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Etiology and Pathophysiology

Ursolic acid and mechanisms of actions on adipose and muscle tissue: a systematic review

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Summary

This systematic review aimed at addressing the ursolic acid actions as an adjunctive treatment of the obesity-mediated metabolic abnormalities. To explore our aims, we used the literature search including clinical and animal studies using the Medline and Google Scholar (up to December 2015). Out of 63 screened studies, 17 presented eligibility criteria, such as the use of ursolic acid on adiposity, energy expenditure and skeletal muscle mass in mice and humans. In the literature, we found that several physiological and molecular mechanisms are implicated in the effects of ursolic acid on obesity, energy expenditure, hepatic steatosis, skeletal muscle mass loss and physical fitness, such as (1) increase of thermogenesis by modulation adipocyte transcription factors, activation of 5′ adenosine monophosphate-activated protein kinase and overexpression of the uncoupling protein 1 thermogenic marker; (2) enhancement of skeletal muscle mass by activation in bloodstream growth hormone and insulin-like growth factor-1 concentrations secretion, as well as in the activation of mammalian target of rapamycin and inhibition of ring-finger protein-1; and (3) improvement of physical fitness by skeletal muscle proliferator-activated receptor gamma co-activator alpha and sirtuin 1 expression. Therefore, supplementation with ursolic acid may be an adjunctive therapy for prevention and treatment of obesity-mediated and muscle mass-mediated metabolic consequences.

Keywords: Energy expenditure, muscle, obesity, ursolic acid.

Eu

Introduction

Ursolic acid (UA) is a pentacyclic triterpene natural compound (1,2) found in the leaves, flowers and fruits of medicinal herbs such as *Rosmarinus officinalis* (rosemary), *Ocimum basilicum* (basil), *Eriobotrya japonica* (medlar), *Eugenia jambolana* (jamelão), *Origanum vulgare* (oregano) leaves, *Eucalyptus globulus* (eucalyptus) leaves and bark, *Coffea arabica* (coffee) leaves and *Pyrus malus* (apple peel) (2). Some of the proven biological effects of UA are anti-inflammatory actions (3), antioxidant effects (4), anti-carcinogenic effects (2,5,6), attenuation of muscle mass atrophy (7) and muscle synthesis (8), thermogenesis activation (9) and anti-obesity effects (1,7–12). The main mechanisms of action of UA involve the following: the activation of uncoupling protein 1 (UCP1) and adenosine monophosphate ratio (AMP)-activated protein kinase (AMPK) in adipose tissue, the reduction of the atrogin and muscle ring-finger protein-1 (MuRF1) in skeletal muscle, and a greater release of growth hormone and insulin-like factor 1 (IGF-1) into the bloodstream, as well as increased muscle protein kinase B (PKB/Akt) and mammalian target of rapamycin (mTOR).

Adiposity has a causal relationship on skeletal muscle mass, which is reflected mainly by decreasing protein synthesis and muscle atrophy induction by inflammation (13). As muscle contraction is crucial for body homeostasis and metabolic function control, this action is considered important for the maintenance of health (14). Additionally, obesity and low muscle mass reflect muscle mass loss and reduced strength, with a prominent risk of physical frailty and locomotion dependency, which leads to weight gain (15,16).

Owing to the consequences of adiposity on health status, several therapeutic strategies are being studied as treatment options for obesity-linked metabolic abnormalities, such as the use of natural bioactive compounds (17,18), e.g. UA (9,19).

This systematic review aims at addressing the mechanisms of action of UA, as an adjuvant treatment for obesity-mediated metabolic abnormalities, such as skeletal muscle mass, muscle strength, adiposity, hepatic steatosis, inflammation, body thermogenesis regulation and physical fitness.

Methods

Data source and search strategy

A structured review was performed using a systematic screening of the literature. The following criteria were used: experimental or clinical research with UA as a treatment for obesity or for the promotion of muscle mass loss and physical fitness.

All clinical and animal studies published in Portuguese, Chinese and English were carried out using the databases Medline and Google Scholar (up to December 2015); the keywords used were as follows: ursolic acid and adiposity/obesity, inflammation, strength/skeletal muscle/muscle mass loss, energy expenditure and/or hepatic steatosis, in either rodents or humans.

The inclusion criteria used were as follows: (1) rat and mouse studies, where UA administration was mixed in either a standard chow or high-fat diet (HFD), in drinking water, oral (gavage) or intraperitoneally, and the main investigation was body-weight change; and (2) human studies that evaluated only men; UA administration was via capsules, and the research was focused on body-weight changes.

The exclusion criteria used were as follows: (1) rodent and human studies related to cancer and (2) any study with *in vitro* assays.

Data extraction and reporting

Two reviewers (C. K. K. and V. R. S.) retrieved potentially eligible papers by searching through the titles and abstracts. After the initial search, data from studies, such as author's last name, publication year, studied model (rodents or humans) and methods (study design, UA outcomes), were obtained. Of the 63 screened studies, 17 fulfilled the eligibility criteria and were relevant for inclusion in the review (Fig. 1). In summary, our eligibility criteria were limited to studies that involved UA supplementation on adiposity, hepatic steatosis, energy expenditure and brown adipose tissue for the development of the following topics: (1) UA and thermogenesis; (2) UA and fat mass, and studies that investigated the effects of UA supplementation on atrophy, fasting and denervation-mediated muscle mass loss, with further elaboration of the topic; (3) UA and skeletal muscle mass and studies that investigated the effects of UA on exercise, physical capacity, muscle fibre type and mitochondrial biogenesis-associated muscle protein expression, for the preparation of the topic; and (4) UA potentiates physical fitness during the exercise.

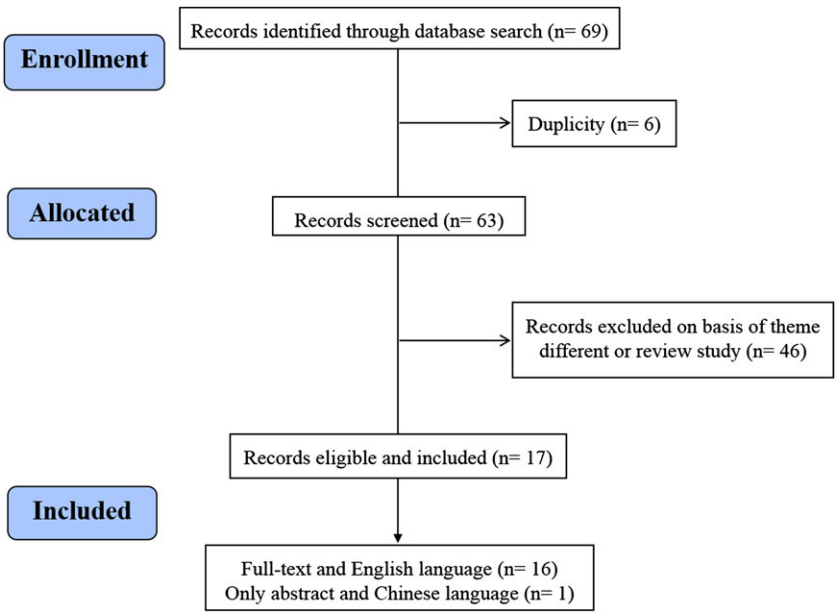

Figure 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses consort flow diagram of included studies. [Colour figure can be viewed at wileyonlinelibrary.com]

Quality and risk-of-bias assessment

A risk-of-bias assessment was systematically performed by two investigators (C. K. K. and V. R. S.), through an evaluation of each study, such as random sequence generation, housekeeping and supplementation conditions for rodents and inclusion and exclusion criteria and supplementation methods for humans. If any discrepancy was found, all findings were debated with another reviewer (G. D. P.), to establish an agreement between the studies.

Discussion

Ursolic acid and thermogenesis

Body thermogenesis control occurs according to food intake and energy expenditure (20,21). Thermogenesis is determined mainly by the functioning of the sympathetic nervous system and hormones, such as leptin and norepinephrine, which can modulate body homeostasis (20–23). Recently, it has been discovered that fat consumption, particularly saturated fatty acids, stimulates inflammatory pathways leading to a breakdown of the thermogenic signals sensitized by anorexigenic or thermogenic hormones, such as leptin, thyroid and glucagon-like peptide 1 in the central nervous system, which then leads to increased body mass (24–26). However, identifying foods that can help increase energy expenditure is considered as a possible therapeutic target because enhanced energy expenditure can stimulate brown adipose tissue, thus promoting the generation of heat and energy expenditure, through increased thermogenesis by the overexpression of UCP1 (20,27). Accordingly, UA has been described as increasing the function of brown fat, which leads to increased energy expenditure and body thermogenesis. Recently, it has been found that the HFD-induced obesity in mice is classically a glucose intolerance and hepatic steatosis model; consequently, after obesity induction, the authors divided the rodents into two groups. One group was fed with a 0.27% UA diet for 3 weeks (acute), and the other group was fed the same diet for 6 weeks (chronic). A monitoring system was used to measure the oxygen consumption and carbon dioxide production of the laboratory animals. The data verified that chronic treatment with UA increased energy expenditure via increased UCP1 protein expression in brown fat (9).

Based on this evidence, it was recently shown that animals treated with UA (0.5%) in their diet for 6 weeks had reduced body weight and triglyceride levels, accompanied by increased energy expenditure via the beta-oxidation of free fatty acids (1).

Some *in vitro* experiments performed in C2C12 muscle cells also showed that UA favours the activation of AMPK and other molecules involved in the beta-oxidation of free fatty acids. Additionally, uncoupling protein 3 in skeletal muscle activated AMPK and increased AMP and adenosine triphosphate, suggesting that UA burns free fatty acids by energy expenditure activation and AMPK-dependent beta-oxidation (1,28).

Recently, a new hormone called irisin or fibronectin type III domain-containing protein 5 (Fndc5) was described. Irisin is responsible for acting upon fat cells and increasing energy expenditure. The production of irisin can occur through physical exercise (27,29,30) because muscle contraction promotes the increased expression of peroxisome proliferator-activated receptor gamma co-activator (PGC1α), a co-activator that transcribes the *Fndc5* gene. The *Fndc5* is a transcript and leads to irisin production. In this context, the irisin hormone appears to be an important mediator for combating metabolic diseases (27,29,31,32) and increasing white adipose tissue thermogenesis. Table 1 describes the studies that evaluated the role of UA in energy expenditure.

Although there is scant current research, studies suggest that UA has an important biological function against obesity pathways via the activation of energy expenditure. Furthermore, evidence shows that UA negatively modulates the adiposity growth maintaining muscle mass. Figure 2 summarizes the findings by which UA acts on thermogenesis.

Ursolic acid and fat mass

Ursolic acid has gained great interest for its anti-obesity actions (1,4,7–12,19,28,33–40). Yan-xiang and co-authors (12) evaluated the effects of UA on body weight, blood glucose, insulin and leptin in HFD-induced obese mice with a dosage of 10 mg kg^{-1} d^{-1} for 20 weeks. Interestingly, the group treated with UA showed reduced body mass gain, serum leptin, insulin and glucose levels, with improved insulin resistance and metabolic disorders (12). Kunkel and colleagues showed lower adiposity (epididymal fat) in mice fed a standard diet supplemented with a 0.14% UA-supplemented diet for 7 weeks. Similar results were obtained when the mice were treated with a 0.27% UA-supplemented diet for 5 weeks; there was a reduction in adiposity and leptin concentrations (8).

Recently, Li and colleagues observed that in HFD-induced obese mice, a UA-supplemented diet (with 0.125%, 0.25% or 0.5%) for 6 weeks showed a decrease in body weight, hepatic steatosis and liver injury, which was accompanied by an increase in peroxisome proliferator-activated receptor-α (PPARα). Additionally, similar results were found *in vitro*, once the PPARα contributed to the anti-steatosis role of UA in liver human cell lines HL-7702. Further, treatment with UA favoured insulin signalling and protected against metabolic disorders, such as systemic oxidative stress and inflammatory mediators such as TNFα, chemokine (C-C motif) ligand 2, monocyte

Table 1 Ursolic acid on modulation of energy expenditure in rodents

Author, year	Study design and studied model	Methods	Results
Rao *et al.* (28) 2011	Male mice (n = 8) Placebo controlled	0.05%, 50 mg L^{-1}, ursolic acid in drinking water for 15 weeks	Increased β-oxidation in liver and energy expenditure.
Kunkel *et al.* (9) 2012	Male mice (n = 6–12) Placebo controlled	0.14% or 0.27%, ursolic acid in high-fat diet for 6 or 17 weeks	Decreased interscapular fat and increased body temperature via uncoupling protein 1 in brown fat and increase energy expenditure.
Chu *et al.* (1) 2015	Male rats (n = 6–12) Placebo controlled	0.5% ursolic acid-supplemented diet for 6 weeks	Increased β-oxidation, increase uncoupling protein 3 and energy expenditure.

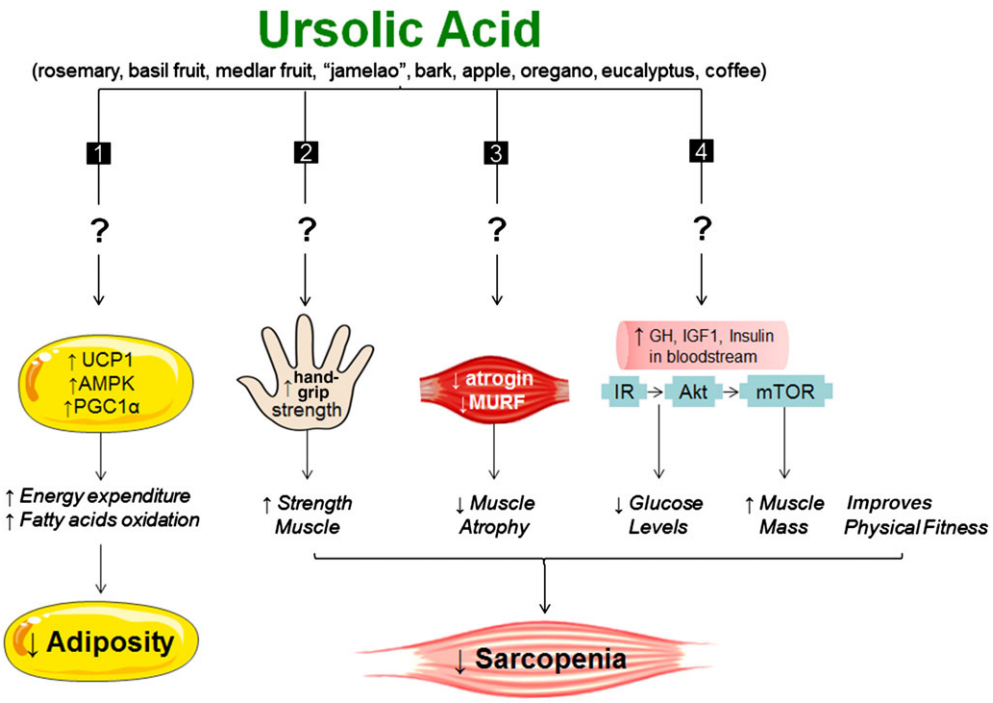

Figure 2 Summary of the findings by which the ursolic acid acts on obesity, skeletal muscle mass and physical fitness. Evidences have shown that ursolic acid (1) activates the uncoupling protein 1 (UCP1) and AMP-activated protein kinase (AMPK) in the tissue adipose enhancing the energy expenditure and fatty acid oxidation; (2) enhances hand grip strength; (3) attenuates the atrogin-1 and MuRF-1 in the skeletal muscle and blocks the muscle atrophy; and (4) induces the growth hormone (GH), insulin-like factor 1 (IGF-1) and insulin release, which binds to insulin receptor (IR) phosphorylating the insulin receptor substrates and protein kinase B attenuating the serum glucose concentrations. Additionally, increase of the Akt expression occurs, which leads to up-regulation of mammalian target of rapamycin (mTOR) into skeletal muscle stimulating the muscle protein synthesis.

chemoattractant protein-1, interleukin (IL)-1 beta, IL2, IL6 and IL8 (4).

In addition to the beneficial effects found by Kunkel *et al.* on adiposity in diet-induced obese mice exposed to a diet supplemented with 0.14% UA for 6 weeks, an increase in the PKB/Akt phosphorylation was also observed. This increase could improve glucose uptake by muscle in the control group. Likewise, it is known that UA (1 μg mL^{-1}) can sensitize the insulin receptor-mediated Akt and extracellular signal-regulated kinase as well as increase glucose transporter type 4 translocation. These effects suggest that UA seems to be beneficial for reducing glucose levels and diabetes (9,41). Additionally, it was observed that the treatment of cell lines with 2.5 to 10 μM UA attenuated in 3T3-L1 cells adipogenic transcription factors, such as PPARγ, enhancer binding protein alpha and beta, and the transcription of sterol regulatory element binding protein 1c, fatty acid synthase and fatty acid-binding protein 4. Moreover, there was an increase in the AMPK level through the inhibition of the liver kinase B1, suggesting a potential anti-obesity effect of UA (40). Although numerous evidence has shown the role of UA on anti-obesity effects and comorbidities (7,11,19,36–38,42), beneficial effects have also been described for various diseases, such as the inhibition of

tumour growth (5,6,43,44), sepsis attenuation (45), ageing (46), human immunodeficiency virus (47), inflammation (19) and rheumatoid arthritis (48,49). Table 2 systematically describes the studies that used UA in the modulation of adiposity in both rodents and humans.

Although UA plays an important role in chronic diseases, understanding how UA and its benefits interact in regulating body thermogenesis and adiposity is of fundamental interest for the development of nutritional strategies aimed at combating excess weight-related metabolic disorders. Figure 2 summarizes the findings that show how UA acts on adiposity.

Ursolic acid and muscle mass

The loss of muscle mass is linked with diabetes, cancer, chronic obstructive pulmonary disease, obesity and renal disease and leads to a marked reduction in muscle strength and function. Additionally, muscle atrophy is enhanced by denervation, immobilization and disuse (50–52). Studies have shown that UA is beneficial for weight loss and increased energy expenditure and can modulate muscle by the activation of protein synthesis and the inhibition of mass atrophy, ageing and denervation-mediated skeletal muscle mass loss (1,8,9,46). Moreover, UA associated with exercise can stimulate mTOR signalling activating protein synthesis (53,54).

Kunkel and colleagues observed that UA attenuates muscle atrophy under fasting conditions and muscle denervation and may increase hypertrophy (8). Intraperitoneal treatment (25 mg mL^{-1}) with UA was responsible for the maintenance of muscle mass and the increased diameter of muscle fibres via increased IGF-1 secretion. Furthermore, muscular atrophy pathway inhibition was observed, which was represented by atrogin-1, MuRF-1 and zinc finger AN1-type domain 5. At the same time, reduced adiposity, body weight, fasting blood glucose, cholesterol and triacylglycerol concentrations were found (8).

In the following year, the same group showed that UA was able to increase muscle mass and strength for HFD-induced obesity and insulin resistance in rodents. Interestingly, UA (0.14% of the diet with UA) was able to reverse the resistance for some intracellular proteins and increased Akt activity in the muscle, enhancing vascular endothelial growth factor and hexokinase II activity. The authors state that these proteins (vascular endothelial growth factor and hexokinase I) are induced by Akt signalling in the muscle, which uses more glucose, and the recruitment of the blood vessel. Moreover, enhanced strength was evaluated by grip strength. It is well known that beyond nutritional strategies, physical stimulus induces increased muscle mass (55,56). Likewise, it has also been verified that resistance exercise training associated with UA supplementation enhances the mTOR complex 1 (mTORC1) activation in rodent muscle (53). In this experiment, animals received electrical stimulation and a single intraperitoneal injection of UA (250 mg kg^{-1}); after the treatment, the animals were euthanized at 1 or 6 h after the treatment. The authors found that in the exercise group, which consumed a placebo, the resistance exercise associated with supplementation activated the p70S6K after 6 h. This finding suggests that UA potentiated the mTOR complex 1 (mTORC1) activation in skeletal muscle for a longer time than in the placebo/exercise group (53). Therefore, these data are of interest because the association of UA supplementation with physical exercise may enhance the mTOR muscle effect. Moreover, these outcomes would be of great importance for nutritional strategies related to ageing, disuse or disease-related muscle mass loss, such as obesity, diabetes, cancer, arthritis, and heart or kidney insufficiency.

Jeong and colleagues showed that in the placebo group, there was a dose-dependent increase in the AMPK, sirtuin 1 (SIRT1) and PGC1α expression in C2C12 cells, with UA extracted from apple leaves (183 mg g^{-1}, diluted 10 μg mL^{-1} and 50 μg mL^{-1}). Additionally, in the placebo group, similar data were found (10 and 50 μg mL^{-1}). However, similar results were also found with a placebo group (54). Similarly, UA increased the IGF-1 (50 μg mL^{-1}), Akt (10 and 50 μg mL^{-1}) and mTOR (50 μg mL^{-1}) expression and muscle hypertrophy-induced proteins, accompanied by the attenuation of the atrogin-1 and MuRF-1 proteins (50 μg mL^{-1}) (classical proteins of muscle atrophy). Both groups had reduced atrogin-1 and MuRF-1 expression levels. The creatine group modulated only the Akt (10 and 50 μg mL^{-1}), MuRF-1 (50 μg mL^{-1}) and atrogin-1 (10 μg mL^{-1}). These data indicate that UA is a compound that is able to regulate the proteins involved in mitochondrial biogenesis, increased hypertrophy and controlled muscle atrophy (54). Chronic treatment with UA for 12 weeks in mice led to an increase in muscle strength and posterior muscle weight in a dose-dependent manner, thus demonstrating that the higher UA concentrations led to higher forelimb grip muscle strength using the grip meter and higher muscle mass via enhanced Akt/mTOR signalling (75, 150 and 300 mg kg^{-1}) and the attenuation of the muscle atrophy pathway, such as atrogin 1 and MuRF-1 (54). Therefore, these data indicate that UA modulates muscle hypertrophy and grip strength by reducing atrophy, whether *in vitro* or *in vivo*.

In healthy humans, resistance training (RT) was performed for 8 weeks, and the participants were divided into two groups: RT and RT plus UA supplementation or placebo (450 mg oral dose). The exercise protocol was established with 26 exercise types, with 10 repetitions at 60–80% of one repetition maximum for 6 d in a week (10). In the trained group supplemented with UA (450 mg oral dose), there was a decrease in the body fat percentage, but the body mass index, lean body mass, and glucose and insulin levels remained unchanged. Additionally, a significant increase between the baseline data and the trained

Table 2 Ursolic acid on adiposity and blood metabolic alterations in rodents and humans

Author, year	Study design and studied model	Methods	Results
Huang *et al.* (33) 2005	Male rats (n = 5) Placebo controlled	Pomegranate flower extract (500 mg kg^{-1}) orally administered for 6 weeks	Decrease hyperglycaemia, hyperlipidemia and lipopolysaccharide.
Jayaprakasam *et al.* (36) 2006	Male mice (n = 5–8) Placebo controlled	(500 mg kg^{-1}) added to high-fat diet for 8 weeks	Decreased lipid in the liver and triacylglycerol Increase the glucose tolerance.
Kim *et al.* (37) 2009	Male rats (n = 4) Placebo controlled	(50–100 mg kg^{-1}) orally administered with 3 mL of lipid emulsion in single	Decrease pancreatic lipase activity. Increase lipolysis in the adipocytes.
Rao *et al.* (28) 2011	Male mice (n = 8) Placebo controlled	(0.05%, 50 mg L^{-1}, in drinking water) for 15 weeks	Decreased body weight, visceral adiposity, blood glucose total cholesterol, triglycerides and ghrelin.
Lu *et al.* (39) 2011	Male mice (n = 5–10) Placebo controlled	(10 mg kg^{-1} d^{-1}) oral administration of the ursolic acid for 20 weeks	Decreased endoplasmic reticulum stress IκB kinase β/ nuclear factor-κB and markers of inflammatory signalling. Restoration of insulin signalling phosphoinositide 3-kinase/ Akt/mammalian target of rapamycin in hippocampus.
Kunkel *et al.* (8) 2011	Male mice (n = 4–10) Placebo controlled	Standard chow supplemented with 0.27% of ursolic acid for 5 weeks or administration of ursolic acid (200 mg kg^{-1}) via intraperitoneal injection twice daily for 7 days	Increased Akt activity in the skeletal muscle. Decreased adiposity, fasting blood glucose, cholesterol and triglycerides.
Sundaresan *et al.* (7) 2012	Male mice (n = 10) Placebo controlled	(5 mg kg^{-1}) oral administration for 5 weeks	Decreased body weight, epididymal fat, cholesterol, triglycerides, blood pressure and increased insulin sensitivity.
Kunkel *et al.* (9) 2012	Male mice (n = 6–12) Placebo controlled	High-fat diet containing 0.14% or 0.27% ursolic acid for 6 or 17 weeks	Increased Akt activity and phosphorylation in the skeletal muscle and decreased resting heart rate, epididymal fat, retroperitoneal fat, hepatic steatosis and body weight.
Yan-xiang *et al.* (12) 2013	Male mice (n = 15) Placebo controlled	Fed ursolic acid every day with 10 mg kg^{-1} for 20 weeks	Attenuated body-weight gain, decreased insulin, leptin, glucose and insulin concentrations.
Kazmi *et al.* (11) 2013	Female mice (n = 6) Placebo controlled	High-Fat Diet containing (1 and 2%) ursolic acid for 9 weeks	Decreased body weight, insulin resistance, lipid parameters, parametrial adipose tissue weight, liver triglyceride and different organ weight.
	Male rats (n = 6) Placebo controlled	(500–1000 mg kg^{-1}) of ursolic acid administered with 1 mL of lipid emulsion orally measured from 0.5 to 5 h	Decreased triglyceride level plasma.

(*Continues*)

Table 2 (Continued)

Author, year	Study design and studied model	Methods	Results
Li *et al.* (4) 2014	Male rats (n = 10–13) Placebo controlled	0.125%, 0.25% and 0.5% ursolic acid-supplemented diet for another 6 weeks	Increased hepatic peroxisome proliferator-activated receptor-α, phospho-AMPK, CPT-1 protein expression in skeletal muscle and adiponectin plasma. Decrease body weight, perirenal and epididymal fat, hepatic steatosis, insulin and serum leptin.
Bang *et al.* (10) 2014	Men-humans (n = 7–9) Single blind and placebo controlled	Leaf extract 450 mg ursolic acid orally ingested 1 capsule 3 times per day for 8 weeks	Decreased body fat percentage, increased serum irisin concentrations.
Chu *et al.* (1) 2015	Male rats (n = 6–12) Placebo controlled	0.5% ursolic acid-supplemented diet for 6 weeks	Increase the AMP-activated protein kinase in muscle Decrease body weight, triglycerides, insulin resistance in the muscle skeletal.

AMPK, AMP-activated protein kinase.

control group, was found in IGF1, irisin concentrations, and maximal isokinetic muscle strength (peak torque using a dynamometer). The authors reported that the elevation of UA-mediated irisin concentrations may be useful for improving skeletal muscle strength during RT (10); however, there was no direct explanation, either physiological or molecular, in support of this finding. Although there is little research that combines exercise and UA, we can partially suggest that this supplement, in conjunction with resistance exercise, is able to fight muscle mass loss-related metabolic diseases, mainly by stimulating anabolic pathways (mTOR and IGF-1) and inhibiting muscular atrophy via MuRF-1 and atrogin. Table 3 describes the studies that evaluated the effects of UA supplementation associated or not associated with resistance exercise on skeletal muscle mass and strength.

Although there are few studies on UA involving skeletal muscle mass, some evidence shows that UA may be an important therapeutic target in muscle atrophy control and in increased hypertrophy and can act as a potent mediator in the attenuation of disorders related to muscular dystrophies, such as sarcopenia and metabolic diseases. Figure 2 summarizes the findings that suggest that UA acts on the skeletal muscle mass.

Ursolic acid potentiates of physical fitness during the exercise

Similar to the experiments performed in obese animals, it was also observed in older rodents that intraperitoneal treatment with UA (200 mg kg^{-1}) for 7 days (46) increased the sirtuin 1 and PGC1α expression responsible for mitochondrial biogenesis in the rectus femoris, tibialis, gastrocnemius and gluteus. Thus, this UA treatment enhanced the proliferation of satellite cells. Additionally, the authors observed that UA stimulated myoglobin expression and increased the type IIA fibre (called oxidative intermediate fibre/glycolytic) amount. These data provide evidence that UA may potentiate physical performance because myoglobin is a classical protein responsible for oxygen transport, and type IIA fibres are more resistant to fatigue (46).

Although there are few studies evaluating the effects of UA and the improvement of physical capacity, some evidence shows that this natural compound can be combined with strength gain and adiposity reduction. Likewise, it was observed that a concentration of 0.27% UA added to the diet for 17 weeks induced muscle hypertrophy by stimulating the muscle Akt pathway and enhanced the size of fast-twitch muscle fibres and oxidative slow-twitch fibres. Therefore, UA was able to increase the physical ability in rodents that underwent treadmill exercise (2 m for every 2 min). Thus, UA improves more the animals physical performance and reduces more resting heart rate than exercise alone (9).

Jeong *et al.* (54) found that treatment with UA for 12 weeks in dependent doses (75, 150 and 300 mg kg^{-1}) increased performance in mice. Further, increased muscle strength and reduced fatigue were verified through endurance tests on crawlers (15–20 meters.min^{-1} up to exhaustion). The animals treated with UA also tolerated longer

Table 3 Summary of studies that evaluated the role of dietary ursolic acid on skeletal muscle mass with focus on muscle anabolism and atrophy pathways in rodents and humans

Author, year	Study design and studied model	Methods	Results
Jeong *et al.* (54) 2015	Male Mice (n = 10) Placebo controlled	Apple pomace extract (183 mg g^{-1}) fed with AIN-93G diets supplemented with 75 mg kg^{-1} for 12 weeks	Reduces lactate, AST, ALT, ALP and creatinine.
	Male mice (n = 10) Placebo controlled	Fed AIN-93G diets supplemented with 150 mg kg^{-1} for 12 weeks	Increase grip strength, muscle weight and performance Reduces atrogin-1.
	Male mice (n = 10) Placebo controlled	Fed AIN-93G diets supplemented with 300 mg kg^{-1} for 12 weeks	Reduces lactate, LDH, AST, ALT, ALP and creatinine Reduces MuRF-1 and atrogin-1 Increase grip strength, muscle weight and performance.
Xu *et al.* (57) 2015	Female rats (n = 5) Placebo controlled	Intraperitoneally administered 20 and 100 mg kg^{-1} d^{-1} from day 7 to day 41	Decrease IL17 Increase IL10 Improvement Experimental autoimmune myasthenia gravis.
Bakhtiari *et al.* (46) 2015	Male mice (n = 5) Placebo controlled	200 mg kg^{-1} twice a day for 7 days	Decrease ATP and ADP Increase S1RT1 and PGC1α Increase myoglobin, fibre IIA type Enhances satellites cell proliferation, skeletal muscle and increase performance.
Bang *et al.* (10) 2014	Men-humans (n = 7–9) Single blind and placebo controlled	By mouth (450 mg) 16 healthy male participants (age, 29.3 ± 5.1 years; body mass index = 27.1 ± 2.1 kg m^{-2})	Decrease fat mass Increase IGF-1, irisin and muscle strength.
Ogasawara *et al.* (53) 2013	Male rats (n = 5) Placebo controlled	Intraperitoneal treatment (250 mg kg^{-1}) in rats (injected intraperitoneally immediately after exercise)	Increase mTORC1/p70S6K and PRAS40Thr246
Kunkel *et al.* (9) 2012	Male mice (n = 5–12) Placebo controlled	0.14% added to high-fat diet in mice for 6 weeks	Increase Akt phosphorylation in muscle Utilization of glucose in muscle, hexokinase II, blood vessel recruitment of vascular endothelial growth factor A Increase IGF-1, muscle mass, fast and slow fibre size Reduce obesity and improve glucose tolerance Decrease liver weight, hepatic triglyceride content.
	Male mice (n = 7–12) Placebo controlled	0.27% added to high-fat diet in mice for 3 days or 6 or 17 weeks	Decrease expression (acetyl-CoA carboxylase 1, fatty acid synthase and stearoyl CoA desaturase-1) Decrease interscapular white fat Decrease hepatocellular steatosis and plasma aminotransferases

(*Continues*)

Table 3 (Continued)

Author, year	Study design and studied model	Methods	Results
			Increase interscapular brown fat, UCP1 and core temperature at 4°C Increase grip strength and exercise capacity.
Kunkel *et al.* (8) 2011	Male mice (n = 16) Placebo controlled	Dissolved in corn oil at a concentration of 20 mg mL^{-1}. The treatment intraperitoneal was (200 mg kg^{-1}) or vehicle (with oil) via intraperitoneal injection twice daily for 7 days	Decrease denervation-induced muscle atrophy and muscle loss Increase in the size of denervated skeletal muscle fibres.
	Male mice (n = 4–10) Placebo controlled	0.27% added to high-fat diet in mice for 5 weeks	Increase grip strength, hypertrophy, IGF-1 and fibre diameter.
	Male mice (n = 10) Placebo controlled	0.14% added to high-fat diet in mice for 7 weeks	Decrease muscle atrophy (MuRF-1 and ZFAND5), epididymal and retroperitoneal fat depots Reduces in plasma leptin levels, triglyceride and cholesterol Increase skeletal muscle weight and Akt activity.

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ATP, adenosine triphosphate; IGF-1, insulin-like factor 1; IL, interleukin; LDH, lactate dehydrogenase; MuRF-1, muscle ring-finger protein-1; UCP1, uncoupling protein 1; ZFAND5, zinc finger AN1-type domain 5.

exercise time (in minutes) and, consequently, greater distance (metres). Compared with the other groups, in animals treated with UA, blood tests also showed that lactate levels, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and creatinine were lower. These findings imply that in addition to improving performance, UA may be effective in controlling muscle fatigue. Figure 2 summarizes the findings that UA acts on physical fitness.

Safety dose of dietary ursolic acid and side effects in humans

Currently, the impact of UA supplementation on muscle atrophy, obesity, strength and aerobic capacity has been appreciated in clinical practice. Thus, Bang and colleagues studied 16 healthy men who were administered UA supplementation. The groups were divided into RT and RT plus UA supplementation (RT + UA). The subjects received oral UA (*rosemary leaf extract*) or placebo (*guar gum*) three times per day, containing 150 mg per capsule, totalling 450 mg d^{-1} for 8 weeks. Compared to RT alone, this supplementation associated with RT was sufficient to increase the IGF-1 and irisin concentrations (10). Accordingly, this study suggests that a daily recommendation of 450 mg UA divided into three doses of 150 mg d^{-1}, together with meals, may be considered appropriate, but additional studies are necessary to establish a plausible dose. However, no data on the side effects of UA in humans have been reported.

By the end of the preparation of this review (up to December 2015), no clinical trial on UA appeared in the literature but only an article about the above-described approach in humans, which is noteworthy. Indeed, UA up to 450 mg d^{-1} did not induce side effects. Dietary UA supplementation may be a new non-drug preventive method, which is probably safe for human health and for use in the prevention and treatment of obesity and muscle low-linked chronic diseases, when consumed in moderation and recommended by a nutritionist.

Data quality and study limitations

Our analysis of the literature covers studies that have been published up to December 2015. Database searches found 69 studies, of which 17 (24.5%) were eligible and included 16 (94%) studies on rodents and only one (6%) on a human (Tables 2 and 3). The human study was placebo controlled and single blinded, and while animal studies were also placebo controlled, these were unblinded.

For studies involving an analysis of thermogenesis and fat mass (1,9,20–32), 94% of studies were performed on mice and rats that received different diets and variable modes of

UA administration (i.e. gavage, drinking water, intraperitoneal injection or UA-enriched food). In addition, UA dosage, source (i.e. apple pomace, rosemary leaf extract or pomegranate flower extract) and administration duration (7 d to 17 weeks) were variable. The sole study on a human (10) revealed a reduction in body fat. Thus, even though 'thermogenesis and fat mass' were mainly investigated in animals, it appears to be more studied and discussed than other topics, such as the impact of UA on muscle mass and physical fitness.

For studies analysing the modulation of the skeletal muscle mass in relation with UA, a considerable heterogeneity was found, mainly owing to different methodologies. For example, a study on rats used electrical stimulation to mimic bouts of RT exercise and associated it with UA supplementation (53), while a second study (54) was performed in vitro (C2C12 cells) and verified that UA treatment stimulates the mTOR pathways and attenuate the atrophy molecules, and a third study in healthy subjects (10) on RT found a reduction in fat mass and an increase in IGF concentration.

This heterogeneity also applies to studies that evaluated the impact of UA on muscle mass and physical fitness: a study performed using a HFD (9), one on ageing (46) and one on rodents fed utilizing the AIN-93G diet (associated or not to exercise on a treadmill) (54). On the other hand, the only study performed on a human had good experimental design (10) and confirmed the evidence found in rodents (i.e. UA reduced fat mass and increased serum IGF concentrations).

In summary, this review showed a low risk of methodological bias among animal studies, a risk that is impossible to assess using human data owing to the unavailability of further test results.

Conclusions

This systematic review shows that UA can to ameliorate obesity and increase muscle mass and physical fitness. The effects on adiposity may occur by an attenuation of adipocyte transcription factors and the activation of AMPK and energy expenditure. Additionally, enhanced muscle mass may be due to serum growth hormone and IGF secretion and the skeletal muscle mTOR pathway, and improved physical fitness may be via an increase in muscle sirtuin 1 and PPARγ co-activator expression and new muscle satellite cell generation.

Author contributors

C. K. K., V. R. S., T. L. G., C. P. and G. D. P. performed the design of the study, researched and discussed the included articles, and all authors wrote the paper. All authors read and approved the final version of the manuscript.

Conflict of interest statement

No conflict of interest was declared.

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References

1. Chu X, He X, Shi Z *et al.* Ursolic acid increases energy expenditure through enhancing free fatty acid uptake and β-oxidation via an UCP3/AMPK-dependent pathway in skeletal muscle. *Mol Nutr Food Res.* 2015; **59**: 1491–1503.
2. Shishodia S, Majumdar S, Banerjee S, Aggarwal BB. Ursolic acid inhibits nuclear factor-kappaB activation induced by carcinogenic agents through suppression of IkappaBalpha kinase and p65 phosphorylation: correlation with down-regulation of cyclooxygenase 2, matrix metalloproteinase 9, and cyclin D1. *Cancer Res.* 2003; **63**: 4375–4383.
3. Ikeda Y, Murakami A, Ohigashi H. Ursolic acid: an anti- and pro-inflammatory triterpenoid. *Mol Nutr Food Res.* 2008; **52**: 26–42.
4. Li S, Liao X, Meng F *et al.* Therapeutic role of ursolic acid on ameliorating hepatic steatosis and improving metabolic disorders in high-fat diet-induced non-alcoholic fatty liver disease rats. *PLoS One.* 2014; **9**: e86724.
5. Lin J, Chen Y, Wei L, Hong Z, Sferra TJ, Peng J. Ursolic acid inhibits colorectal cancer angiogenesis through suppression of multiple signaling pathways. *Int J Oncol.* 2013; **43**: 1666–1674.
6. Wang X, Zhang F, Yang L *et al.* Ursolic acid inhibits proliferation and induces apoptosis of cancer cells in vitro and in vivo. *J Biomed Biotechnol.* 2011; **2011**: 419343.
7. Sundaresan A, Harini R, Pugalendi KV. Ursolic acid and rosiglitazone combination alleviates metabolic syndrome in high fat diet fed C57BL/6 J mice. *Gen Physiol Biophys.* 2012; **31**: 323–333.
8. Kunkel SD, Suneja M, Ebert SM *et al.* mRNA expression signatures of human skeletal muscle atrophy identify a natural compound that increases muscle mass. *Cell Metab.* 2011; **13**: 627–638.
9. Kunkel SD, Elmore CJ, Bongers KS *et al.* Ursolic acid increases skeletal muscle and brown fat and decreases diet-induced obesity, glucose intolerance and fatty liver disease. *PLoS One.* 2012; **7**: e39332.
10. Bang HS, Seo DY, Chung YM *et al.* Ursolic acid-induced elevation of serum irisin augments muscle strength during resistance training in men. *Korean J Physiol Pharmacol.* 2014; **18**: 441–446.
11. Kazmi I, Afzal M, Rahman S, Iqbal M, Imam F, Anwar F. Antiobesity potential of ursolic acid stearoyl glucoside by inhibiting pancreatic lipase. *Eur J Pharmacol.* 2013; **709**: 28–36.
12. Yan-xiang L. Research on the anti-obesity effect of ursolic acid with experimental hyperlipidemia mice. *West China Journal of Pharmaceutical Sciences.* 2013; **28**: 264–266.
13. Kob R, Bollheimer LC, Bertsch T *et al.* Sarcopenic obesity: molecular clues to a better understanding of its pathogenesis? *Biogerontology.* 2015; **16**: 15–29.
14. Woźniak Ł, Skąpska S, Marszałek K. Ursolic acid—a pentacyclic triterpenoid with a wide spectrum of pharmacological activities. *Molecules.* 2015; **20**: 20614–20641.

15. Doherty TJ. Invited review: aging and sarcopenia. *J Appl Physiol (1985)* 2003; **95**: 1717–1727.
16. Stenholm S, Harris TB, Rantanen T, Visser M, Kritchevsky SB, Ferrucci L. Sarcopenic obesity: definition, cause and consequences. *Curr Opin Clin Nutr Metab Care*. 2008; **11**: 693–700.
17. Bastos DH, Rogero MM, Arêas JA. Effects of dietary bioactive compounds on obesity induced inflammation. *Arq Bras Endocrinol Metabol*. 2009; **53**: 646–656.
18. Torres-Fuentes C, Schellekens H, Dinan TG, Cryan JF. A natural solution for obesity: bioactives for the prevention and treatment of weight gain. A review. *Nutr Neurosci*. 2015; **18**: 49–65.
19. Zhang WL, Zhu L, Jiang JG. Active ingredients from natural botanicals in the treatment of obesity. *Obes Rev*. 2014; **15**: 957–967.
20. Lowell BB, Spiegelman BM. Towards a molecular understanding of adaptive thermogenesis. *Nature*. 2000; **404**: 652–660.
21. van der Klaauw AA, Farooqi IS. The hunger genes: pathways to obesity. *Cell*. 2015; **161**: 119–132.
22. Schwartz MW, Woods SC, Porte D, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature*. 2000; **404**: 661–671.
23. Claussnitzer M, Dankel SN, Kim KH *et al*. FTO obesity variant circuitry and adipocyte browning in humans. *N Engl J Med*. 2015; **373**: 895–907.
24. van de Sande-Lee S, Pereira FR, Cintra DE *et al*. Partial reversibility of hypothalamic dysfunction and changes in brain activity after body mass reduction in obese subjects. *Diabetes*. 2011; **60**: 1699–1704.
25. López M, Lage R, Saha AK *et al*. Hypothalamic fatty acid metabolism mediates the orexigenic action of ghrelin. *Cell Metab*. 2008; **7**: 389–399.
26. Drucker DJ. Minireview: the glucagon-like peptides. *Endocrinology*. 2001; **142**: 521–527.
27. Boström P, Wu J, Jedrychowski MP *et al*. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature*. 2012; **481**: 463–468.
28. Rao VS, de Melo CL, Queiroz MG *et al*. Ursolic acid, a pentacyclic triterpene from *Sambucus australis*, prevents abdominal adiposity in mice fed a high-fat diet. *J Med Food*. 2011; **14**: 1375–1382.
29. Wu J, Boström P, Sparks LM *et al*. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell*. 2012; **150**: 366–376.
30. Jedrychowski MP, Wrann CD, Paulo JA *et al*. Detection and quantitation of circulating human irisin by tandem mass spectrometry. *Cell Metab*. 2015; **22**: 734–740.
31. Tsuchiya Y, Ando D, Takamatsu K, Goto K. Resistance exercise induces a greater irisin response than endurance exercise. *Metabolism*. 2015; **64**: 1042–1050.
32. Nygaard H, Slettaløkken G, Vegge G *et al*. Irisin in blood increases transiently after single sessions of intense endurance exercise and heavy strength training. *PLoS One*. 2015; **10**: e0121367.
33. Huang TH, Yang Q, Harada M *et al*. Pomegranate flower extract diminishes cardiac fibrosis in Zucker diabetic fatty rats: modulation of cardiac endothelin-1 and nuclear factor-kappaB pathways. *J Cardiovasc Pharmacol*. 2005; **46**: 856–862.
34. Na M, Yang S, He L *et al*. Inhibition of protein tyrosine phosphatase 1B by ursane-type triterpenes isolated from *Symplocos paniculata*. *Planta Med*. 2006; **72**: 261–263.
35. Zhang W, Hong D, Zhou Y *et al*. Ursolic acid and its derivative inhibit protein tyrosine phosphatase 1B, enhancing insulin receptor phosphorylation and stimulating glucose uptake. *Biochim Biophys Acta*. 1760; **2006**: 1505–1512.
36. Jayaprakasam B, Olson LK, Schutzki RE, Tai MH, Nair MG. Amelioration of obesity and glucose intolerance in high-fat-fed C57BL/6 mice by anthocyanins and ursolic acid in Cornelian cherry (*Cornus mas*). *J Agric Food Chem*. 2006; **54**: 243–248.
37. Kim J, Jang DS, Kim H, Kim JS. Anti-lipase and lipolytic activities of ursolic acid isolated from the roots of *Actinidia arguta*. *Arch Pharm Res*. 2009; **32**: 983–987.
38. Li Y, Kang Z, Li S, Kong T, Liu X, Sun C. Ursolic acid stimulates lipolysis in primary-cultured rat adipocytes. *Mol Nutr Food Res*. 2010; **54**: 1609–1617.
39. Lu J, Wu DM, Zheng YL *et al*. Ursolic acid improves high fat diet-induced cognitive impairments by blocking endoplasmic reticulum stress and IκB kinase β/nuclear factor-κB-mediated inflammatory pathways in mice. *Brain Behav Immun*. 2011; **25**: 1658–1667.
40. He Y, Li Y, Zhao T, Wang Y, Sun C. Ursolic acid inhibits adipogenesis in 3T3-L1 adipocytes through LKB1/AMPK pathway. *PLoS One*. 2013; **8**: e70135.
41. Jung SH, Ha YJ, Shim EK *et al*. Insulin-mimetic and insulin-sensitizing activities of a pentacyclic triterpenoid insulin receptor activator. *Biochem J*. 2007; **403**: 243–250.
42. Liu JJ, Toy WC, Liu S *et al*. Acetyl-keto-β-boswellic acid induces lipolysis in mature adipocytes. *Biochem Biophys Res Commun*. 2013; **431**: 192–196.
43. Yie Y, Zhao S, Tang Q *et al*. Ursolic acid inhibited growth of hepatocellular carcinoma HepG2 cells through AMPKα-mediated reduction of DNA methyltransferase 1. *Mol Cell Biochem*. 2015; **402**: 63–74.
44. Pathak AK, Bhutani M, Nair AS *et al*. Ursolic acid inhibits STAT3 activation pathway leading to suppression of proliferation and chemosensitization of human multiple myeloma cells. *Mol Cancer Res*. 2007; **5**: 943–955.
45. Chen X, Wan Y, Zhou T, Li J, Wei Y. Ursolic acid attenuates lipopolysaccharide-induced acute lung injury in a mouse model. *Immunotherapy*. 2013; **5**: 39–47.
46. Bakhtiari N, Hosseinkhani S, Tashakor A, Hemmati R. Ursolic acid ameliorates aging-metabolic phenotype through promoting of skeletal muscle rejuvenation. *Med Hypotheses*. 2015; **85**: 1–6.
47. Kashiwada Y, Nagao T, Hashimoto A *et al*. Anti-AIDS agents
38. Anti-HIV activity of 3-*O*-acyl ursolic acid derivatives. *J Nat Prod*. 2000; **63**: 1619–1622.
48. Baek SY, Lee J, Lee DG *et al*. Ursolic acid ameliorates autoimmune arthritis via suppression of Th17 and B cell differentiation. *Acta Pharmacol Sin*. 2014; **35**: 1177–1187.
49. Kang SY, Yoon SY, Roh DH *et al*. The anti-arthritic effect of ursolic acid on zymosan-induced acute inflammation and adjuvant-induced chronic arthritis models. *J Pharm Pharmacol*. 2008; **60**: 1347–1354.
50. Milan G, Romanello V, Pescatore F *et al*. Regulation of autophagy and the ubiquitin–proteasome system by the FoxO transcriptional network during muscle atrophy. *Nat Commun*. 2015; **6**: 6670.
51. Wall BT, Snijders T, Senden JM *et al*. Disuse impairs the muscle protein synthetic response to protein ingestion in healthy men. *J Clin Endocrinol Metab*. 2013; **98**: 4872–4881.
52. Wall BT, Dirks ML, Snijders T, Senden JM, Dolmans J, van Loon LJ. Substantial skeletal muscle loss occurs during only 5 days of disuse. *Acta Physiol (Oxf)*. 2014; **210**: 600–611.
53. Ogasawara R, Sato K, Higashida K, Nakazato K, Fujita S. Ursolic acid stimulates mTORC1 signaling after resistance exercise in rat skeletal muscle. *Am J Physiol Endocrinol Metab*. 2013; **305**: E760–E765.
54. Jeong JW, Shim JJ, Choi ID *et al*. Apple pomace extract improves endurance in exercise performance by increasing

strength and weight of skeletal muscle. *J Med Food*. 2015; **18**: 1380–1386.
55. Oates BR, Glover EI, West DW, Fry JL, Tarnopolsky MA, Phillips SM. Low-volume resistance exercise attenuates the decline in strength and muscle mass associated with immobilization. *Muscle Nerve*. 2010; **42**: 539–546.
56. Phillips SM. A brief review of critical processes in exercise-induced muscular hypertrophy. *Sports Med*. 2014; **44**(Suppl 1): S71–S77.
57. Xu H, Zhang M, Li XL *et al*. Low and high doses of ursolic acid ameliorate experimental autoimmune myasthenia gravis through different pathways. *J Neuroimmunol*. 2015; **281**: 61–67.