through the coupling of the amino acid transporter system 1 (LAT1 or SLC7A5), which carries leucine in exchange for glutamine [23]. The availability of amino acids (especially leucine and glutamine) is determined by its uptake by the cell, which appears to play an essential role in the entrance of Leu into the cell and the maintenance of a high intracellular concentration of glutamine [24]. Some evidence suggests that leucine uses the insulin signalling pathway, but the exact mechanism of triggering the mTOR complex remains under debate [25]. Nutritional supplementation with leucine stimulated the incorporation of phenylalanine in muscle in an experimental cachexia model, confirming an increased protein synthesis and also an increasing muscle mass [26, 27]. Subunits of the mTORC1 complex (i.e., Raptor and GβL) and substrates belonging to the downstream pathway (i.e., 4E-BPs, eIF4A, eIF4B, eIF4E, including S6K1), which represent the key points within the metabolism of proteins through mTOR, are highly increased in the muscle of Walker-256 tumour-bearing animals subjected to leucine nutritional supplementation [27–29]. *In vitro* cell culture studies have generated evidence relevant to the mechanism through which leucine affects mTOR [30, 31]. As such, leucine supplementation can stimulate protein synthesis and, consequently, might lead to a positive protein net balance, even within a high rate of protein degradation. In addition to increasing the protein synthesis in skeletal muscle, a leucine-rich diet has a protective effect in other tissues. In our previous works, we also observed improvement of protein synthesis in placenta tissue since leucine acted by improving the cell-signalling activity, thereby increasing placental protein synthesis and also reducing the placental proteolytic process [32, 33].

Interestingly, our previous works and other experimental studies have shown that leucine supplementation can work as an excellent nutritional strategy to treat or prevent muscle wasting in cancer cachexia. In this way, leucine also emerged as a potent stimulator of metabolism, leading to improvements in both oxidative metabolism and mitochondrial biogenesis [34]. Recently, our research group used metabolomic and proteomic analyses in an experimental cachexia model to better understand the benefits of leucine supplementation. Compared to a non-supplemented group, tumour-bearing rats under leucine supplementation showed metabolic pathways diverted to ketone bodies and butyrate metabolism [35]. Since an excess of leucine might provide ketone precursors being utilised by muscle tissue as energy sources, this likely diverted the metabolism to improve muscle protein synthesis [35]. The ketone bodies could provide additional energy to skeletal muscle and host tissues; this energy source is not available to the non-leucine-supplemented group. Besides acting as a fuel source to supply energy for the cellular activity of several tissues, ketone bodies, especially acetoacetate, can also promote muscle cell proliferation [36], probably accounting for the benefits of leucine nutritional supplementation [35]. We also made important findings as part of our proteomic analysis of the muscle tissue of tumour-bearing rats fed a leucine-rich diet (data in print). These results show a significant action of leucine on modulation of the mitochondrial membrane proteins involving the production of ATP, such as the ATP synthase complex family. Proteins associated with ATP synthase (e.g., F1F0 or Complex V) participate in the synthesis of ATP from ADP in the presence of a proton gradient across the mitochondrial membrane. One protein from this family that stood out in our studies is the ATP5a1 synthase subunit alpha. The tumour-bearing group showed a higher concentration of ATP5a1, which indicates a higher mitochondrial activity for the production of ATP, which is associated with a greater availability of glucose from the gluconeogenesis process. In contrast, the leucine tumour-bearing group showed lower muscle ATP5a1 content, likely indicating that the production of ATP must be derived from other metabolic processes. Therefore, the presence of tumour factors interferes in the cellular processes involved with obtaining energy. Since cell proliferation depends on the constant use of ATP for the duplication of all cellular machinery, the interference of tumour factors in the muscle mitochondria and the electron transport chain leads to less availability to energy for muscle cell activity. With leucine supplementation, a stimulating pathway occurs to obtain energy, thus contributing to the maintenance of adequate ATP supply and the ability maintain muscle activities. Since leucine is a ketogenic amino acid, its entire carbon skeleton is converted to keto acid or acetyl-CoA, which can be directed to participate in the Krebs cycle and beta-oxidation processes, both of which produce ATP as the final product. According to our results, the metabolic pathways in cachectic-tumour-bearing animals are related to ammonia recycling and the urea cycle, likely associated with protein degradation and directly associated with the futile cycle of energy production. In parallel, the metabolic activity of the leucine-tumour-bearing group was affected, which was related to ketone body and butyrate metabolism [35]. These points confirm the relationship to the increase in the muscle tissue's energy needs in tumour-bearing animals, which are minimised/modulated when the animal's diet is supplemented with leucine. Our proteomic results show that, in muscle tissue, mitochondria dysfunction occurs in the tumour-bearing host; however, under leucine supplementation, there are muscle mitochondrial biogenesis and activities improvements (data not published). Thus, we know that both insulin and leucine can independently affect the activation of mRNA translation and, consequently, the protein synthesis process [17, 37]. However, the real effect of amino acid signalling, especially leucine, on protein synthesis via the mTOR pathway remains complex and less understood, and there is a need for further studies, especially *in vivo* models. Moreover, as mentioned previously, our data for the CatWalk analysis showed an improvement in muscular functional activity; i.e., when rats with tumours were fed a leucine-rich diet, muscle function improved (data in print). Moreover, a leucine-supplemented maternal diet can influence and ameliorate the adult host response in tumour-bearing rats. In this way, an improved understanding of muscle protein synthesis and how leucine influences it is essential when developing new targets and strategies to restore muscle in muscle wasting diseases.

4. Muscle protein degradation and leucine

4.1 Skeletal muscle wasting in cachexia

Muscle homeostasis is important because muscle makes up a large part of the whole organism and performs many functions and activities. Moreover, as one of the main structures of the body, it is the most significant source of the protein turnover process. As noted above, muscle maintenance occurs by the intense activity of both protein synthesis and degradation [38]. Accordingly, proteolytic systems also play a key role in the regulation of cellular homeostasis and cell recycling, differentiation, cell cycle, abnormal protein degradation, and amino acid supply for gluconeogenesis [39]. Myofibrillar protein degradation is performed by the following four different pathways: ubiquitin proteasome system (UPS), autophagy, Ca^{2+}-dependent proteolysis, and caspase pathway [40]. The increase of protein catabolism in skeletal muscle contributes to a worse prognosis in cancer patients, especially those in a cachexia state, which is one of the most important causes of morbidity and mortality in these patients [41]. In cancer patients, the loss of either skeletal or cardiac muscle mass might lead to cardiac and respiratory failure, in addition to the fact that it decreases the host's response after conventional treatments, such as chemotherapy or radiotherapy [42]. Moreover, it is well-established that the ubiquitin-proteasome system is a very important pathway in skeletal muscle degradation during cancer cachexia [43]. Furthermore, multiple studies have identified released factors that contribute to an increase in muscle protein degradation during cancer. The main factors that lead to protein degradation during cancer cachexia syndrome produced by the host are tumour necrosis factor alpha (TNF-α), interleukin-6 (IL-6), interleukin-1 (IL-1), interferon gamma (IFN-γ) [44]. Meanwhile, the main factors produced by cancer cells are proteolysis-inducing factor (PIF), lipid-mobilising factor (LMF), and anaemia-inducing factor (AIS) [44].

Among the factors produced by a tumour, the proteolysis-inducing factor (PIF) has central importance. This protein, first described by Todorov et al. [6], is a 24 kDa glycoprotein isolated from the adenocarcinoma MAC16 tissue, an experimental model of cancer cachexia. Similarly, some studies found a PIF like those that were also verified in other cachexia models, such as in Walker-256 carcinosarcoma [31], in patients with gastrointestinal [45], pancreatic [46], and other types of cancer [47]. The injection of PIF in mice induces an intense loss of lean body mass, similar to that associated with MAC16 tumour growth [6]. After being synthesised and released by tumour cells, PIF reaches the bloodstream and binds to its cell membrane receptor in muscle cells [48], leading to activation of the ubiquitin-proteasome pathway and a decrease in protein synthesis by stimulating the double-stranded RNA-dependent protein kinase (PKR) [49]. The activated PKR leads to phosphorylation of eukaryotic translation initiation factor (eIF2α) and, consequently, inhibition of protein synthesis [50]. Many studies have shown that, unlike starvation, a decrease in food intake is not sufficient to cause muscle mass wasting in cancer patients, such as that which occurs during cancer cachexia [51].

As mentioned above, despite the fact that leucine stimulates protein synthesis, leucine and its metabolite β-hydroxy-β-methylbutyrate (HMB) can also decrease the rate of protein degradation apparently by reducing the expression of proteins from the ubiquitin-proteasome system [52, 53] and the other proteolytic pathways, i.e., mainly autophagy [54]. This characteristic makes leucine a great tool in cancer-induced cachexia therapy. In fact, leucine or HMB, i.e., alone [55] or in combination with other nutrients [56], can prevent the decrease of lean mass in cancer patients; this has been verified by our group in an experimental cachexia model [32, 35].

4.1.1 Proteolytic pathways and leucine

As noted above, the UPS is responsible for degrading proteins and might be responsible for up to 80% of proteolysis during skeletal muscle wasting [57, 58]. Since the UPS depends on linking the target protein to a ubiquitin tag and subsequent recognition and degradation by the proteasome core, leucine cell signalling can affect multiple steps. Ubiquitin conjugation to target proteins involves the action of a ubiquitin-activating enzyme (E1), which uses ATP to form thioester ubiquitin; conjugating to the ubiquitin-conjugating protein family of enzymes (E2), which in concert with ubiquitin protein ligase (E3), mediates the binding of the ubiquitin C terminal end to the targeted protein. The specificity of the substrate recognition is mainly dependent on E3 interaction with the targeted protein, giving relevance to this class of enzymes in studies of muscle atrophy affected by tumour evolution [59]. Our previous studies have shown that leucine supplementation can minimise the E2 activity in the muscle of Walker-256 tumour-bearing rats, suggesting a beneficial effect of this cell signal (data in print). Although approximately 1000 members of the E3 ligase family have been described, MuRF1 and

Atrogin have been reported to be specifically expressed and increased in skeletal muscle under many catabolic conditions [60, 61]. Interestingly, acute or chronical leucine supplementation prevented the upregulation of proteasomal proteolysis in fasted aged rats as compared to younger adult controls [62]. We have recently verified that ageing causes additional proteasomal activity in an experimental model of cancer-induced cachexia (unpublished data). Thus, leucine supplementation might be a valuable tool to counteract higher susceptibility to cachexia in senescence. Also, MuRF1, atrogin, and other E3 ligases, such as MUSA1 and SMART1, have been associated with enhanced proteolysis during muscle wasting [63]. Moreover, the degradation of ubiquitin-tagged proteins occurs in proteasome 26S, formed by regulatory (19S) and catalytic (20S) subunits. Interaction of the 19S subunit with ubiquitin drives the target protein to the core of the proteasome, a cylindrical protein complex formed by two external alpha rings (alpha 1–7) and two central beta rings (beta 1–7), in which beta 1, 2, and 5 present caspase-like, trypsin, and chymotrypsin protease activity, respectively [64]. Proteasome degradation results in 7–9 amino acid peptides, which are subsequently degraded by cytosolic proteases. During muscle wasting, the activity of chymotrypsin is increased, as is the expression of 19S, 11S, and 20S, all of which are modulated by the nutritional supplementation of leucine [27, 65, 66].

In addition to UPS in muscle wasting, autophagy is a degradation process led by lysosomes, and it manages the catabolism of long-life proteins, defective organelles, and protein aggregates. Three different autophagy pathways have been described, i.e., microautophagy, chaperone driven autophagy, and macroautophagy (herein referred as autophagy)—extensively reported as a key regulator of muscle mass. Autophagy involves complex protein machinery, including ATGs (autophagy-related genes); ultimately, autophagy leads to the formation of phagophores, i.e., the formation of autophagosomes by the elongation of the lipid membrane, which is followed by a fusion of the autophagosome to the lysosome, generating the autolysosome with many hydrolases and proteases (i.e., cathepsins). The first step of autophagy is the activation of ULK1, which, in turn, phosphorylates Beclin1, promoting its interaction with VPS34, VPS15, and ATG14. This complex activates VPS34, assembling the phagophore rich in PI3K class III enzyme to form PI3P, a signal to recruit other ATGs. The ubiquitin-like ATG5 brings together the final complex, i.e., ATG5/ATG12/ATG16, initiating and expanding the membrane extension of the phagophore. In parallel, the conjugated form of ATG8, also a homologue to LC3 in muscle, is tightly bound to the autophagosome membrane and later cleaved by ATG4, thereby converting LC3I to LC3II, which is necessary for the fusion of the autophagosome with the endocytic compartments, thus forming the lysosome. Inside the autolysosome, cargoes are degraded with cathepsins L and B being especially important for the degradation of myofibrils proteins [67]. Interestingly, the treatment of C2C12 myotubes with PIF-like increased the cathepsin B and chymotrypsin-like activity. The previous exposition of leucine PIF-like-treated myotubes prevented not only cathepsins and chymotrypsin enzymes activity but also proteasome activity [31], thereby highlighting another role for leucine in cancer-cachexia reversal. Indeed, the inhibition of cathepsin activity has been suggested as a useful approach to treat cancer cachexia [68].

Calcium-dependent proteolysis is composed by cysteine-proteases, which are dominated by calpains and the endogenous inhibitor calpastatin [69]. Among the 14 calpains described, striated muscle contains considerable amounts of μ-calpain, m-calpain and calpain 3, which are activated by the intracellular concentration of calcium [70]. Above a certain threshold, Ca^{2+} intracellular levels interact with the C-terminal domain of the calpain large subunit, thereby promoting N-terminal autocleavage and leading to maximal protease activity. Therefore, there is a correlation between calpain activity and protein turnover in muscle, thereby suggesting an important role in muscle mass maintenance and partially accounting for muscle wasting in some pathologies [71]. Cancer patients and tumour-bearing rats present higher calcium-dependent proteolysis, which is linked with increased calpain and decreased calpastatin protein content [72, 73]. Moreover, there is evidence that calpains, mainly μ-calpain, is localised to the Z-disk in the sarcomere, which is anchored by myofibrils such as p35, nebulin, troponin-T, α-activin, and desmine. Thus, Ca^{2+} dependent proteolysis seems essential to the initial disaggregation of the sarcomere structure, thereby releasing contractile proteins for further degradation by other proteolytic systems. Similarly, leucine supplementation improves muscle mass, thereby minimising the muscle wasting by inhibiting the calpain activity.

Other points of the proteolysis processes that should be mentioned include those related to other intracellular pathways that govern cancer cachexia. For example, AKT phosphorylation causes FoxOs inactivation and translocation from the nucleus to the cytosol [74]. FoxOs (Foxo1, 3 and 4) are transcription factors that regulate energy metabolism, the cell cycle, antioxidant defence, cell death, and longevity [75]. Repression of FoxOs by AKT is a key step in the anticatabolic action of insulin/IGF1 signalling [76]. Therefore, genetic ablation of FoxOs specifically in skeletal muscle reverses muscle atrophy caused by starvation and denervation, indicating that FoxOs are necessary to the expression of several atrophic genes, such as atrogin1, MuRF1, proteasome subunits, and lysosomal enzymes [75]. FoxO directly upregulated many proteasome subunits and E3 ligases in cancer-induced muscle wasting. Interestingly, FoxOs also increased autophagy in tumour-bearing mice [77], inducing the expression of such genes as Cathepsin-L and other genes related to the lysosomal/autophagy pathway (e.g., Gabarapl1 and Bnip3). Thus, FoxOs seem to mediate crosstalk between proteasomal and autophagy-dependent proteolysis in cancer-induced cachexia since the inhibition of FoxO3 or FoxO1 by RNA interference entirely prevents muscle loss [78]. Also, pro-inflammatory cytokines contribute to mass muscle decline under several conditions [79]. TNFα increases muscle protein degradation by activating the transcription factor NFκB [38]. The blockade of NFκB signalling in tumour-bearing mice partially attenuated cancer-induced muscle loss, thereby enhancing longevity [80]. Activation of NFκB enhanced atrophy by the transcription of MuRF1, ubiquitin, UbcH2 (E2), proteasome subunits, and autophagy-related genes [81]. Likewise, higher levels of myostatin and activin-A (i.e., members of the TGF-B family, share the receptor ACTRIIβ, and are known to regulate muscle mass) are related to muscle atrophy [82, 83]. Moreover, the inhibition of the bioactivity of activin-A and myostatin by inhibin and follistatin prevents muscle loss independent of tumour growth [84]. Additionally, several human tumour cell lines secrete considerable amounts of myostatin and activin-A [85], which are correlated with muscle strength loss [86]. Moreover, myostatin acting due to ACTRIIβ downstream effectors Smad2/3 activity also enhances skeletal muscle loss by phosphorylating the Smad2/3 and transcription of MuRF1, atrogin1, and autophagy induction [87], which corroborates our data, thereby highlighting the modulatory effect of leucine supplementation in C2C12 cells treated with PIF-like. Interestingly, myostatin effects might depend on the suppression of PI3K/Akt signalling [88], where we also find some beneficial effects of leucine supplementation, such as restoring the inhibitory effect of Akt and minimising proteolysis in PIF-like-treated C2C12 cells.

5. Myocardial muscle in cachexia and leucine

Recently, the number of studies addressing cancer and cardiac failure has increased. This is because cancer has significant effects on skeletal muscle, causing a catabolic state and resulting in widespread and progressive atrophy, including myocardial tissue. Studies have shown that cardiac atrophy can be a result of cancer evolution and its treatments [89–91]. These damages result in symptoms that can include breathlessness, lethargy, reduced exercise tolerance, congestive cardiac failure, and mortality [92]. Because the alterations in cardiac muscle structure and metabolism induced by cancer cachexia are poorly understood, cardiologists and oncologists are working together to explore models of care to improve outcomes. Some findings show that pancreatic, lung, and colorectal cancer patients have a reduced heart mass with a reduced left ventricular (LV) and wall thickness and are associated with smaller heart cell size and numbers and increased extracellular stroma surrounding the myocytes [92]. Indeed, this cardiac atrophy is part of a complex systemic metabolic syndrome caused by cancer damage, resulting in severe muscle wasting, including of the myocardium. Rodent models of cancer cachexia also show characteristics of cardiac atrophy, including decreased heart weight and LV mass; the thinning of septal, interventricular, and posterior walls; and chamber dilation as demonstrated by echocardiography [91, 93]. Cardiac atrophy in cancer cachexia is likely driven by cellular atrophy, including the activation of UPS [93] and the imbalance of protein turnover [91]. Cancer cachectic mice presented decreased cardiac contractile function and heart rate with concomitantly increased heart tissue fibrosis, which was associated with higher pro-inflammatory cytokine content and enhanced oxidative stress [94, 95]. Therefore, the use of an experimental cancer cachexia model allows us to evaluate how leucine supplementation counteracts cachexia damage in the heart. Recently, we reported relevant data related to the benefits of leucine supplementation for reverting/maintaining cardiac mass for both tumour-bearing rats fed a leucine-rich diet [91] and adult offspring whose mothers had been fed a leucine-rich diet (data not published). More interestingly, we observed improvement in enzyme activities related to the heart function via electrocardiography as a positive effect of leucine in tumour-bearing rats [91]. We know that leucine stimulates protein synthesis through activation of the mTOR pathway, thereby stimulating the intracellular signalling pathways that modulate cellular metabolism and apoptosis; this supports our data since the activation of mTOR is also essential for mediating physiologic cardiac hypertrophy and preventing cardiac dysfunction in the face of pressure overload [91, 96], thus supporting the cardioprotective effects of leucine over the cancer-cachexia-induced cardiac damages [91].

6. Leucine maternal diet influence over muscle wasting

Since the number of new cases of cancer is increasing every year, and most of these are attributed to environmental factors and lifestyle, prevention is a major target of cancer studies [97, 98]. In addition to maintaining a balanced diet throughout life, the influence of maternal diet on offspring's adulthood has been widely studied [99].

Among environmental factors, nutritional composition is the main factor in the modulation of gene expression, especially those related to metabolic pathways. The periods of gestation and lactation are considered crucial because the maternal diet exerts influence on the development and the plasticity of organs and tissues of the foetus/newborn [100]. The energy composition of foods, fatty acid composition, proteins, and micronutrients can modify several aspects of metabolism. Poor or imbalanced maternal diet, e.g., undernutrition, might contribute to a change in the metabolic programming of the offspring [101, 102], thereby increasing the risk of metabolic diseases (e.g., insulin resistance, obesity, type II diabetes), cardiovascular disorders (e.g., hypertension and atherosclerosis), hormonal imbalance, and even cancer incidence in the offspring [103]. Thus, some amount of prevention may be achieved through a balanced maternal diet, considering not only the proper nutrition but the nutritional scheme; this can be viewed as a long-term investment that benefits both the current generation and its descendants, i.e., one that can minimise the risk of diseases (e.g., cancer) in the mother and her adult children [104, 105]. Thereby, due to foetal and lifetime nutrition there are epigenetic modifications [101], which are stable heritable patterns of gene expression in the DNA and histone proteins [106], and may result in DNA methylation, histone modifications, and RNA interference. Global hypomethylation, global miRNA downregulation, specific promoter hypermethylation, histone deacetylation, and upregulation of epigenetic machinery have been reported in cancer [107, 108], which are related to epigenetic silencing of detoxifying enzymes, suppressor tumour genes, cellular cycle regulators, apoptosis inducers, and DNA repair genes [109].

Knowing the benefits of leucine for attenuating the cachectic state and preventive interventions [110–112], previous studies using animal models of cachexia indicate that maternal nutrition affects the development of cancer cachexia and its effects in offspring adulthood [113, 114]. In our previous work, a maternal diet supplemented with leucine had a positive impact on the adult offspring's ability to respond to a Walker-256 tumour, diminishing the cachexia index, modulating markers of hepatic damage functions, and increasing the antioxidant response of the liver [112]. In this same experimental procedure concerning muscle wasting, our unpublished data show that maternal leucine supplementation can minimise the cachectic index by preserving the skeletal muscle mass in adult offspring. These results are confirmed by the stimulatory effect on the expression of mTOR pathway proteins. We observed a significant activation of mTOR and p70S6K, which indicates the preservation of protein synthesis and a decrease in proteolysis (i.e., we also verified less tyrosine release in the perfusion procedure) in the gastrocnemius muscle of these adulthood Walker-256 tumour-bearing rats subjected to a leucine enriched maternal diet (data in print). In fact, these findings indicate that leucine supplementation can modulate the mTOR pathway, resulting in the preservation of protein synthesis (data in print) and protection against the damaging effects of the Walker-256 tumour. Thus, maternal leucine supplementation shows promise in terms of improving the response to cachexia, i.e., preventing muscle loss, and further studies are needed to better understand the epigenetic mechanisms involved in this modulation and how the parental influence can counteract the damages caused by cachexia.

7. Conclusion

In summary, in **Figure 1**, we present evidence demonstrating the key role of leucine in improving skeletal muscle protein synthesis and minimising muscle degradation; we also report some metabolomic and proteomic findings, which are ameliorated by a diet supplemented with leucine. Also, these data show the benefits of leucine supplementation in cases of cardiac cachexia and the potential that a leucine supplemented maternal diet has for improvement of the host response to cancer-cachexia-induced muscle damage. As found in our studies and reported by other research groups, leucine is a suitable co-adjuvant treatment in an experimental model of cancer cachexia. However, more translation human studies are needed to determine whether leucine supplementation is capable of modulating muscle mass in cancer cachexia patients.

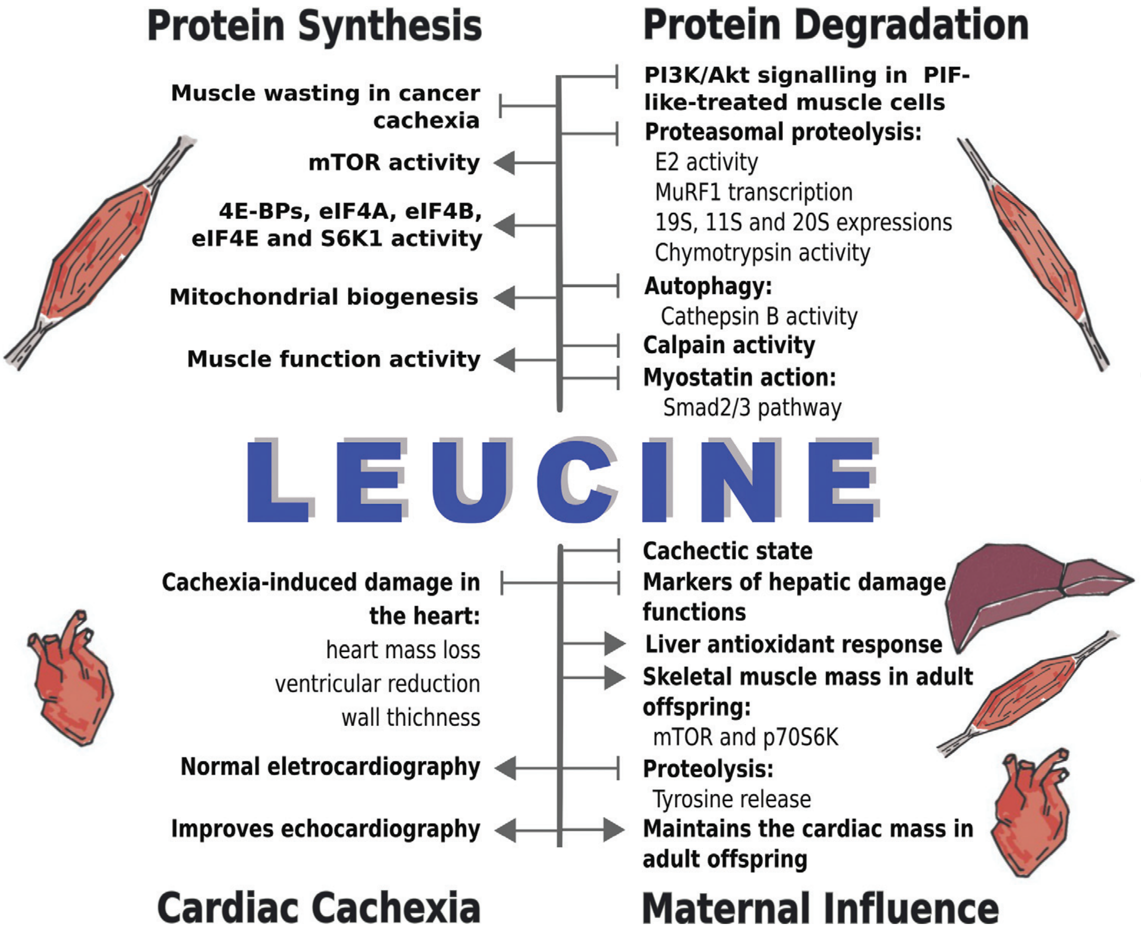

Figure 1.
Summary of the effects of leucine (and maternal diet) on protein synthesis, degradation, and cardiac cachexia. The arrows indicate the principal impact of leucine over the process, and the bar-headed lines show the inhibitory effect of leucine. All cited processes mentioned in the main text are summarised in these four blocks.

Acknowledgements

The authors are thankful for the financial support of Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq #302524/2016-9), and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP #2014/13334-7; #2015/06766-0; #2015/21890-0; #2017/02739-4; #2017/10809-2; #2017/23065-1). The authors gratefully thank Biol. R.W. Santos for technical support and A.C.G. Marcondes for the graphic art of tissue and organs. The main text has been edited by native speakers from American Manuscript Editors (Editing Certificate # 241-061-990-754-255).

Conflict of interest

The authors declare that there are no conflicts of interest.

Author details

Andre Gustavo Oliveira†, Bread Cruz†, Sarah Christine Pereira de Oliveira†,
Lais Rosa Viana†, Natalia Angelo Da Silva Miyaguti†, Luiz Alberto Ferreira Ramos†,
Rafael Rossi Valentim† and Maria Cristina Cintra Gomes-Marcondes*
Department of Structural and Functional Biology, Laboratory of Nutrition and Cancer, Institute of Biology, University of Campinas (UNICAMP), Campinas, Sao Paulo, Brazil

*Address all correspondence to: cintgoma@unicamp.br

† All authors are considered the first author, as all contributed equally to write, research, and develop data from experimental procedures for this work.

References

[1] Tisdale MJ. Are tumoral factors responsible for host tissue wasting in cancer cachexia? Future Oncology. 2010;**6**:503-513. DOI: 10.2217/fon.10.20

[2] Johns N, Stephens NA, Fearon KC. Muscle wasting in cancer. The International Journal of Biochemistry & Cell Biology. 2013;**45**:2215-2229. DOI: 10.1016/j.biocel.2013.05.032

[3] Skipworth RJE, Stewart GD, Dejong CHC, Preston T, Fearon KCH. Pathophysiology of cancer cachexia: Much more than host-tumour interaction? Clinical Nutrition. 2007;**26**: 667-676. DOI: 10.1016/j.clnu.2007.03.011

[4] Argilés JM, López-Soriano FJ, Busquets S, Argiles JM, Lopez-Soriano FJ, Busquets S. Mechanisms and treatment of cancer cachexia. Nutrition, Metabolism, and Cardiovascular Diseases. 2013;**23**(Suppl 1):S19-S24. DOI: 10.1016/j.numecd.2012.04.011

[5] Deans DAC, Wigmore SJ, Gilmour H, Paterson-Brown S, Ross JA, Fearon KCH. Elevated tumour interleukin-1beta is associated with systemic inflammation: A marker of reduced survival in gastro-oesophageal cancer. British Journal of Cancer. 2006; **95**:1568-1575. DOI: 10.1038/sj.bjc.6603446

[6] Todorov P, Cariuk P, McDevitt T, Coles B, Fearon K, Tisdale M. Characterization of a cancer cachectic factor. Nature. 1996;**379**:739-742. DOI: 10.1038/379739a0

[7] Maschke J, Kruk U, Kastrati K, Kleeberg J, Buchholz D, Erickson N, et al. Nutritional care of cancer patients: A survey on patients' needs and medical care in reality. International Journal of Clinical Oncology. 2017;**22**:200-206. DOI: 10.1007/s10147-016-1025-6

[8] Columbus DA, Fiorotto ML, Davis TA. Leucine is a major regulator of muscle protein synthesis in neonates. Amino Acids. 2015;**47**:259-270. DOI: 10.1007/s00726-014-1866-0

[9] Moro T, Ebert SM, Adams CM, Rasmussen BB. Amino acid sensing in skeletal muscle. Trends in Endocrinology and Metabolism. 2016; **27**:796-806. DOI: 10.1016/j.tem.2016.06.010

[10] Cohen S, Nathan JA, Goldberg AL. Muscle wasting in disease: Molecular mechanisms and promising therapies. Nature Reviews. Drug Discovery. 2015; **14**:58-74. DOI: 10.1038/nrd4467

[11] Argilés JM, Busquets S, Stemmler B, López-Soriano FJ. Cancer cachexia: Understanding the molecular basis. Nature Reviews. Cancer. 2014;**14**: 754-762. DOI: 10.1038/nrc3829

[12] Wang XJ, Yang X, Wang RX, Jiao HC, Zhao JP, Song ZG, et al. Leucine alleviates dexamethasone-induced suppression of muscle protein synthesis via synergy involvement of mTOR and AMPK pathways. Bioscience Reports. 2016;**36**(3):1-11. DOI: 10.1042/BSR20160096

[13] Mahoney SJ, Narayan S, Molz L, Berstler LA, Kang SA, Vlasuk GP, et al. A small molecule inhibitor of Rheb selectively targets mTORC1 signaling. Nature Communications. 2018;**9**(1): 548-559. DOI: 10.1038/s41467-018-03035-z

[14] Mieulet V, Roceri M, Espeillac C, Sotiropoulos A, Ohanna M, Oorschot V, et al. S6 kinase inactivation impairs growth and translational target phosphorylation in muscle cells maintaining proper regulation of protein turnover. American Journal of Physiology. 2007;**293**:C712-C722. DOI: 10.1152/ajpcell.00499.2006

[15] Gran P, Cameron-Smith D. The actions of exogenous leucine on mTOR signalling and amino acid transporters in human myotubes. BMC Physiology. 2011;**11**:10-19. DOI: 10.1186/1472-6793-11-10

[16] Hang YZ, Obayashi HK, Awatari KM, Ato JS, Ajotto GB, Itaura YK, et al. Effects of branched-chain amino acid supplementation on plasma concentrations of free amino acids, insulin, and energy substrates in young men. Journal of Nutritional Science and Vitaminology. 2011;**57**: 114-117

[17] Kimball SR, Jefferson LS. New functions for amino acids: Effects on gene transcription and translation. The American Journal of Clinical Nutrition. 2006;**83**:500S-507S

[18] Hernandez-García AD, Columbus DA, Manjarín R, Nguyen HV, Suryawan A, Orellana RA, et al. Leucine supplementation stimulates protein synthesis and reduces degradation signal activation in muscle of newborn pigs during acute endotoxemia. American Journal of Physiology. Endocrinology and Metabolism. 2016; **311**:E791-E801. DOI: 10.1152/ajpendo.00217.2016

[19] Kimball SR, Jefferson LS. Branched-chain amino acids: Metabolism, physiological function, and application signaling pathways and molecular mechanisms through which branched-chain amino acids mediate translational control of protein synthesis. The Journal of Nutrition. 2006;**136**:227-231

[20] Kitsy A, Carney S, Vivar JC, Knight MS, Pointer MA, Gwathmey JK, et al. Effects of leucine supplementation and serum withdrawal on branched-chain amino acid pathway gene and protein expression in mouse adipocytes. PLoS One. 2014;**9**:e102615. DOI: 10.1371/journal.pone.0102615

[21] Kimball SR, Shantz LM, Horetsky RL, Jefferson LS. Leucine regulates translation of specific mRNAs in L6 myoblasts through mTOR-mediated changes in availability of eIF4E and phosphorylation of ribosomal protein S6. The Journal of Biological Chemistry. 1999. DOI: 10.1074/jbc.274.17.11647

[22] Anthony JC, Yoshizawa F, Anthony TG, Vary TC, Jefferson LS, Kimball SR. Leucine stimulates translation initiation in skeletal muscle of postabsorptive rats via a rapamycin-sensitive pathway. The Journal of Nutrition. 2000;**130**:2413-2419

[23] Nicklin P, Bergman P, Zhang B, Triantafellow E, Wang H, Nyfeler B, et al. Bidirectional transport of amino acids regulates mTOR and autophagy. Cell. 2009;**136**:521-534. DOI: 10.1016/j.cell.2008.11.044

[24] Dodd KM, Tee AR. Leucine and mTORC1: A complex relationship. The American Journal of Clinical Nutrition. 2004;**79**:418-423. DOI: 10.1152/ajpendo.00525.2011

[25] Shimobayashi M, Hall MN. Making new contacts: The mTOR network in metabolism and signalling crosstalk. Nature Reviews. Molecular Cell Biology. 2014;**15**:155-162. DOI: 10.1038/nrm3757

[26] Ventrucci G, Mello MAR, Gomes-Marcondes MCC. Proteasome activity is altered in skeletal muscle tissue of tumour-bearing rats fed a leucine-rich diet. Endocrine-Related Cancer. 2004; **11**(4):887-895. DOI: 10.1677/erc.1.00828

[27] Cruz B, Oliveira A, Gomes-Marcondes MCC. L-leucine dietary supplementation modulates muscle protein degradation and increases pro-inflammatory cytokines in tumour-bearing rats. Cytokine. 2017; **96**:253-260. DOI: 10.1016/j.cyto.2017.04.019

[28] Soliman GA. The mammalian target of rapamycin signaling network and gene regulation. Current Opinion in Lipidology. 2005;**16**:317-323

[29] Ventrucci G, Mello MAR, Gomes-Marcondes MCC. Leucine-rich diet alters the eukaryotic translation initiation factors expression in skeletal muscle of tumour-bearing rats. BMC Cancer. 2007;**7**:42. DOI: 10.1186/1471-2407-7-42

[30] Gonçalves EM, Gomes-Marcondes MCC. Leucine affects the fibroblastic Vero cells stimulating the cell proliferation and modulating the proteolysis process. Amino Acids. 2010;**38**:145-153. DOI: 10.1007/s00726-008-0222-7

[31] Gonçalves EM, Salomão EM, Gomes-Marcondes MCC. Leucine modulates the effect of Walker factor, a proteolysis-inducing factor-like protein from Walker tumours, on gene expression and cellular activity in C2C12 myotubes. Cytokine. 2013;**64**:343-350. DOI: 10.1016/j.cyto.2013.05.018

[32] Viana LR, Gomes-Marcondes MCC. A leucine-rich diet modulates the tumor-induced down-regulation of the MAPK/ERK and PI3K/Akt/mTOR signaling pathways and maintains the expression of the ubiquitin-proteasome pathway in the placental tissue of NMRI mice. Biology of Reproduction. 2015;**92**(2):49-56. DOI: 10.1095/biolreprod.114.123307

[33] Cruz BLG, da Silva PC, Tomasin R, Oliveira AG, Viana LR, Salomao EM, et al. Dietary leucine supplementation minimises tumour-induced damage in placental tissues of pregnant, tumour-bearing rats. BMC Cancer. 2016;**16**:58. DOI: 10.1186/s12885-016-2103-x

[34] Vaughan RA, Garcia-Smith R, Gannon NP, Bisoffi M, Trujillo KA, Conn CA. Leucine treatment enhances oxidative capacity through complete carbohydrate oxidation and increased mitochondrial density in skeletal muscle cells. Amino Acids. 2013;**45**:901-911. DOI: 10.1007/s00726-013-1538-5

[35] Viana LR, Canevarolo R, Luiz ACP, Soares RF, Lubaczeuski C, Zeri AC de M, et al. Leucine-rich diet alters the ^{1}H-NMR based metabolomic profile without changing the Walker-256 tumour mass in rats. BMC Cancer. 2016;**16**:764. DOI: 10.1186/s12885-016-2811-2

[36] Zou X, Meng J, Li L, Han W, Li C, Zhong R, et al. Acetoacetate accelerates muscle regeneration and ameliorates muscular dystrophy in mice. The Journal of Biological Chemistry. 2016;**291**(5):2181-2195. DOI: 10.1074/jbc.M115.676510

[37] Proud CG. Role of mTOR signalling in the control of translation initiation and elongation by nutrients. Current Topics in Microbiology and Immunology. 2004;**279**:215-244

[38] Porporato PE. Understanding cachexia as a cancer metabolism syndrome. Oncogene. 2016;**5**:e200. DOI: 10.1038/oncsis.2016.3

[39] Koepp DM. Cell cycle regulation by protein degradation. Methods in Molecular Biology. 2014;**1170**:61-73. DOI: 10.1007/978-1-4939-0888-2_4

[40] Sandri M. Protein breakdown in muscle wasting: Role of autophagy-lysosome and ubiquitin-proteasome. The International Journal of Biochemistry & Cell Biology. 2013;**45**:2121-2129. DOI: 10.1016/j.biocel.2013.04.023

[41] Del Fabbro E. Current and future care of patients with the cancer anorexia-cachexia syndrome. American Society of Clinical Oncology Educational Book. American Society of Clinical Oncology Meeting. 2015;**35**:e229-e237. DOI: 10.14694/EdBook_AM.2015.35.e229

[42] Aversa Z, Costelli P, Muscaritoli M. Cancer-induced muscle wasting: Latest findings in prevention and treatment. Therapeutic Advances in Medical Oncology. 2017;**9**:369-382. DOI: 10.1177/1758834017698643

[43] Khal J, Hine AV, Fearon KC, Dejong CH, Tisdale MJ. Increased expression of proteasome subunits in skeletal muscle of cancer patients with weight loss. The International Journal of Biochemistry & Cell Biology. 2005;**37**: 2196-2206. DOI: 10.1016/j.biocel.2004.10.017

[44] Argilés JM, Busquets S, Rodrigo Moore-Carrasco FJL-S. The role of cytokines in cancer cachexia. In: Mantovani G, AS D, Akio I, MJ E, Rossi FF, Daniele S, et al., editors. Cachexia Wasting A Mod. Approach. Milano: Springer; 2006. pp. 467-475. DOI: 10.1007/978-88-470-0552-5_43

[45] Cabal-Manzano R, Bhargava P, Torres-Duarte A, Marshall J, Bhargava P, Wainer IW. Proteolysis-inducing factor is expressed in tumours of patients with gastrointestinal cancers and correlates with weight loss. British Journal of Cancer. 2001;**84**:1599-1601. DOI: 10.1054/bjoc.2001.1830

[46] Wigmore SJ, Todorov PT, Barber MD, Ross JA, Tisdale MJ, Fearon KCH. Characteristics of patients with pancreatic cancer expressing a novel cancer cachectic factor. The British Journal of Surgery. 2000;**87**: 53-58. DOI: 10.1046/j.1365-2168.2000.01317.x

[47] Williams ML, Torres-Duarte A, Brant LJ, Bhargava P, Marshall J, Wainer IW. The relationship between a urinary cachectic factor and weight loss in advanced cancer patients. Cancer Investigation. 2004;**22**:866-870

[48] Todorov PT, Wyke SM, Tisdale MJ. Identification and characterization of a membrane receptor for proteolysis-inducing factor on skeletal muscle. Cancer Research. 2007;**67**:11419-11427. DOI: 10.1158/0008-5472.CAN-07-2602

[49] Eley HLL, Russell STT, Tisdale MJJ. Effect of branched-chain amino acids on muscle atrophy in cancer cachexia. The Biochemical Journal. 2007;**407**:113-120. DOI: 10.1042/BJ20070651

[50] Eley HL, Russell ST, Tisdale MJ. Mechanism of activation of dsRNA-dependent protein kinase (PKR) in muscle atrophy. Cellular Signalling. 2010;**22**:783-790. DOI: 10.1016/j.cellsig.2010.01.002

[51] Mendes MCS, Pimentel GD, Costa FO, Carvalheira JBC. Molecular and neuroendocrine mechanisms of cancer cachexia. The Journal of Endocrinology. 2015;**226**:R29-R43. DOI: 10.1530/JOE-15-0170

[52] Smith HJ, Mukerji P, Tisdale MJ. Attenuation of proteasome-induced proteolysis in skeletal muscle by {beta}-hydroxy-{beta}-methylbutyrate in cancer-induced muscle loss. Cancer Research. 2005;**65**:277-283

[53] Holeček M. Beta-hydroxy-beta-methylbutyrate supplementation and skeletal muscle in healthy and muscle-wasting conditions. Journal of Cachexia, Sarcopenia and Muscle. 2017;**8**:529-541. DOI: 10.1002/jcsm.12208

[54] Girón MD, Vílchez JD, Shreeram S, Salto R, Manzano M, Cabrera E, et al. β-Hydroxy-β-methylbutyrate (HMB) normalizes dexamethasone-induced autophagy-lysosomal pathway in skeletal muscle. PLoS One. 2015;**10**: e0117520. DOI: 10.1371/journal.pone.0117520

[55] Wilkinson DJ, Hossain T, Hill DS, Phillips BE, Crossland H, Williams J, et al. Effects of leucine and its metabolite β-hydroxy-β-methylbutyrate on human skeletal muscle protein metabolism. The Journal of Physiology.

2013;**591**:2911-2923. DOI: 10.1113/jphysiol.2013.253203

[56] May PE, Barber A, D'Olimpio JT, Hourihane A, Abumrad NN. Reversal of cancer-related wasting using oral supplementation with a combination of beta-hydroxy-beta-methylbutyrate, arginine and glutamine. American Journal of Surgery. 2002;**183**:471-479

[57] Tawa NE, Odessey R, Goldberg AL. Inhibitors of the proteasome reduce the accelerated proteolysis in atrophying rat skeletal muscles. The Journal of Clinical Investigation. 1997;**100**(1):197-203. DOI: 10.1172/JCI119513

[58] Senf SM, Dodd SL, Judge AR. FOXO signaling is required for disuse muscle atrophy and is directly regulated by Hsp70. American Journal of Physiology-Cell Physiology. 2010;**298**(1):C38-C45. DOI: 10.1152/ajpcell.00315.2009

[59] Oliveira AG, Gomes-Marcondes MCC. Metformin treatment modulates the tumour-induced wasting effects in muscle protein metabolism minimising the cachexia in tumour-bearing rats. BMC Cancer. 2016;**16**:418. DOI: 10.1186/s12885-016-2424-9

[60] Gomes MD, Lecker SH, Jagoe RT, Navon A, Goldberg AL. Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy. Proceedings of the National Academy of Sciences of the United States of America. 2001;**98**:14440-14445. DOI: 10.1073/pnas.251541198

[61] Bodine SC, Latres E, Baumhueter S, Lai VK, Nunez L, Clarke BA, et al. Identification of ubiquitin ligases required for skeletal muscle atrophy. Science. 2001;**294**:1704-1708. DOI: 10.1126/science.1065874

[62] Combaret L, Dardevet D, Rieu I, Pouch M-N, Béchet D, Taillandier D, et al. A leucine-supplemented diet restores the defective postprandial inhibition of proteasome-dependent proteolysis in aged rat skeletal muscle. The Journal of Physiology. 2005;**569**:489-499. DOI: 10.1113/jphysiol.2005.098004

[63] Milan G, Romanello V, Pescatore F, Armani A, Paik J-H, Frasson L, et al. Regulation of autophagy and the ubiquitin–proteasome system by the FoxO transcriptional network during muscle atrophy. Nature Communications. 2015;**6**:6670-6683. DOI: 10.1038/ncomms7670

[64] Arendt CS, Hochstrasser M. Identification of the yeast 20S proteasome catalytic centers and subunit interactions required for active-site formation. Proceedings of the National Academy of Sciences of the United States of America. 1997;**94**:7156-7161

[65] Ventrucci G, Mello MARAR, Gomes-Marcondes MCCCC. Proteasome activity is altered in skeletal muscle tissue of tumour-bearing rats a leucine-rich diet. Endocrine-Related Cancer. 2004;**11**:887-895. DOI: 10.1677/Erc.1.00828

[66] Cruz B, Gomes-Marcondes MCC. Leucine-rich diet supplementation modulates foetal muscle protein metabolism impaired by Walker-256 tumour. Reproductive Biology and Endocrinology. 2014;**12**:2. DOI: 10.1186/1477-7827-12-2

[67] Morris CA, Morris LD, Kennedy AR, Sweeney HL. Attenuation of skeletal muscle atrophy via protease inhibition. Journal of Applied Physiology. 2005;**99**:1719-1727. DOI: 10.1152/japplphysiol.01419.2004

[68] Sudhan DR, Siemann DW. Cathepsin L targeting in cancer treatment. Pharmacology & Therapeutics. 2015;**155**:105-116. DOI: 10.1016/j.pharmthera.2015.08.007

[69] Goll DE, Thompson VF, Li H, Wei W, Cong J. The calpain system. Physiological Reviews. 2003;**83**:731-801. DOI: 10.1152/physrev.00029.2002

[70] Goll DE, Neti G, Mares SW, Thompson VF. Myofibrillar protein turnover: The proteasome and the calpains. Journal of Animal Science. 2008;**86**:E19-E35. DOI: 10.2527/jas.2007-0395

[71] Bartoli M, Richard I. Calpains in muscle wasting. The International Journal of Biochemistry & Cell Biology. 2005;**37**:2115-2133. DOI: 10.1016/j.biocel.2004.12.012

[72] Costelli P, De Tullio R, Baccino FM, Melloni E. Activation of Ca^{2+}-dependent proteolysis in skeletal muscle and heart in cancer cachexia. British Journal of Cancer. 2001;**84**:946-950. DOI: 10.1054/bjoc.2001.1696

[73] Smith IJ, Aversa Z, Hasselgren P-O, Pacelli F, Rosa F, Doglietto GB, et al. CALPAIN activity is increased in skeletal muscle from gastric cancer patients with no or minimal weight loss. Muscle & Nerve. 2011;**43**:410-414. DOI: 10.1002/mus.21893

[74] Gan L, Zheng W, Chabot J-G, Unterman TG, Quirion R. Nuclear/cytoplasmic shuttling of the transcription factor FoxO1 is regulated by neurotrophic factors. Journal of Neurochemistry. 2005;**93**:1209-1219. DOI: 10.1111/j.1471-4159.2005.03108.x

[75] Link W, Fernandez-Marcos PJ. FOXO transcription factors at the interface of metabolism and cancer. International Journal of Cancer. 2017;**141**:2379-2391. DOI: 10.1002/ijc.30840

[76] O'Neill BT, Lee KY, Klaus K, Softic S, Krumpoch MT, Fentz J, et al. Insulin and IGF-1 receptors regulate FoxO-mediated signaling in muscle proteostasis. The Journal of Clinical Investigation. 2016;**126**:3433-3446. DOI: 10.1172/JCI86522

[77] Judge SM, Wu C-L, Beharry AW, Roberts BM, Ferreira LF, Kandarian SC, et al. Genome-wide identification of FoxO-dependent gene networks in skeletal muscle during C26 cancer cachexia. BMC Cancer. 2014;**14**:997. DOI: 10.1186/1471-2407-14-997

[78] Liu C-M, Yang Z, Liu C-W, Wang R, Tien P, Dale R, et al. Effect of RNA oligonucleotide targeting Foxo-1 on muscle growth in normal and cancer cachexia mice. Cancer Gene Therapy. 2007;**14**:945-952. DOI: 10.1038/sj.cgt.7701091

[79] de Matos-Neto EM, JDCC L, de Pereira WO, Figuerêdo RG, DMDR R, Radloff K, et al. Systemic inflammation in cachexia—Is tumor cytokine expression profile the culprit? Frontiers in Immunology. 2015;**6**:629. DOI: 10.3389/fimmu.2015.00629

[80] Macpherson PCD, Wang X, Goldman D. Myogenin regulates denervation-dependent muscle atrophy in mouse soleus muscle. Journal of Cellular Biochemistry. 2011;**112**:2149-2159. DOI: 10.1002/jcb.23136

[81] Cornwell EW, Mirbod A, Wu C-L, Kandarian SC, Jackman RW. C26 cancer-induced muscle wasting is IKKβ-dependent and NF-kappaB-independent. PLoS One. 2014;**9**:e87776. DOI: 10.1371/journal.pone.0087776

[82] Zhou X, Wang JL, Lu J, Song Y, Kwak KS, Jiao Q, et al. Reversal of cancer cachexia and muscle wasting by ActRIIB antagonism leads to prolonged survival. Cell. 2010;**142**:531-543. DOI: 10.1016/j.cell.2010.07.011

[83] Li Q, Kumar R, Underwood K, O'Connor AE, Loveland KL, Seehra JS, et al. Prevention of cachexia-like

syndrome development and reduction of tumor progression in inhibin-deficient mice following administration of a chimeric activin receptor type II-murine Fc protein. Molecular Human Reproduction. 2007; **13**:675-683. DOI: 10.1093/molehr/gam055

[84] Chen JL, Walton KL, Hagg A, Colgan TD, Johnson K, Qian H, et al. Specific targeting of TGF-β family ligands demonstrates distinct roles in the regulation of muscle mass in health and disease. Proceedings of the National Academy of Sciences of the United States of America. 2017;**114**: E5266-E5275. DOI: 10.1073/pnas.1620013114

[85] Gold E, Marino FE, Harrison C, Makanji Y, Risbridger G. Activin-β(c) reduces reproductive tumour progression and abolishes cancer-associated cachexia in inhibin-deficient mice. The Journal of Pathology. 2013; **229**:599-607. DOI: 10.1002/path.4142

[86] Loumaye A, de Barsy M, Nachit M, Lause P, van Maanen A, Trefois P, et al. Circulating Activin A predicts survival in cancer patients. Journal of Cachexia, Sarcopenia and Muscle. 2017;**8**:768-777. DOI: 10.1002/jcsm.12209

[87] Sandri M. Protein breakdown in cancer cachexia. Seminars in Cell & Developmental Biology. 2016;**54**:11-19. DOI: 10.1016/j.semcdb.2015.11.002

[88] Seiliez I, Sabin N, Gabillard J-C. FoxO1 is not a key transcription factor in the regulation of myostatin (mstn-1a and mstn-1b) gene expression in trout myotubes. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology. 2011;**301**:R97-R104. DOI: 10.1152/ajpregu.00828.2010

[89] Loncar G, Omersa D, Cvetinovic N, Arandjelovic A, Lainscak M. Emerging biomarkers in heart failure and cardiac cachexia. International Journal of Molecular Sciences. 2014;**15**: 23878-23896. DOI: 10.3390/ijms151223878

[90] Rajagopalan V, Zhao M, Reddy S, Fajardo G, Wang X, Dewey S, et al. Altered ubiquitin-proteasome signaling in right ventricular hypertrophy and failure. American Journal of Physiology. Heart and Circulatory Physiology. 2013; **305**:H551-H562. DOI: 10.1152/ajpheart.00771.2012

[91] Toneto AT, Ferreira Ramos LA, Salomão EM, Tomasin R, Aereas MA, Gomes-Marcondes MCC. Nutritional leucine supplementation attenuates cardiac failure in tumour-bearing cachectic animals. Journal of Cachexia, Sarcopenia and Muscle. 2016;**7**:577-586. DOI: 10.1002/jcsm.12100

[92] Murphy KT. The pathogenesis and treatment of cardiac atrophy in cancer cachexia. American Journal of Physiology. Heart and Circulatory Physiology. 2016;**310**:H466-H477. DOI: 10.1152/ajpheart.00720.2015

[93] Musolino V, Palus S, Tschirner A, Drescher C, Gliozzi M, Carresi C, et al. Megestrol acetate improves cardiac function in a model of cancer cachexia-induced cardiomyopathy by autophagic modulation. Journal of Cachexia, Sarcopenia and Muscle. 2016;**7**:555-566. DOI: 10.1002/jcsm.12116

[94] Tian M, Asp ML, Nishijima Y, Belury MA. Evidence for cardiac atrophic remodeling in cancer-induced cachexia in mice. International Journal of Oncology. 2011;**39**:1321-1326. DOI: 10.3892/ijo.2011.1150

[95] Springer J, Tschirner A, Haghikia A, von Haehling S, Lal H, Grzesiak A, et al. Prevention of liver cancer cachexia-induced cardiac wasting and heart failure. European Heart Journal. 2014;

35:932-941. DOI: 10.1093/eurheartj/eht302

[96] Huang Y, Zhou M, Sun H, Wang Y. Branched-chain amino acid metabolism in heart disease: An epiphenomenon or a real culprit? Cardiovascular Research. 2011;**90**:220-223. DOI: 10.1093/cvr/cvr070

[97] Bail J, Meneses K, Demark-Wahnefried W. Nutritional status and diet in cancer prevention. Seminars in Oncology Nursing. 2016;**32**:206-214. DOI: 10.1016/j.soncn.2016.05.004

[98] World Health Organization. Global Status Report on Noncommunicable Diseases 2010. World Health Organization; 2011. p. 176. DOI: ISBN: 978 92 4 156422 9

[99] Remely M, Stefanska B, Lovrecic L, Magnet U, Haslberger AG. Nutriepigenomics. Current Opinion in Clinical Nutrition and Metabolic Care. 2015;**18**:328-333. DOI: 10.1097/MCO.0000000000000180

[100] Eberle C, Ament C. Diabetic metabolic programming: Mechanisms altering the intrauterine milieu. ISRN Pediatrics. 2012;**2012**:1-11. DOI: 10.5402/2012/975685

[101] Vanden Berghe W. Epigenetic impact of dietary polyphenols in cancer chemoprevention: Lifelong remodeling of our epigenomes. Pharmacological Research. 2012;**65**:565-576. DOI: 10.1016/j.phrs.2012.03.007

[102] Mathias PCF, Elmhiri G, De Oliveira JC, Delayre-Orthez C, Barella LF, Tófolo LP, et al. Maternal diet, bioactive molecules, and exercising as reprogramming tools of metabolic programming. European Journal of Nutrition. 2014;**53**:711-722. DOI: 10.1007/s00394-014-0654-7

[103] Wu G, Bazer FW, Cudd TA, Meininger CJ, Spencer TE. Maternal nutrition and fetal development. The Journal of Nutrition. 2004:2169-2172

[104] Victora CG, Adair L, Fall C, Hallal PC, Martorell R, Richter L, et al. Maternal and child undernutrition: Consequences for adult health and human capital. Lancet. 2008;**371**: 340-357. DOI: 10.1016/S0140-6736(07) 61692-4

[105] Perera F, Herbstman J. Prenatal environmental exposures, epigenetics, and disease. Reproductive Toxicology. 2011;**31**:363-373. DOI: 10.1016/j.reprotox.2010.12.055

[106] Tammen SA, Friso S, Choi SW. Epigenetics: The link between nature and nurture. Molecular Aspects of Medicine. 2013;**34**:753-764. DOI: 10.1016/j.mam.2012.07.018

[107] Taby R, Issa JJ. Cancer epigenetics. CA: A Cancer Journal for Clinicians. 2010;**60**:376-392. DOI: 10.3322/caac.20085

[108] Esteller M. Cancer epigenomics: DNA methylomes and histone-modification maps. Nature Reviews. Genetics. 2007;**8**:286-298

[109] Gerhauser C. Cancer chemoprevention and nutri-epigenetics: State of the art and future challenges. In: Pezzuto MJ, Suh N, editors. Natural Products in Cancer Prevention and Therapy. Berlin, Heidelberg: Springer Berlin Heidelberg; 2012. pp. 73-132. DOI: 10.1007/128_2012_360

[110] Hunter DC, Weintraub M, Blackburn GL, Bistrian BR. Branched chain amino acids as the protein component of parenteral nutrition in cancer cachexia. The British Journal of Surgery. 1989;**76**:149-153

[111] Tayek JA, Bistrian BR, Hehir DJ, Martin R, Moldawer LL, Blackburn GL. Improved protein kinetics and albumin

synthesis. Cancer. 1986:147-157. DOI: 10.1002/1097-0142

[112] Miyaguti NA da S, de Oliveira SCP, Gomes-Marcondes MCC. Maternal nutritional supplementation with fish oil and/or leucine improves hepatic function and antioxidant defenses, and minimizes cachexia indexes in Walker-256 tumor-bearing rats offspring. Nutrition Research. 2018;**51**:29-39. DOI: 10.1016/j.nutres.2017.12.003

[113] Togni V, Ota CCC, Folador A, Júnior OT, Aikawa J, Yamazaki RK, et al. Cancer cachexia and tumor growth reduction in Walker 256 tumor-bearing rats supplemented with N-3 polyunsaturated fatty acids for one generation. Nutrition and Cancer. 2003; **46**:52-58. DOI: 10.1207/S15327914NC4601_07

[114] Folador A, Hirabara SM, Bonatto SJR, Aikawa J, Yamazaki RK, Curi R, et al. Effect of fish oil supplementation for 2 generations on changes in macrophage function induced by Walker 256 cancer cachexia in rats. International Journal of Cancer. 2007;**120**:344-350. DOI: 10.1002/ijc.22333