Effect of apple peel extract supplementation in skeletal muscle of aged mice

by

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Abstract

Apple peels contain naturally occurring bioactive compounds called triterpenoids that process properties that can enhance muscle mass and function. Triterpenoids exhibit a wide range of biological functions including antioxidant, anti-microbial, anti-inflammatory, and other anticancer activities. Triterpenoids also possess anabolic properties without androgenic side effects, therefore they might be a viable treatment for promoting muscle maintenance and growth. The expanded literature review, focused on the effects of apple peel extract on muscle health, replaced my research project. The molecular pathways were examined to understand how atrophy and hypertrophy occur in skeletal muscle. Several studies discussed the effects triterpenoids paired with resistance exercise, with a high fat diet, or alone can cause skeletal muscle to increase in mass, strength by increasing IGF-1, and the PKI3/Akt/mTORC signaling pathway.

Introduction

Ageing is a multifactorial process involving biochemical and morphological changes at the cellular level that affects the organism as a whole (Goljanek-Whysall, 2016). Some effects of aging are morphological changes (it affects cells, organs and tissues), changes in the functioning of all body systems, loss of the ability to adapt to stress situations and to recover (Pinzón-Ríos). An organism's quality of life is altered typically around 55 years of age from age-induced muscle tissue loss (muscle atrophy) with the subsequent loss of strength called sarcopenia (Sebastián, 2016).

Sarcopenia and Aging

Muscle mass and force decreases as age increases, and is a normal part of the biological aging process (Goljanek-Whysall, 2016). Sarcopenia can happen in a wide range of elderly adults. There is a prevalence of sarcopenia in 15% of heathy older adults, 76% of acutely hospitalized patients, and up to 69% of patients admitted post-acute geriatric rehabilitation (Pacifico, 2020). Individuals with dementia and cardiovascular disease have the highest prevalence of sarcopenia (Pacifico, 2020). Age-related sarcopenia can arise from a multitude of factors including inactivity, poor nutrition, and chronic illness and is associated with disability, hospital admissions, and death (Giallauria, 2015). It complicates a wide range of severe human illnesses, including diabetes, cancer, chronic renal failure, congestive heart failure, chronic respiratory disease, acute critical illness, chronic infections such as HIV/ AIDS, spinal cord injury (SCI), muscle denervation, and many other medical and surgical conditions that limit muscle use (Kunkle, 2011).

Strength, stamina, balance, and the ability to adequately move around is dependent on skeletal muscle (Janssen, 2002). Elderly patients who have difficulties balancing, moving around, and standing up are directly linked with sarcopenia. This can lead to falling and possible hospitalization. There has also been a link between aging and a decrease in anabolic hormones such as growth hormone and insulin-like growth factor-1 (IGF-1), which can lead to sarcopenia (Sitt, 2014). Muscle fibers can be divided into slow twitch such as the

soleus and fast twitch such as the extensor digitorum longus (EDL, Ciciliot, 2013). Muscle atrophy occurs preferentially in certain fiber types or is accompanied by shifts in fiber type profile, as muscle disuse can cause a slow to fast shift in fiber type (Ciciliot, 2013). Patients who have denervation, are bed ridden, or have hind limp immobilization experienced fiber type shifts from slow to fast (Ciciliot, 2013). Muscle atrophy in eldery patients continues to be an area that lacks the necessary treatments. However, it has been suggested that interventional strategies, such as exercise and nutrition should be implemented by the fifth decade of life to help prevent some of these adverse effects (Janssen, 2002).

Exercise Interventions for Combating Sarcopenia

Exercise and nutrition have been demonstrated as interventions to slow down sarcopenia. In age groups older than 60 years the prevalence of sarcopenia is at 10%, and in adults older than 80, sarcopenia prevalence rises to >50% (Fragala, 2019). The rate of decline in muscle strength with age is 2-5 times greater than declines in muscle size (Fragala, 2019). Exercise has been found to slow down the effects of sarcopenia through the homeostatic equilibrium of skeletal muscle by the stimulation of anabolic hormones, up-regulation of antioxidant enzymes, reduced inflammation, improved muscle insulin sensitivity, and increased protein synthesis (Giallauria, 2015). The maintenance of skeletal muscle mass is a delicate balance between muscle protein synthesis and breakdown. Markers of muscle protein synthesis are elevated and autophagy is depressed after bouts of resistance exercise (Fry, 2013).

The previously mentioned mechanisms are benefits of exercise on the muscular system to combat sarcopenia among older people. A form of exercise that has been well documented for the prevention of sarcopenia in the elderly is resistance training. Resistance training is a form of weight lifting that improves muscular strength and endurance, as well as to stimulate muscle protein synthesis. Properly designed resistance training programs may counteract changes in contractile function, atrophy, and morphology of aging human skeletal

muscle (Fragala, 2019). Resistance training in adults 85 years and older increased skeletal muscle mass by 4-33% (Fragala, 2019).

In aging adults, muscular strength, power, and neuromuscular functioning have the possibility to be enhanced through training programs (Fragala, 2019). When performed 2-3 days per week, favorable neuromuscular adaptations in adults can occur through resistance exercise training by improving muscle mass, function, and power output (Fragala, 2019; Hakkinen, 1998). Hypertrophy, the increase of muscle mass, is suggested to be a benefit from consistent resistance exercise training. Muscle cross sectional area (CSA) of the legs have been shown to increase in size after resistance exercise training programs in both younger and older men (Hakkinen, 1998). Studies have found that maximal peak force and explosive strength training increase both muscle mass and maximal isometric strength in older adults (Hakkinen, 1998).

Nutritional Interventions for Combating Sarcopenia

Nutrition is an important part of maintaining proper bodily functions especially in the skeletal muscle system. Age has been linked to a decrease in intake of food quantity, most likely due to changes in appetite hormones, loss in taste, smell, and chewing abilities (Wakimoto, 2001). Poor nutrition intake (monogamous diets and decreased food intake), has been suggested as a link to sarcopenia and frailty in older adults (Robinson, 2017). Fueling the body with proper nutrition can help prevent sarcopenia from happening. While resistance exercise is an effective means to combat sarcopenia and promote hypertrophy in skeletal muscle, it can be a barrier for older adults for reasons including safety, fear, health concerns, pain, fatigue, and lack of social support (Fragala, 2019). These reasons might be why only 8.7% of adults 75 years and older in the United states participate in muscle-strengthening activities in their free time (Fragala, 2019). The low percentage of older adults participating in resistance exercise calls for a new approach, and nutrition may be the answer.

Nutritional Interventions: Protein and Amino Acids

There are 21 amino acids which are organic compounds made up of a carbon surrounded by a phosphate group, nitrogen group, and an R group which make up a protein. Protein is a macronutrient that makes up the functional and structural components of many cells (Dietary Guidelines, 2015). There are 9 essential amino acids that cannot be synthesized by humans and must be obtained from the diet (Dietary guidelines, 2015). These 9 essential amino acids cannot be produced by the body in physiological amounts and are a crucial component of a balanced diet (Wolfe, 2017). Consumption of branched-chain amino acids such as leucine, isoleucine, and valine, have been shown to increase skeletal muscle protein synthesis (Robinson, 2018). Leucine is one of the essential branched chain amino acids that is not only a precursor for protein synthesis, but also plays a role as a regulator of intracellular signaling pathways and is involved in the process of protein synthesis (Wolfe, 2017). Amino acids can become depleted quickly and have no physiological significance as protein synthesis cannot be sustained (Wolfe, 2017). Amino acids are provided by dietary protein to synthesize muscle protein and act as an anabolic stimulus (Robinson, 2018). Frequently consuming meals with at least 30g grams of protein has shown to be effective at increasing lean mass and muscle strength (Robinson). The Dietary Guidelines for Americans (2015) suggest that males and females 51 years + should have 10-35% of their caloric intake be protein. One study found that protein intake in frail elderly subjects increased muscle strength, but did not have a significant impact on muscle mass (Tieland, 2012). About 10% of older adults do not meet the recommended protein intake, which may decrease muscle mass (Pacifico, 2020).

Many studies have linked protein intake and resistance exercise with increased muscle mass. One study found that consuming lean red meats, combined with resistance training, resulted in an increase of lean muscle mass, muscle strength, and insulin-like growth factor I (IGF-1; Daly, 2014). Exercising before protein intake maximizes the muscle protein anabolic response in the young and elderly (Pennings, 2010). Half of the dietary

protein derived amino acids become available in the body post-exercise protein intake (Pennings, 2010). Physical activity sensitizes skeletal muscle tissue to the anabolic properties of amino acids (Robinson, 2018). Protein has been shown to be a nutritional intervention for increasing muscle mass and strength when paired with resistance exercise.

Nutritional Interventions: Apples and Apple Peels

Apple peels contain triterpenoids, a bioactive compound that can enhance muscle mass and function. Triterpenoids exhibit a wide range of biological functions including antioxidant, anti-microbial, anti-inflammatory, and other anti-cancer activities (Frighetto, 2008; Ikeda, 2008). Triterpenoids also possess anabolic properties without androgenic side effects, therefore they might be a viable treatment for promoting muscle maintenance and growth. Ursolic acid, a triterpenoid found in apple peel extract has been found to increase skeletal muscle mass by inhibiting atrophy-associated skeletal muscle gene expression (Kunkle, 2011). A few other studies have demonstrated that nutrition interventions such as apple peel extract as a supplementation is useful for combating sarcopenia. Ursolic acid supplementation in mice demonstrated increased muscle weight, increased muscle fiber diameter, and increased grip strength (Kunkel, 2012). These results demonstrated that ursolic acid can cause muscle hypertrophy. It is possible that apple peels, containing triterpenoids like ursolic acid, may be beneficial in combating sarcopenia.

The purpose of this study was to determine whether apple peel extract supplementation could restore muscle function in aged mouse skeletal muscle. This was accomplished by analyzing muscle function (i.e. strength) of the dorsiflexor muscle group (including the tibialis anterior, extensor digitorum longus, and extensor hallucis longus) of the mouse hindlimb before and after supplementation. I hypothesized that muscle function and strength will either stay the same or increase, but not decline, in mice consuming apple peel extract daily compared to the placebo. Results from this study could be beneficial to increase knowledge on the effects of apple peel extract on aged muscle. These findings may provide data which would allow for future use of apple peel extract supplementation in aged

humans. For now, the use of mice in this study allows for a better understanding of how the supplementation affects aged muscle.

Methods

The following procedures for the mouse model were approved for by the Institutional Animal Care and Use Committee at Appalachian State University (IACUC Protocol #20-03). All experiments were performed in the ASU vivarium in the Rankin Science Building on the campus of Appalachian State University. The animals used for this research were old (24-36 months) male C57BL/6 mice that were kept under a reverse 12-hour light and 12-hour dark cycle in the vivarium. Mice were randomly assigned to 1 of 4 experimental or control groups and underwent the following a) baseline skeletal muscle function testing b) daily supplementation with apple peel extract or placebo, and c) post- supplementation skeletal muscle function testing.

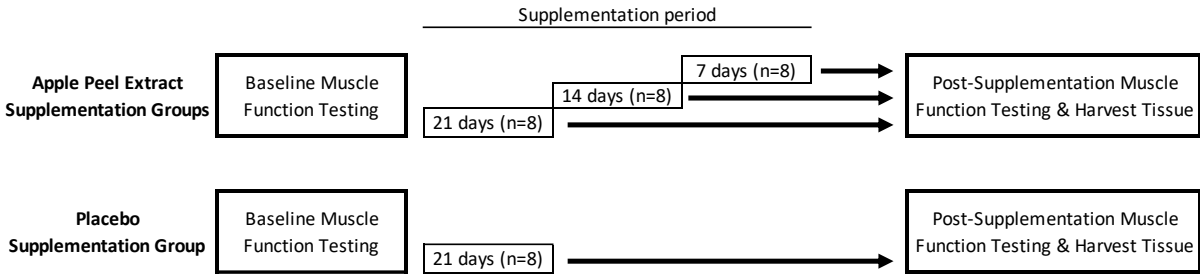

Figure 1. Protocol schematic for experimental design for apple peel extract supplementation groups and placebo supplementation groups.

Baseline Muscle Function Testing Protocol

All mice underwent a baseline muscle function testing before apple peel extract or placebo supplementation. The body weights of each mouse were recorded daily. Mice were anesthetized via inhalation of 4% isoflurane with 800 mL/min O2 and maintained at 2% inhaled isoflurane with 500 mL/min O2. The left hind limb was aseptically prepared by removing the hair with a dilapidating cream the mouse was under anesthesia.

The mouse, while still under anesthesia, was placed on a heated platform with the knee braced and the foot placed on an aluminum foot pedal using flexible medical wrapping. The foot pedal is connected to Aurora Scientific dual mode servmotor (Model- 305B-LR) in order to measure torque. Sterile 23ga needle electrodes were slightly inserted into the skin to create an electric field and the peroneal nerve was stimulated to induce muscle contractions of the anterior dorsiflexor muscles. Optimal electrode placement was found using 100 Hz stimulation and adjusting the voltage to equal close to 100% using the equation ((torque / body mass) x 100). The left anterior dorsiflexor muscle group function testing *in vivo* was accessed by measuring peak isometric torque from the electrode stimulation frequency (1-300 Hz) with a 2-minute rest in between stimuli (see Figure 2 below). After the skeletal muscle function testing, the mouse was placed in an empty cage on a heating bed for at least 30 minutes to recover from anesthesia. Once fully recovered, the mouse was gavaged with either placebo or apple peel extract and placed back into its original cage.

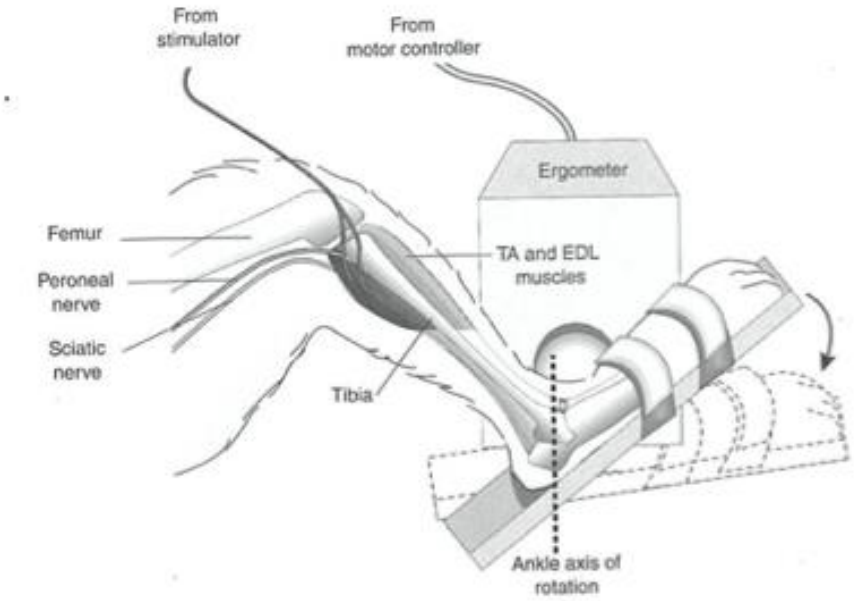

Figure 2. Position of the mouse hindlimb in the Aurora Scientific Muscle Testing System. The left foot of the anesthetized mouse is secured to the foot pedal and then sterile percutaneous needle electrodes are used to stimulate the common peroneal nerve during forced ankle plantar flexion (see arrow). In this model, the dorsiflexor muscles are contracted as a result (from Lieber et al. J Appl Physiol 1994).

Apple Peel Extract Supplementation

The apple peel extract was prepared in the BCHS Biochemistry lab by Dr. Zwetsloot, under the guidance of Dr. Martin Root in the Dept. of Nutrition using optimized techniques (Frighetto, 2008). Triterpenoids, found in apple peel extract, were extracted from freeze-dried apple peels using the organic solvent dichloromethane (DCM). The DCM was evaporated, then the lipid-soluble, triterpenoid-rich extract was dissolved with ethanol and finally mixed with corn oil for solubility. The ethanol was evaporated off leaving a triterpenoid-rich corn oil suspension of 20 mg of triterpenoids per mL of solution, identified as the “apple peel extract”. Liquid chromatography (Waters HPLC) was used to analyze the solution to determine the concentration and purity.

Each mouse was supplemented daily via oral gavage with 200 mg of triterpenoids per kg of body mass. Oral gavage administration of the extract into the lower esophagus or stomach was accomplished using a stainless steel ball-tip feeding needle. For example, A 30g mouse would receive 0.3 mL volume via gavage, as the oral gavage guidelines for administration of volume in a mouse are 10 mL/kg. The same requirements are upheld for the placebo mice which receive an oral gavage of plain corn oil daily.

Post-Supplementation Muscle Function Testing Protocol

After the supplementation period of 21, 14, or 7 days, all mice underwent a second muscle function testing to determine if apple peel extract supplementation enhances muscle function, compared to placebo. The same exact muscle function testing protocol was utilized as before in the baseline muscle function testing. The mouse was again placed under anesthesia with the left hind leg aseptically prepared. The right leg did not undergo the *in vivo* muscle function testing or injury protocol and served as a contralateral control.

After the mouse completed the post-supplementation muscle function testing, each mouse received a single IP injection of puromycin 30 minutes prior to euthanasia to assess skeletal muscle protein synthesis. Puromycin is an analog to the amino acid tyrosine that is incorporated into the polypeptide chain during protein synthesis when injected systemically.

Puromycin, dissolved in sterile PBS was IP injected into each mouse at a volume of 10 mL/kg BW at a dose of 0.04 μmol/g BW. Using the SUnSET technique, detection of puromycin via injection can be used as an indicator of protein synthesis in skeletal muscle (Goodman and Hornberger 2013).

Tissue Harvesting

Thirty minutes after injecting puromycin, the mouse was euthanized by cervical dislocation while still under anesthesia and euthanasia was confirmed by removal of the heart. Once euthanasia was confirmed, the mouse was prepared for the tissue harvest procedure. The tibialis anterior (TA) and the extensor digitorum longus (EDL) muscles of the left hindlimb were harvested first, followed by the right hindlimb. Subsequently, the muscles were weighed, placed in labeled cryovials, and then snap frozen in liquid nitrogen. Frozen muscle samples were stored at -80°C until further analysis.

Results

I began this study at the beginning of the Spring 2020 semester, but we were unable to complete the study due to the COVID-19 pandemic. A total of three mice in the 21-day supplementation group were completed before the university was closed to research in early March. In lieu of presenting data on only three samples, it was determined that an *expanded literature review* on the potential health benefits of apples and apple peels would replace the remainder of the thesis.

Expanded Literature Review

Ageing is a multifactorial process involving biochemical and morphological changes at the cellular level that affects the organism as a whole (Goljanek-Whysall, 2016). Muscle atrophy in eldery patients (sarcopenia) continues to be an area that lacks the necessary treatments. However, it has been suggested that interventional strategies, such as exercise and nutrition should be implemented by the fifth decade of life to help prevent sarcopenia.

(Janssen, 2002). Triterpenoids possess anabolic properties without androgenic side effects; therefore they might be a viable treatment for promoting muscle maintenance and growth.

Nutritional and Health Benefits of Apples

Fruits are an important part of maintaining healthy eating patterns. The nutritional guidelines for Americans suggest that consuming fruits, especially whole fruits are part of a healthy eating pattern (Dietary guidelines,2015). Fruit consumption is recommended at 2 cup per day (Dietary Guidelines, 2015). My plate suggests that half of our plate should be fruits and vegetables (Dietary Guidelines, 2015). Apples, with skins, in a standard portion contain 95 calories and are a nutrient dense fruit containing about 4.4 g of dietary fiber (Dietary Guidelines, 2015). According to the U.S Department of Agriculture, one medium apple (182 grams) contains 0.473 g of protein, 156 g of water, 397 kJ of energy, 18.9 g of sugar, 21.5g of carbohydrates, and 0.309 g total lipid (fat) (Food data cent., 2019). Apples also contains 107 mg Vitamin K and 4.6 mg of Vitamin C (Food data cent, 2019).

Old age is a critical time to intake food with nutritional properties that help prevent illness and frailty. Consuming foods that contain protein, vitamin D, antioxidants, and long-chain polyunsaturated fatty acids is beneficial during older age to prevent and manage sarcopenia (Robinson, 2018). Apples contain antioxidants, polyphenols, and compounds in their skin have been identified such as organic acids, phenolic acids, flavonoids, triterpene acids, coumarly fatty acid esters, and sesquiterpenes (McGhie, 2011). Antioxidants have been suggested to counterbalance reactive oxidative species (ROS) from decreasing muscle mass and strength in older age (Robinson, 2018). This suggests that apples, which contain many beneficial bioactive components, may be a possible intervention to prevent sarcopenia and preserve skeletal muscle health.

Ursolic acid is a triterpenoid that is found in high concentration within apple peels. Research has provided insight on the possible health benefits from ursolic acid. One of the benefits found from ursolic acid was its lipase-inhibitory activity (McGhie, 2011). LC-ESI-QTOR-HRMS was used to analyze apple peels which contain a large number of ursenoic

acids with different chemical structures. Flavanols, flavanals, and procyanides make up 90% of the total antioxidant activity in apple peels (Biedrzycka, 2008).

Molecular components involved in skeletal muscle atrophy and hypertrophy

Mammalian target of rapamycin complex 1 (mTORC1) acts as an environmental sensor for promotion of critical cell functions (Ekim, 2011). Among these are cell growth, protein synthesis, and proliferation in response to growth factors and nutrients (Ekim, 2011). Insulin-like growth factor-1 (IGF-1) production is increased during skeletal muscle hypertrophy (Glass, 2003). In a study on transgenic mice overexpressing IGF-1, skeletal muscle mass was two times greater in IGF-1 mice than normal mice (Glass, 2003). IGF-1 binds to its receptor, which phosphorylates phosphatidylinositol 3-kinase (PI3K) and allows protein kinase B also known as Akt, to bind and become activated (Lei, 2004). Akt mediates the response from growth factors, insulin, and IGF-1 (Lei, 2004). Downstream targets of Akt include mTORC1 and regulatory proteins involved in protein synthesis (Lei, 2004). Muscle hypertrophy is observed in mice with short term activation of Akt in skeletal muscle, leading to mTORC1 downstream activation of protein synthesis (Lei, 2004). The Akt/mTOR pathway is upregulated during muscle hypertrophy and downregulated during muscle atrophy (Bodine, 2001). As shown in Figure 1, an increase in Akt phosphorylation inhibits tuberous sclerosis complex (TSC) which is a heterodimer containing Tsc1(hamartin) and Tsc2(tuberin) proteins (Ekim, 2011). Inhibition of either Tsc1 or Tsc2 causes strong mTORC1 activation. Tsc2 contains a GTPase activating protein that acts on ras homologue enriched in brain (Rheb) which activates mTORC1 through unknown mechanisms (Ekim, 2011). The Akt phosphorylation of Tsc2 suppresses its inhibitory effect of Tsc1 and 2 on mTORC and Rheb can be activated (Ekim, 2011).

Amino acids can also play a part in activating mTORC (Fig.1) as they bypass the Akt signaling pathway (Huang, 2014). The growth factor mediated activation of mTORC1 requires sufficient levels of amino acids and energy, as their diminish inactivates mTORC1 signaling rapidly (Huang, 2014). Amino acids, especially Leucine in particular are essential

for basal mTORC1 signaling in response to growth factor signals (Huang, 2014). Studies have suggested that Rag GTPases, the Rag C/D, RAG A/B and v-ATPases contribute to mTORC1 signaling in response to amino acids (Huang, 2014). However, for mTORC1 to associate with lysosomes and become activated, both amino acids and insulin must be present (Huang, 2014).

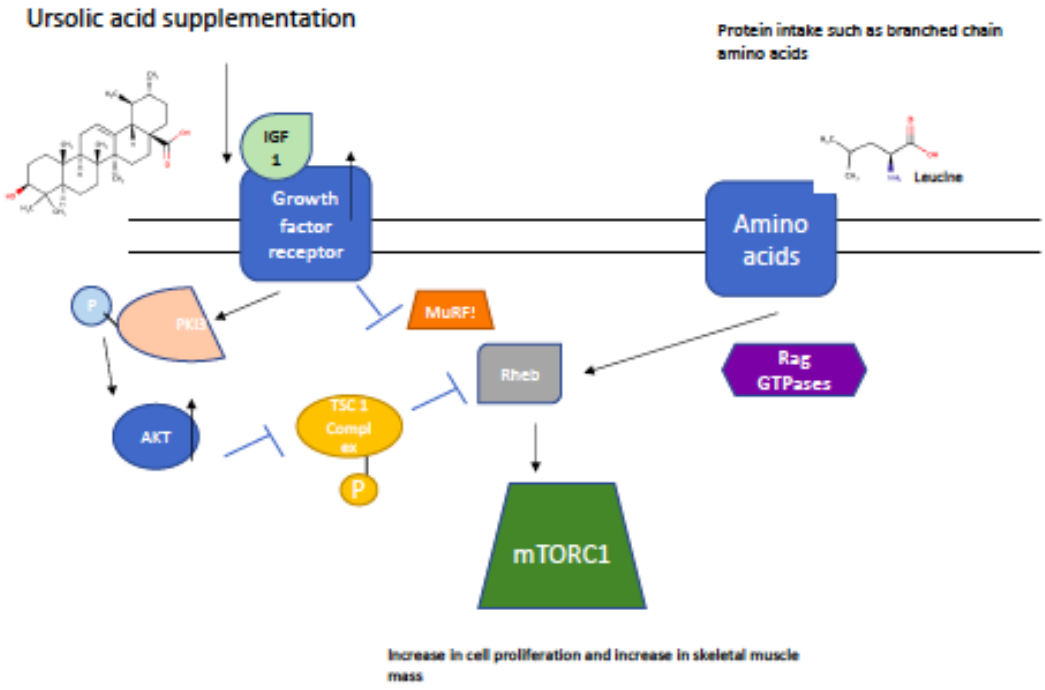

Figure 3. Ursolic acid supplementation causes an increase in growth factors such as IGF-1 and insulin. This in turn causes an increase in PI3K and Akt phosphorylation in the skeletal muscle. Akt can phosphorylate Tsc 1 and 2, inhibiting it which allows Rheb to become active and mTORC1 to become activated. Ursolic acid supplementation inhibits *MuRF1* mRNAs from being express, therefore decreasing muscle atrophy. Amino acids, such as the branched chain amino acid Leucine from protein consumption, can bypass the PI3K/Akt pathway and directly activate Rheb which will then activate mTORC1. When mTORC1 Is activated, there is an increase in cell cycle continuation, protein synthesis, which can lead to cell proliferation (Ekim, 2011).

mTORC1 signaling increased from ursolic acid and resistance exercise

The molecular aspect of the effect of ursolic acid on skeletal muscle after resistance exercise is important to analyze. In a study by Ogasawara et al (2013), twenty 10 week-old

male Sprague-Dawley rats were exercised after a 12-hour overnight fast and ursolic acid (in corn oil) or placebo (only corn oil) was injected intraperitoneally immediately after electrically-stimulated resistance exercise (Ogasawara, 2013). Target tissues were removed 1 to 6 hours after the completion of resistance exercise.

Ursolic acid was found to stimulate protein synthesis via PI3K/Akt signaling which indicates that ursolic acid can increase Akt-dependent (mTORC1) activation leading to enhanced muscle protein synthesis and muscle hypertrophy (Ogasawara, 2013). The mTORC1 activity is induced by resistance exercise, which can be sustained by ursolic acid (Ogasawara, 2013). Contracted skeletal muscle from resistance exercise has been shown in various studies to activate mTORC1 through PI3k/Akt signaling (Ogasawara, Kobayashi 2013). This study also found that ursolic acid increased muscle IGF-1 concentrations 6 hours after resistance exercise, and increased Akt phosphorylation 1 hour and 6 hours after injection (Ogasawara, 2013). The results from this study suggest that ursolic acid promotes the same molecular pathways as resistance exercise. Ursolic acid could potentially be used as an alternative means to activate the PI3K/Akt/mTORC1 signaling pathways leading to stimulation of protein synthesis.

The growth factor-mediated mTORC signaling requires sufficient levels of amino acids (Ekim, 2011). Akt seems to promote protein synthesis by phosphorylation of glycogen synthesis kinase 3β (GSK-3β) leading to inhibition and upregulation of protein synthesis (Bodine, 2001). When the hindlimb of mice was atrophied, Akt protein and phosphorylation levels decreased (Bodine, 2001). Rapamycin, an m-TOR inhibitor, blocked muscle hypertrophy for 7 days when the atrophy limb was used again (Bodine, 2001).

Effects of Ursolic acid on molecular machinery in skeletal muscle

Kunkel et al (2012) hypothesized that ursolic acid might increase skeletal muscle Akt activity in a mouse model of diet-induced obesity, leading to muscle hypertrophy and increased energy expenditure (Kunkel, 2012). This study used 8-week-old male C57BL/6 mice with *ad libitum* access to a high-fat diet supplemented with 0.14% ursolic acid for 6

weeks to investigate the effects of ursolic acid in diet-induced obese mice (Kunkel, 2012). The tricep muscle was examined 6 weeks after the diets, and steady-state Akt phosphorylation was examined. Ursolic acid supplementation was found to increase Akt phosphorylation more than two-fold, repress muscle-atrophy-associated gene expression and promote muscle hypertrophy by increasing activity of IGF-I, Akt, and insulin receptors. Furthermore, adipose weight was decreased by reducing the adipocyte size (Kunkel, 2012).

Ursolic acid has been shown to increase muscle mass, but not body weight as it increases Akt signaling activity. Akt activity inhibits muscle atrophy, while promoting muscle hypertrophy and in skeletal muscle energy expenditure (Bodine, 2001). In addition, increases in skeletal muscle IGF-1 mRNA, an autocrine/paracrine mechanism that increases skeletal muscle protein synthesis, significantly increased in high fat-fed mice that had been given ursolic acid (Kunkel, 2012). The high-fat diet (HFD) in conjunction with ursolic acid (UA) supplementation, showed an increase in the fiber cross sectional area of both fast and slow skeletal muscle fibers (Kunkel, 2012).

Ursolic acid supplementation on recovery of skeletal muscle atrophy

A study by Kang et al (2019), investigated if ursolic acid could enhance recovery of skeletal muscle atrophy. The left hindlimb of 8-week-old Sprague- Dawley (SD) rats was immobilized for 10 days to induce muscle atrophy, while the right leg was used as a control (Kang, 2019). Rats were split into sedentary and ursolic acid supplementation groups. The recovery rate of skeletal muscle mass after 8 weeks of ursolic acid treatment was 4.5% to 11.3% higher than sedentary controls (Kang, 2019) with the most significant increase observed in the gastrocnemius muscle. This study demonstrates that ursolic acid is also effective at enhancing the recovery of skeletal muscle mass from immobilization-induced atrophy, a situation that may be very similar to what elderly patients with sarcopenia may encounter.

Ursolic acid potentially decreases fasting-induced muscle atrophy

Fasting, or nutrient deprivation, has been shown to induce skeletal muscle atrophy by increasing skeletal muscle degradation and reducing skeletal muscle protein synthesis. Kunkel et al (2012) identified multiple skeletal muscle atrophy genes that displayed increased expression in response to one atrophy-inducing stress (i.e. 24-hour fasting) in both humans and mouse skeletal muscle. The authors hypothesized that compounds contained in apple peels, primarily ursolic acid, might inhibit fasting-induced skeletal muscle atrophy. Ursolic acid was administered to fasting mice via i.p injection. After 12 hours of fasting the mice received a second dose of ursolic acid and were examined after a total of 24 hours of fasting (Kunkel, 2011). Muscle weight was reduced 9% in control mice with fasting, but in the presence of ursolic acid, muscle weight increased by 7% (Kunkel, 2011). The *MuRF1* gene, an atrophy gene, was upregulated by atrophy-inducing fasting, while *PGC-1α* mRNA that encodes a protein that inhibits atrophy-associated gene expression and skeletal muscle atrophy in mice was decreased (Kunkel, 2011). Ursolic acid supplementation decreased *MuRF1* gene expression, inhibitors of PI3K, and rapamycin which is an inhibitor of mTORC1 in skeletal muscle mRNA expression (Kunkel, 2011). Furthermore, skeletal muscle specific IGF-1 was increased following ursolic acid supplementation (Kunkel, 2011). Increased IGF-1 expression has been shown to reduce muscle atrophy (Barton, 1998). These results suggest that ursolic acid can prevent expression of atrophy associated gene expression by decreasing muscle atrophy.

Ursolic acid potentially increases muscle hypertrophy and strength

Since ursolic acid has been reported to decrease muscle atrophy, it is also possible to induce muscle hypertrophy. Mice were used to test this by feeding them either control chow or chow containing 0.27% ursolic acid for 5 weeks (Kunkel, 2012). Larger skeletal muscles were observed in mice fed ursolic acid versus control mice (Kunkel, 2012). Ursolic acid-supplemented mice increased muscle fiber diameter over 5 weeks compared to control

mice (Kunkel, 2011). Muscle fiber diameter was higher in ursolic acid diet mice at 40-55 μm compared to control mice mostly between 35-45 μm (Kunkel, 2012). Furthermore, mice given ursolic acid for 5 weeks increased grip strength compared to controls. These results suggest that ursolic acid can be reparative by inducing muscle hypertrophy and increasing strength.

Ursolic acid supplementation combined with resistance exercise

As demonstrated previously, ursolic acid supplementation and resistance exercise are effective methods to increase skeletal muscle mass and function. Combining ursolic acid supplementation in conjunction with resistance exercise could be a promising intervention to increase muscle mass, muscle function, and decrease sarcopenia. In a study by Bang et al. (2014), had human subjects, 16 males with an average age of 29 years, perform an exercise regime that included 8 weeks of supervised resistance training with or without ursolic acid supplementation. This study found that pairing resistance exercise with ursolic acid supplementation significantly increased muscle strength from baseline, compared to resistance training alone (Bang, 2014). After resistance training plus ursolic acid, IGF-1 levels significantly increased (Bang, 2014). It is also notable that this combined treatment decreased body fat percentage, but did not change muscle mass (Bang, 2014). The results from this study indicate that ursolic acid supplementation combined with resistance exercise is beneficial for increasing muscle strength and IGF-1 levels in skeletal muscle.

Summary

Ursolic acid appears to be a common triterpenoid used in experiments to study its effects on skeletal muscle. As shown in Table 1, multiple studies have found that ursolic acid increase IGF-1 expression, increases muscle mass, muscle strength, and muscle fiber CSA. Ursolic acid supplementation has been shown to increase muscle hypertrophy, decrease muscle atrophy, and decrease body fat (Kang, 2019; Kunkel, 2012; Bang, 2014). The combination of resistance training with ursolic acid can increase muscle strength (Bang,

2014) and in some cases muscle mass (Kunkel, 2011). The molecular machinery such as PI3K/Akt and IGF-1 were found to stimulate mTORC1 signaling leading to increased protein synthesis in response to ursolic acid supplementation post resistance training (Ogasawara, 2013). Molecular pathways are an important part of understanding how triterpenoids cause changes physiologically.

Table 1. Summary of the effect of Ursolic acid supplementation on muscle mass, strength/function, muscle fiber cross-sectional area (CSA), and IGF-1 expression from multiple studies. Some studies gave Ursolic acid supplementation in combination with resistance exercise or alone.

Study (author, year)	Supplementation regimen	Route of administration	In combination with resistance exercise	Muscle mass (lower limb)	Strength/ function	Muscle fiber CSA	IGF-1 expression
Kunkel, 2011	Chow containing 0.27% ursolic acid	chow with ad libtum access	no	550 mg	6.5 g/kg body weight	45-55 µm	increased
Kunkel, 2012	0.14% ursolic acid	chow with ad libtum access	no	400 mg	190 g	slow muscle: 30 µm. Fast muscle: 55µm	increased
Kang, 2019	5 mg/kg once a day of ursolic acid	intraperitoneal injection once a day	no	increased 4.5% to 11.3%	N/A	N/A	N/A
Ogasawara, 2013	250 mg/kg body weight	injected intraperitoneally once a day	yes	N/A	N/A	N/A	increased
Bang, 2014	450 mg ursolic acid	3 capsules daily	yes	no significant results	left extension: 256.44 N/m. left flexion: 161.00 N/m	N/A	221.04 ng/mL

Conclusion

The research project for my thesis was unable to continue due to the COVID-19 pandemic and the university closing for research, so the results from the potential effects of apple peel extract on muscle mass and function in aged mice is inconclusive at this time. However, the expanded literature review should demonstrate the great potential for apple peel extract/ursolic acid to be a viable countermeasure against sarcopenia and the age-related loss of muscle function. It was my intention to highlight the importance of further

investigation in this area as it could have major health benefits for the general public, particularly the aging population.

Future Directions

To the best of my knowledge, we are the first lab to examine the effects of apple peel extract on skeletal muscle mass and function in older mice (24-30 months old). Many other studies have examined the combined effects of ursolic acid and resistance exercise, but not the effects of apple peel extract alone on aged mice. Further research can be conducted to continue this study and obtain results in order to gain more information on how apple peel extract affects skeletal muscle. Apple peel extract supplementation may be beneficial to the medical community due its potential nutritional benefits for sarcopenia prevention in elderly adults. Overall the results from these studies provide insight on a new way to combat skeletal muscle sarcopenia, atrophy, and the possibility for skeletal muscle hypertrophy.

Limitations

There were limitations to this study including the time frame in which it was conducted and the age of the mice used for the study. Further, having a longer time frame to test more mice would be beneficial to obtain greater amounts of viable data . The age of the mice is also a limitation. The mice in the experiment were 24-36 months old, therefore some died due to old age, tumors, or over-stimulation of the peroneal nerve. It is important to take precautions when handling or experimenting with older mice especially when administering gavuage treatments to help in keeping older mice alive and healthy.

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