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Ketogenic Diet and Skeletal Muscle Hypertrophy: a Frenemy Relationship?

by

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Ketogenic diet (KD) is a nutritional regimen characterized by a high-fat and an adequate protein content and a very low carbohydrate level (less than 20 g per day or 5% of total daily energy intake). The insufficient level of carbohydrates forces the body to primarily use fat instead of sugar as a fuel source. Due to its characteristic, KD has often been used to treat metabolic disorders, obesity, cardiovascular disease, and type 2 diabetes. Skeletal muscle constitutes 40% of total body mass and is one of the major sites of glucose disposal. KD is a well-defined approach to induce weight loss, with its role in muscle adaptation and muscle hypertrophy less understood. Considering this lack of knowledge, the aim of this review was to examine the scientific evidence about the effects of KD on muscle hypertrophy. We first described the mechanisms of muscle hypertrophy per se, and secondly, we discussed the characteristics and the metabolic function of KD. Ultimately, we provided the potential mechanism that could explain the influence of KD on skeletal muscle hypertrophy.

Key words: *ketogenic diet, skeletal muscle hypertrophy, resistance training, signaling, muscle protein synthesis.*

Introduction

Interactions between nutrition and skeletal muscle hypertrophy have been widely investigated. Several studies have analysed the effects of different macronutrients (Escobar et al., 2016; Phillips, 2017; Witard et al., 2016) and the energy intake amount (Hector et al., 2018) on muscle mass variations. Indeed, the size of skeletal muscle is the result of the balance between muscle protein synthesis (MPS) and muscle protein breakdown (MPB). The algebraic sum of MPS and MPB results in the net protein balance (NPB). Only with a positive NPB it is possible to achieve muscle protein accretion and, consequently, growth of muscle fiber size (Stokes et al., 2018). Many factors influence NPB (Figure 1), and among the others, nutrition together with resistance training play a pivotal role (Witard et al., 2016). Amino acids (AA) and protein ingestion lead to an increase (though transient) in the MPS' rate (Morton et al., 2015). The importance of protein quality and quantity in MPS has been widely investigated throughout the last 20 years (Mitchell et al., 2016; Phillips, 2011; Tipton and Phillips, 2013; van Loon and Gibala, 2011). Nowadays, it is well established that a minimal level of protein intake is mandatory to stimulate significant muscle growth (Churchward-Venne et al., 2016; Paddon-Jones et al., 2008; Phillips, 2012, 2017; Reidy and Rasmussen, 2016). However, this minimal level is still upon debate and relates also to body size and typology of training (Macnaughton et al., 2016; Moore et al., 2009)

Considering the paramount importance of protein intake as a skeletal muscle protein

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synthesis stimulus, it is somewhat surprising that a relatively high protein diet such as the very low carbohydrate ketogenic diet, more simply called ketogenic diet (KD) (Paoli, 2018), has been, till now, poorly investigated as far as its effects on skeletal muscle hypertrophy are concerned. KD is a nutritional approach consisting of high-fat and an adequate protein content, but of an insufficient level of carbohydrates for metabolic needs (less than 20 g per day or 5% of total daily energy intake; Paoli et al., 2015a), thus forcing the body to primarily use fat as a fuel source. Given this lack of knowledge, our aim was to review all scientific evidence about the effects of KD on muscle hypertrophy, dissecting the mechanisms of muscle hypertrophy *per se*, then discussing the characteristics and the metabolic effects of KD and finally, hypothesizing the potential influence of KD on muscle hypertrophy.

Skeletal Muscle Hypertrophy

Skeletal muscle tissue plays a pivotal role in the regulation of whole body metabolism, functional work capacity and exercise performance (Egan and Zierath, 2013). Muscle plasticity is an essential feature that makes this tissue able to react to different stimuli. Muscle hypertrophy is, for example, the accretion of muscle fiber size that may occur in response to resistance exercise. Understanding the molecular mechanisms of this muscle adaptation is important to counteract the opposite mechanism such as muscle atrophy that is related to many pathological conditions (disuse, cancer, denervation injury, aging) (Glass, 2003).

As previously stated, muscle mass ensures dynamic equilibrium between muscle loss/catabolism (MPB) and muscle growth/anabolism (MPS) (Figure 1). Indeed, balance between the rates of synthesis and degradation of muscle protein or net protein balance yields changes in the amount of muscle myofibrillar proteins therefore, influencing muscle mass. Exercise and nutrition are two interventions that influence rates of MPS and MPB and, ultimately, the NPB. It is well known that nutrition influences, per se, both MPS and MPB.

Measuring muscle protein synthesis (MPS) and muscle protein breakdown (MPB)

Protein Turnover

Physiological and metabolic homeostasis is strictly influenced by the regulation of MPS and MPB. Indeed, 80% of the daily protein synthesis comes from the recycling of amino acids from MBP, while only 20% seem to be induced by protein intake with diet (Wolfe and Chinkes, 2005). Under the post-absorptive condition, MPB is approximately 30% higher compared to MPS (Biolo et al., 1994), thus the nitrogen balance is, at basal fasting condition, normally negative. Anabolic stimuli, such as consumption of AA or exercise, can increase MPS by improving the efficacy of recycling EAA from MPB (Wolfe, 2017). Thus, understanding the mechanism of both protein synthesis and breakdown is a fundamental step to assess adequate strategy of intervention to regulate muscle protein turnover.

MPS

Muscle protein synthesis could be defined as the process in which new proteins are produced. MPS is directly activated via the mammalian/mechanistic target of rapamycin complex 1 (mTORC1) system (Dickinson et al., 2011; Drummond et al., 2009), containing the regulatory protein mTOR (Saxton and Sabatini, 2017). When appropriately stimulated by an increased concentration of AA, insulin or subsequent to mechanical stress induced by muscle contraction, mTORC1 complex translocates into the lysosome where it can interact with the G-protein Ras homolog enriched in the brain (Rheb) (Bar-Peled and Sabatini, 2012). The translation initiation and elongation process are then triggered: S6K1(Thr389) phosphorylation activates eIF4B (Holz et al., 2005; Saxton and Sabatini, 2017) which regulates the translation initiation and the kinase eEF2k (Wang et al., 2006) to promote the translation elongation process; simultaneously, the eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) is inhibited by mTORC1 by its phosphorylation (at Thr37/46), allowing the dissociation of eIF4F and mRNA translation (Thoreen et al., 2012).

MPB

Muscle protein breakdown is an important metabolic process that results in protein degradation. MPB function is to enable protein and amino acids availability that could be used during stress, such as nutrient depravation or exercise. MPB is regulated by three different protein-dependent systems that can work independently or synergistically: autophagy, the

ubiquitin-proteasomal pathway (UPP) and the calpain Ca^{2+}-dependent cysteine proteases.

Autophagy is a lysosomal degradation system sensitive to energy accessibility, particularly efficient to disrupt bound proteins, intracellular organelles and damaged proteins (Masiero et al., 2009). Autophagy uses the autophagosome to surround and sequester the lysosome, where the targeted protein complex is degraded.

The UPP system is the primary ATP-dependent mechanism of protein degradation and targets short-lived proteins. The UPP regulation of protein breakdown depends on the 26kDa proteasome, which attracts those proteins conjugated with ubiquitin molecules via an E3 ubiquitin ligase, such as the ubiquitin ligases muscle RING finger protein 1 (MuRF1) and Atrogin 1 (Bodine and Baehr, 2014; Murton et al., 2008).

Sarcolemmal, cytoskeletal and myofibrillar proteins are finally degraded by the calpain Ca^{2+}-dependent cysteine proteases. This process requires specific levels of Ca^{2+} to activate the enzyme that leads to proteolysis (Dargelos et al., 2008). Therefore, it seems that the calpain system is activated during muscle injuries, when cytosolic Ca^{2+} levels rises (Goll et al., 1992).

Methods for assessing muscle protein metabolism

Dynamic assessment of MPS and MPB can be determined using stable isotope methodologies (Wolfe and Chinkes, 2005). Stable isotopes are non-radioactive molecules, naturally enriched with heavier atoms, which results in a different molecular mass, but functionally identical to the endogenous counterparts. Thanks to the mass spectrometry technique, it is then possible to measure the incorporation of the isotopic labelled molecules (usually, not metabolized essential amino acids such as phenylaniline) into blood or muscle tissue, thus measuring the rate of muscle protein turnover. The two most common methods used to asses MPS and MPS are the two-pool (or AV balance, arterial and venous poll) and the three-pool (arterial, venous and muscle) model. In both methodologies it is assumed that a physiological and isotopic steady state is achieved by a constant infusion of the tracer.

Then, the differences between arterial and venous enrichment of the labeled tracer are calculated, and the amount of the tracer disappearing from the arterial blood is assumed to represent MPS, while the amount of the tracer released in the vein represents MPB or the outward flux of amino acids from the muscle to the venous pool (Biolo et al., 1994; Wolfe and Chinkes, 2005). However, the arteriovenous blood balance does not consider the portion of the amino acids that is recycled in the intracellular pool and the rate of their transportation. For this reason, the three-pool model is probably a better method to asses muscle protein turnover as it includes the isotopic enrichment of the intracellular amino acids obtained by a muscle biopsy (Biolo et al., 1995).

Despite the important and unique information that can be obtained by tracer methodology, its limitations including the invasivity of the technique, the numerous assumptions that are not always possible to be supported (such as a physiological steady state) and the remarkable cost of the material (Pacy et al., 1989; Thompson et al., 1989; Wolfe and Chinkes, 2005) have to be taken into consideration.

MPS and MPB can also be measured via changes in molecular signals of specific muscle proteins. Measuring RNA via the quantitative real-time polymerase chain (qRT-PCR) or the most recent RNA-sequencing (RNA-seq) technique, protein levels or western blotting, provides indices of the protein signaling pathway at individual time points. The most common proteins studied as markers of MPS are linked to the mTOR and the insulin signaling pathway. mTORC1 downstream that targets S6 kinase 1 (S6K1) and 4E-binding protein 1 (4E-BP1), together with the stimulation of the insulin-signaling pathway through phosphatydilinositol 3-kinase (PI3K) and phosphorylated protein kinase B (Akt/PKB), has been widely investigated in nutrition and exercise studies (Avruch et al., 2006; Bodine et al., 2001; Dickinson et al., 2011; Drummond et al., 2009). On the other hand, expression of MuRF1 and atrogin 1 as well as other E3-ubiquitin ligases are the molecular pathway for MPB (Fry et al., 2013; Pasiakos and Carbone, 2014).

The major limitation of these static methodologies for measuring muscle protein turnover is related to their time sensitivity. Those

are, in fact, a sort of a "snap-shot" of what is happening in the muscle, and each protein has its own time course. Moreover, an increase in the mRNA expression not always translates in an increase in protein production (Atherton et al., 2016).

Molecular machineries involved in skeletal muscle hypertrophy

Protein balance is, at a deeper level, controlled by a complex molecular apparatus that has either a negative or a positive muscle growth effect. There are three main pathways involved in the skeletal muscle mass control, mitochondrial biogenesis and metabolic homeostasis signalling pathways (Sandri et al., 2013): 1) IGF-1/Akt/mTOR, 2) FOXO/MuRF1, and 3) AMPK/PGC1α pathways. While the IGF-1/Akt/mTOR and the FOXO/MuRF1 pathways are involved in the first control mechanisms, the latter functions are under the control of the AMPK/PGC1α pathway.

As described above, skeletal muscle protein synthesis following exercise is mainly regulated by mTORC1 that is part of the IGF-Akt-mTOR signalling pathway (Schiaffino and Mammucari, 2011; Wackerhage et al., 2019). Specifically, IGF-1 binds to its receptor that in turn phosphorylases the insulin receptor substrate (IRS) which activates phosphatidylinositol-3-kinase (PI3K). PI3K phosphorylates membrane phospholipids, leading to formation of phosphoinositide-3,4,5-triphosphate (PIP3) that acts as a docking site for two kinases, phosphoinositide dependent kinase 1 (PDK1) and Akt. PDK1 activates Akt which on one hand inhibits protein degradation by repressing the members of the Forkhead box class O family member proteins (FoxO), and on the other stimulates protein synthesis via mTOR and glycogen synthase kinase 3b (GSK3b). mTOR forms two protein complexes, the rapamycin sensitive mTORC1 with Raptor, and rapamycin insensitive mTORC2 with Rictor (Zoncu et al., 2011). mTORC2 phosphorylates Akt at serine 473 for its maximum activation, while mTORC1 phosphorylates the S6K1 that activates the ribosomal protein S6 and other proteins to stimulate protein synthesis (Schiaffino and Mammucari, 2011).

One of the crucial actions of Akt is also the inhibition of FoxO. Skeletal muscle presents three transcriptional factors of the FoxO family: Foxo1, Foxo3 and Foxo4. All these three factors can be inhibited by Akt, through phosphorylation (Brunet et al., 1999; Sandri et al., 2013). FoxOs control numerous genes related to muscle atrophy. In particular, under stress conditions such as fasting or disuse, FoxOs promote the expression of Murf1 and Atrogin-1 genes, and consequently rule out their protein abundance (Bodine and Baehr, 2014). As described above, these two ligases are linked to the UPP system and regulate protein breakdown.

On the other hand, 5'-adenosine monophosphate-activated protein kinase (AMPK) is a key regulator of muscle homeostasis (Merrill et al., 1997; Winder and Thomson, 2007). AMPK is an intracellular sensor of ATP, activated when the intramuscular AMP:ATP ratio increases, to promote fuel utilization.

Due to its function, AMPK inhibits muscle growth and has a negative impact on those processes that consume ATP, such as protein synthesis (Romanello et al., 2010; Weerasekara et al., 2014). The inhibitory action of AMPK on muscle protein synthesis mainly occurs through the inhibition of mTORC1: AMPK can directly phosphorylate mTOR in one or both of its two sites (Thr2446 and Ser2448) (Cheng et al., 2004; Figueiredo et al., 2017); or indirectly by the phosphorylation of the tuberous sclerosis complex 2 (TSC2) (Long et al., 2005); or phosphorylating RAPTOR, a regulatory-associated protein required for mTORC1 activity (Gwinn et al., 2008). Moreover, AMPK can exercise its inhibitory action blocking the downstream binding of the elongation factor eEF2 to the ribosome. This process can occur via mTOR inhibition or directly, phosphorylating eEF2k, an eEF2 kinase, that has a negative control on the elongation process (Horman et al., 2002; Rose and Richter, 2009). Thus, AMPK could be considered a negative regulator of muscle hypertrophy, as its phosphorylation is correlated with a reduction in muscle protein synthesis (Dreyer et al., 2006; Gordon et al., 2008; Thomson and Gordon, 2005; Thomson et al., 2008). In addition, it seems that AMPK activation could promote the shift from the glycolytic to the more oxidative fiber status, via the peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1a), which promotes mitochondrial biogenesis (Garcia and

Shaw, 2017; Mounier et al., 2015).

Ketogenic Diet

KD is a high fat and moderately high protein diet in which the level of carbohydrate must be below 30 g/day or 5% of total energy intake (Paoli et al., 2015a). During KD an important shift at the metabolic level occurs. The lack or the reduction of dietary carbohydrates decreases plasma insulin and increases glucagon; this state promotes hepatic glycogenolysis and gluconeogenesis as well as lipolysis of adipose tissue through the increase of HSL (hormone sensitive lipase). Thus, after a period of CHO restriction (in general 4-7 days), glycogenolysis becomes not sufficient anymore and the increased levels of free fatty-acids and of acetyl CoA (through mitochondrial beta-oxidation), lead to ketogenesis. The end-products of this process are the ketone bodies: acetoacetate (AcAc), beta-hydroxybutyrate (BHB) and acetone (McPherson and McEneny, 2012; Paoli, 2014). The production of ketone bodies is stimulated by the overproduction of acetyl-CoA (increased lipolysis and beta-oxidation) without concomitant production of an adequate amount of oxaloacetic acid (Paoli et al., 2015a). It is thus worthy to underline that the reduction of glucose flux, due to the nutritional carbohydrate restriction, leads to a lower level of oxaloacetate. Oxaloacetate is an intermediate of the TCA (tricarbossilic acid cyle, or Kreb's cycle) and it is unstable at average body's temperature. Thus, it is necessary to re-fuel an adequate amount of oxaloacetate through the (almost) only feasible way in mammalian: the conversion of piruvate into oxaloacetate. The imbalance between the two fluxes: production of FFA and production of oxaloacetate, leads to an excess of FFAs that are converted in the liver into the three KBs (Paoli et al., 2013). This process is called ketogenesis and principally occurs in the mitochondrial matrix of the liver (Veech, 2004). When glycogen's stores are depleted, glycaemia is maintained stable mainly through the so-called gluconeogenesis process. In humans, gluconeogenesis usually derivates from the concession of a carbonic skeleton from those few amino acids with gluconeogenetic properties (ie. Alanine, Glutamine, Glycine). In overweight and obese people also glycerol derived from triglycerides provides an important source of glucose (Bortz et al., 1972). These two sources maintain a stable glycaemia index and avoid hypoglycaemia during fasting or a ketogenic diet. Ketone bodies are then captured by extrahepatic tissues from circulation and used as an energy source. Anyway, 10-20% of KBs could be lost in the urine. KBs are used as an alternative source of energy through a pathway that firstly converts 3HB back to AcAc which is then transformed into acetoacetyl-CoA. The latter is finally divided into two molecules of acetyl-CoA, which are subsequently used in the Krebs cycle. Furthermore, compared with glucose, the energy produced from KBs is greater (Fukao et al., 2004; Paoli et al., 2014). During KD, the low amount of carbohydrate intake stabilizes glycaemia at a physiological range around 3.61-4.44 mmol/L (65-80mg/dL), while the level of ketone bodies (KBs) reaches a maximum level of 3-4 mmol/L (usually 1.2-2 mmol/L) with low insulin levels (Paoli et al., 2013). This situation is known as "Ketosis" (increase in ketone bodies) which also occurs physiologically after prolonged fasting and exercise.

In the physiological context blood pH does not change; conversely in uncontrolled diabetic ketoacidosis, ketonemia can exceed 20mmol/l with concomitant lowering of blood pH, that can lead to potentially lethal ketoacidosis. To differentiate this pathological condition from ketosis induced by fasting or KD, already in 1966, Hans Krebs called the latest condition "physiological ketosis" (Krebs, 1966).

If properly managed, the "physiological ketosis" is not dangerous and can be a useful tool to treat different pathological conditions. Historically, the VLCKD has been used to treat epilepsy, in particular in children who were non-responders to pharmacological interventions (Rho, 2017). More recently, the therapeutic use of this diet type in other diseases such as metabolic disorders, obesity, cardiovascular disease, type 2 diabetes and also some types of cancer, has raised great interest. In the last 10-15 years there has been a dramatic increase in the number of published research on ketogenic diet and different diseases. Indeed in 2013, we published a review suggesting some strong emerging evidence about the role of KD in different diseases (Paoli et al., 2013) which has been mostly confirmed so far. Many articles have been published indicating positive effects of KD on neurological diseases

such as Alzheimer's diseases (Ota et al., 2019; Taylor et al., 2018), ALS (Ari et al., 2014), brain trauma (concussion) (Zhang et al., 2018), migraine (Di Lorenzo et al., 2018), PCOS (Mavropoulos et al., 2005), and cancer (Klement, 2019). Moreover, new scenarios are opening such as the effects of BHB on epigenetics changes (Newman and Verdin, 2013; Ruan and Crawford, 2018), inflammation (Paoli et al., 2015b) and microbiota (Olson et al., 2018).

Table 1

Characteristics of studies performed on KD and resistance training. RT = resistance training

Study	Effects on LBM	RT (Yes/No)	Athletes (Yes/No)	Protein g/kgbw/day reported	Protein g/kgbw/day calculated
Wilson et al. (2017)	↑	Yes	Yes	No	1.73
Vargas et al. (2018)	=	Yes	Yes	2.0	/
Kephart et al (2018)	=	Yes	Yes	No	~ 1.0
Greene et al. (2018)	↓	Yes	Yes	No	~ 1.6
Paoli et al. (2012)	=	Yes	Yes	2.2	/
Wood et al. (2012)	↓	Yes	No	No	~ 1.18
Jabekk et al. (2010)	=	Yes	No	No	~ 1.0

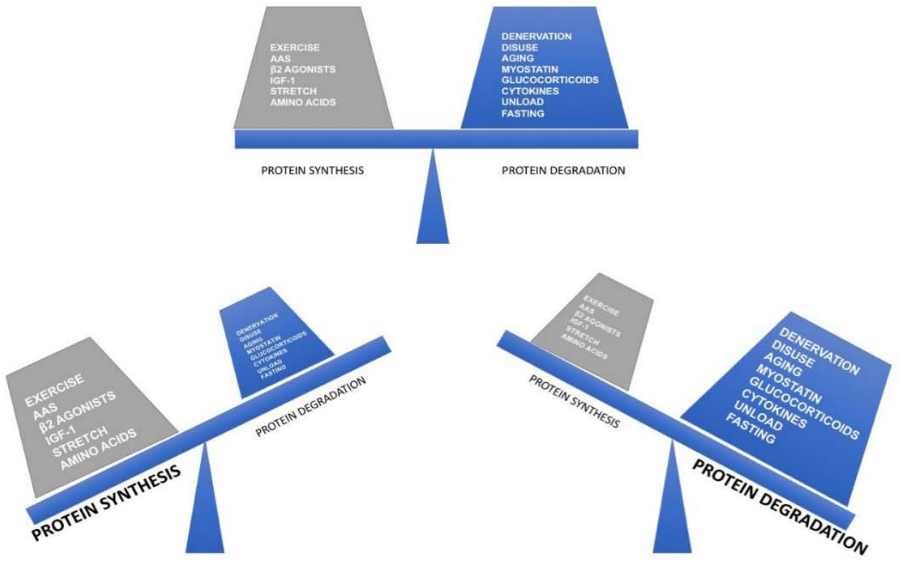

Figure 1

Factors that influence skeletal muscle protein balance (AAS=Androgen Anabolic Steroids). An increase in positive protein balance's factors stimulates protein synthesis, on the opposite an increase in negative protein balance's factors stimulates protein degradation. The sum of these two parts of the scale defines the net protein balance (NPB).

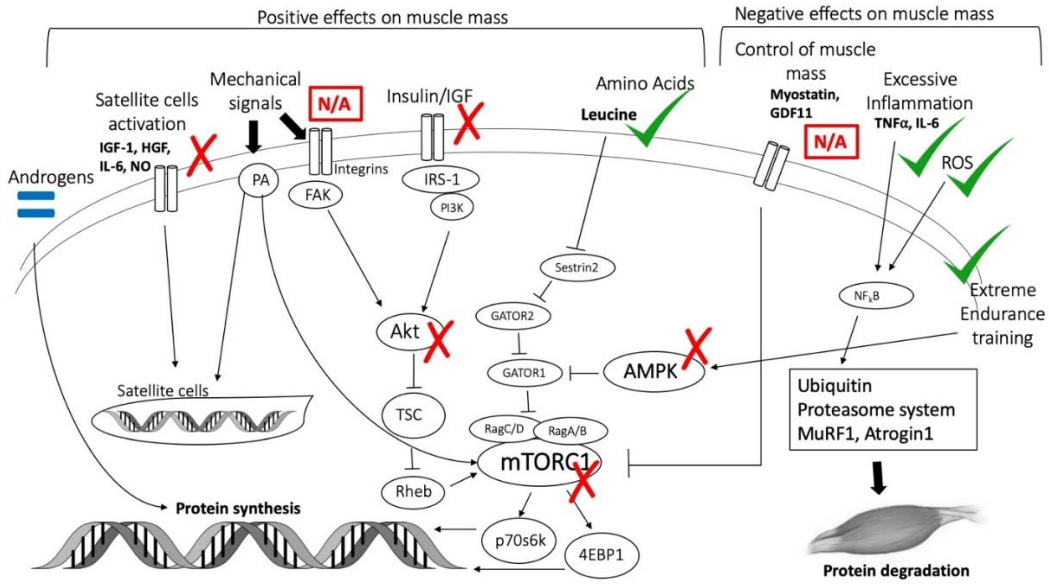

Figure 2

Effects of KD on muscle mass control pathways. IGF-1 = insulin-like growth factor-1; HGF = Hepatocyte growth factor; IL-6=Interleukin-6; NO = Nitric Oxide; PA = phosphatidic acid; FAK = Focal adhesion kinase; IRS-1 = Insulin receptor substrate-1; PI3K = Phosphoinositide 3-kinase; AKT = protein kinase B; TSC = Tuberous sclerosis protein; Gap activity toward Rags 1; GATOR2, Gap activity toward Rags 2; AMPK = 5' adenosine monophosphate- activated protein kinase; Rheb = Ras homolog enriched in brain; mTORC1 = mammalian/mechanistic target of rapamycin complex 1; p70s6k = S6 kinase beta- 1; 4EBP1 = 4E-binding protein 1; GDF11 = Growth differentiation factor 11; TNFα = Tumor necrosis factor α; ROS = *Reactive oxygen species*; NF κB = *nuclear factor kappa-light-chain-enhancer of activated B cells*; MuRF1 = *Muscle RING-finger protein-1.*

Ketogenic diet and muscle mass

There are conflicting data about the effect of chronic very low carbohydrate (ketogenic) diet on lean body mass, muscle mass and exercise. Wilson and collaborators (2017) recently showed an increase in muscle mass after 10 weeks of KD and 2 weeks of carbohydrate reintroduction in resistance trained males, while Vargas et al. (2018), Paoli et al. (2012), Kephart et al. (2018), and Jabekk et al. (2010) found a general conservation of muscle mass with no significant increases.

In another study, authors showed a similar decrease in lean body mass during KD with resistance training, KD without training and low fat diet with resistance training, while the loss of muscle mass was greater after a low fat diet without resistance training suggesting a protective effect of KD on muscle mass compared to a low fat diet in absence of training (Wood et al., 2012). Moreover, a recent paper investigating the effects of KD on body composition and performance in weight lifters found a decrease in lean body mass without negative effects on specific sport's performance (Greene et al., 2018).

One confounding factor might be the amount of daily protein intake expressed as grams of protein per kilogram of body mass (Table 1) and the fitness level of subjects (sedentary, overweight or active/athletes).

Effects of ketogenic diet on skeletal muscle protein balance

Muscle protein balance is clearly influenced, directly or indirectly, by many nutritional factors. We can summarize, according to different authors (Hoppeler et al., 2011) these factors into positive regulators such as growth factors, AA signalling, hormones, mechanical stimuli, and negative regulators such as Myostatin, GDF11, chronic low-grade inflammation and continuous repeated contractions.

Hormones

It is well known that some hormones exert anabolic effects on skeletal muscle; among others, the most powerful are testosterone, growth hormone (and its downstream: IGF-1) and insulin. Data available on mice showed that KD *per se* did not influence testosterone levels, while testosterone was reduced by a caloric restriction regimen and, instead, increased by a high fat diet (Kennedy et al., 2007). In humans, less data are available: Volek and colleagues (2001) found no effects on testosterone levels of a high fat-low carbohydrate diet. On the contrary, recently, an increase in testosterone levels has been shown after KD (Wilson et al., 2017). It should be mentioned that in the study of Wilson and colleagues (2017), the final measurements were made after one week of carbohydrate reintroduction that could have modified the final results through glycogen replenishment. While caloric restriction, fasting and ketogenic diet share many mechanisms (Paoli et al., 2019), the effects of KD on testosterone levels seem negligible, opposite to caloric restriction (Cangemi et al., 2010) and fasting (Moro et al., 2016) that both reduce the main androgenic hormone.

The majority of studies performed on human subjects demonstrated a strong decrease in insulin levels after KD (Bueno et al., 2013; Fery et al., 1982), with the notable exception of the aforementioned Wilson et al.'s study (Wilson et al., 2017). Decreases in insulin levels are clearly related to the dramatic reduction of carbohydrate intake and it could be stated that one of the main effects of ketogenic diet relies on this reduction (Paoli et al., 2015a).

Muscle's mass regulatory pathways

Mechanisms underlying the skeletal muscle protein balance during KD and resistance training are still not clear. One of the more often advocated mechanism is the activation of the AMP-activated protein kinase. AMPK phosphorylation blunts Akt/mTOR pathways. But, although it has been demonstrated that fasting induces muscle AMPK activation in animals' models (Lee et al., 2017), in humans available data do not confirm these results; indeed, fasting in humans seems to reduce activation of Akt pathways without affecting AMPK (Wijngaarden et al., 2014). Regarding the effects of KD on these pathways, Kennedy and colleagues (2007) demonstrated a threefold increase in AMPK activity in the soleus muscle of rats fed with KD, but no relevant data are available in humans.

Indeed, little data are available about how KD influences skeletal muscle's mass regulatory pathways, and most of them are only available in animal models. Roberts and colleagues (2016) demonstrated that in rats a LCKD induced only mild ketosis. In that study, LCKD did not affect basal muscular signalling and moreover, it did not affect acute muscle response to exercise measured with MPS. Furthermore, in that study, even during chronic resistance exercise, LCKD did not impair glycogen, total RNA, myofibril protein levels, or mRNA expression patterns in skeletal muscle. Again, in a further study of the same authors, it was demonstrated that 1 month of KD was able to improve the preservation of the relative mass of gastrocnemius and other hind limb muscles in aging mice. Interestingly, mice that subjected to KD did not show the age-related decrease in grip strength compared to control mice, suggesting that ketogenic diet may improve and preserve forelimb grip strength during aging (Roberts et al., 2017). It has been shown that in mice skeletal muscle, KD induced an increase in p-4E-BP1 (downstream of mTOR) levels with no changes in phosphorylated AMPK, p-Akt or p-Erk1/2 compared to control. Those authors suggested that their results might be linked to higher protein intake compared to other KD in animal models (Roberts et al., 2016; Roberts et al., 2017). Another target of metabolic changes

induced by KD is Akt. Akt is involved in different cellular processes such as cell growth, proliferation, apoptosis, transcription, angiogenesis, migration, and glucose metabolism and it is also activated following a great number of stimuli among which insulin and insulin-like growth factor are of greatest importance (Sandri et al., 2013). In turn, Akt acts on target proteins that include glycogen synthase kinase 3, mammalian target of rapamycin (mTOR), p70S6K, PHAS-1 (4EBP-1), and Foxo family (Sharples et al., 2015). Akt shows three isoforms, Akt1, Akt2 and Akt3; the first isoform regulates the cell growth process and recent research indicates that it is a potential target to treat obesity because of its role in energy metabolism (Matheny et al., 2018). In this regard, BHB inhibits directly AKT (Yamada et al., 2010) and this mechanism may be responsible for the temporary KD-induced insulin resistance found in different tissues such as muscle and heart that preserves glucose for brain's use (Grandl et al., 2018; Rojas-Morales et al., 2016). On the other hand, this inhibition may negatively affect muscle mass.

Ketogenic diet and skeletal muscle glycogen

One important component of muscle mass is glycogen. Glycogen is stored in skeletal muscles at a rate of 500 g (Murray and Rosenbloom, 2018) and each gram of glycogen is bound to 3 grams of water (Fernandez-Elias et al., 2015), thus glycogen and associated water may significantly contribute to the whole muscle mass in athletes, especially in activities such as competitive body building (Robinson et al., 2015; Schoenfeld, 2010). Despite intuitive reasoning: no carbohydrate from food = less glycogen storage, research has indicated that during endurance exercise, nutritional ketosis alters substrate competition and reduces glycolysis, promoting the preservation of glycogen resources to enhance muscle performance (Cox et al., 2016). More generally, ketosis related to both chronic high-fat, low-carbohydrate diet, or KE intake, decreases glycogen breakdown at rest as well as during exercise (Cox et al., 2016; Vandoorne et al., 2017; Volek et al., 2016; Webster et al., 2016).

Histone acetylation mechanisms

Histone acetylation plays numerous roles in muscle control and different classes of histone deactylase have different effects on skeletal muscle such as control of contracting proteins, calcium channels and, in general, muscle health. BHB mediates inhibition of histone deacetylase (HDAC) activity in vivo (Roberts et al., 2017). HDAC inhibitors increased the lifespan by a still not very well known mechanism, yet associated with hyperacetylation of histones and a large number of other proteins (Roberts et al., 2016). Moreover, KD inhibits HDAC activity modifying related gene transcription and leads to reduced oxidative damage that could help the maintenance of muscle mass (Wang et al., 2017). Also acetoacetate may play an important role as a signalling molecule in muscle cells and there is some evidence suggesting that this ketone body can improve muscular dystrophy and accelerate muscle regeneration (Zou et al., 2016). However, more studies need to address the potential function of these two KBs on the acetylation process.

Conclusions

Nutrients, growth factors, hormones, and muscle activity generate cellular signals that have the capacity to maintain and/or grow muscle mass. The regulation of skeletal muscle mass, in fact, reflects changes in protein synthesis and protein degradation, and several stimuli lead to protein synthesis and subsequent muscle hypertrophy. Indeed, nutrition is a key factor for muscle mass balance and macronutrients' distribution, and the quantity may influence muscle growth and muscle response to training. The question that should be addressed is whether a diet in which one of the macronutrients is restricted, i.e. carbohydrates in KD, may affect skeletal muscle hypertrophy.

KD theoretically may affect skeletal muscle mass control pathways in several ways (Figure 2), but data provided by scientific literature suggest a negligible or no effect of KD on muscle mass with concomitant resistance training. KD may instead exert a protective effect against muscle mass loss during aging or during low calorie diets. The total effect seems to consist in the maintenance of muscle mass rather than a net hypertrophic effect.

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