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Longer Life Foundation Final Research Report

Abstract

Loss of muscle mass is a normal consequence of aging, worsened by chronic illness, poor appetite and diet, and reduced physical activity in many older adults. The ensuing decline in physical function is a major cause of frailty, disability and death. Approximately half of the population over 60 y of age is considered sarcopenic (i.e., muscle mass one standard deviation or more below the sex-specific value for young adults). Treatments that can reverse or reduce the age-associated loss of muscle mass are therefore much needed. Anabolic resistance, i.e., the inability of aging muscle to adequately increase muscle protein synthesis and decrease muscle breakdown in response to nutritional anabolic stimuli (e.g., amino acids and insulin) is considered a major cause for the loss of muscle mass in advanced age. Evidence is emerging that long-chain omega-3 polyunsaturated fatty acid (LCn-3PUFA) consumption may be important for maintenance of muscle mass and physical function throughout the life-span. For example, feeding fish oil, which is rich in LCn-3PUFA, was found to increase whole-body disposal of amino acids in growing steers. And, fatty fish consumption was found to be the most important independent dietary factor in relation to grip strength in the Hertfordshire, UK study, including 2983 men and women aged 59 to 73 y. These findings have led us to hypothesize that dietary LCn-3PUFA supplementation stimulates muscle protein anabolism. To test this hypothesis, we measured the protein fractional synthetic rate (FSR) and the phosphorylation of elements of the anabolic pathway (Akt, mTOR, and p70s6k) during basal, postabsorptive conditions and during a hyperinsulinemic-hyperaminoacidemic clamp and muscle protein, RNA, and DNA concentrations before and after eight weeks of LCn-3PUFA supplementation (4 $g \cdot d^{-1}$ of Lovaza™) in two groups of older adults who were randomized to receive LCn-3PUFA or an isoenergetic LCn-3PUFA free control-oil for 8 weeks. Our preliminary results indicate that dietary LCn-3PUFA supplementation stimulates protein anabolism in human muscle and might therefore be useful for the prevention and treatment of sarcopenia.

Lay Summary

Defects in the processes responsible for building muscle, in particular the building of muscle after eating, is a major cause for loss of muscle mass in older people which leads to physical impairments and frailty, loss of independence, admission to assisted living facilities and often premature death. Our main goal was to determine whether consumption of long-chain omega-3 polyunsaturated fatty acids (LCn-3PUFA; i.e., fish oil) can help build muscle in older adults. We found that LCn-3PUFA can help build muscle. Increased consumption of oily fish or LCn-3PUFA supplementation could therefore provide a safe, simple, and relatively cheap intervention to reduce the loss of muscle mass and its consequences.

Introduction

Long-chain n-3 polyunsaturated fatty acids (LCn-3PUFA) are essential nutrients that have anti-inflammatory properties[1] and reduce the risk for cardiovascular disease[1]. Evidence for a potentially anabolic effect of LCn-3PUFA is also emerging. For example, supplementation with LCn-3PUFA increased whole-body protein synthesis and whole-body protein net balance in burned rats[2]. Moreover, in a recent study conducted in growing steers it was found that when they ate feed enriched in menhaden oil, a fish oil rich in LCn-3PUFA, the insulin-stimulated non-oxidative whole-body disposal of amino acids (a marker of increased whole-body protein synthesis) doubled while activation of the Akt-mTOR-p70s6k signaling pathway in muscle was increased significantly[3]. The purpose of the present study therefore was to determine the effect of LCn-3PUFA supplementation on the rate of muscle protein synthesis *in vivo* in human muscle. The following Specific Aims were investigated in healthy young and older adults.

Aim 1. *Evaluate the effect of LCn-3PUFA on skeletal muscle protein synthesis*.

We hypothesize that LCn-3PUFA increase the stimulatory effect of combined hyperinsulinemia-hyperaminoacidemia on muscle protein synthesis.

Aim 2. *Evaluate the effect of LCn-3PUFA on anabolic signalling pathways in skeletal muscle*.

We hypothesize that LCn-3PUFA increase the activation of anabolic signalling pathways in muscle by hyperinsulinemia-hyperaminoacidemia.

Aim 3. *Evaluate the effect of LCn-3PUFA on inflammatory cytokines in the systemic circulation and inflammatory signalling pathways in skeletal muscle*.

We hypothesize that LCn-3PUFA decrease the plasma concentration of pro-inflammatory cytokines and decrease the activity of inflammatory pathways in muscle.

Methods

We measured the fractional synthesis rate (FSR) of muscle proteins (by using stable isotope labeled tracer techniques) during basal, post-absorptive conditions and during hyperinsulinemia-hyperaminoacidemia (within the range normally seen after meal consumption[4, 5]), the concentrations of protein, RNA, and DNA in muscle (to obtain indices of the protein synthetic capacity, translational efficiency[6, 7] and cell size[8]), the activation (as phosphorylation) of elements of intracellular signaling pathways involved in the regulation of muscle protein synthesis (Akt; mTOR; p70s6k; eEF2)[9, 10], and markers of inflammation in plasma (C-reactive protein [CRP], interleukin 6 [IL-6], tumor necrosis factor alpha [TNF-α]) and muscle (nuclear factor kappa-light-chain-enhancer of activated B cells [NFκB]) in two groups (n = 8 each) of older adults who were randomized to receive LCn-3PUFA (4 $g \cdot d^{-1}$ of Lovaza™) or an isoenergetic LCn-3PUFA free control-oil for 8 weeks and in nine 25-45 y old healthy subjects who received LCn-3PUFA (4 $g \cdot d^{-1}$ of Lovaza™) for 8 weeks (Table 1).

Results

To date we have completed all data analyses for nine young and middle-aged subjects and eight older adults (Table 1). The basal muscle protein fractional synthesis rate (FSR) before supplementation was not different in young and old subjects; during amino acid, glucose and insulin infusion, the muscle protein FSR increased in both groups but to a greater extent in the young than in the old subjects (Figure 1). These results are consistent with earlier observations made by ourselves and other investigators who found no difference in the basal rate of muscle protein synthesis in healthy young and old subjects[11-15], but resistance to the anabolic effect of nutritional stimuli (amino acids, insulin)[11, 14, 16-18].

Table 1. Subjects' age, sex and BMI.

	Young	Old	
	LCn-3PUFA	Cornoil	LCn-3PUFA
N (male/female)	9 (5/4)	4 (3/1)	4 (2/2)
Age (years)	39.7 ± 1.7	70 ± 3	71 ± 2
BMI (kg/m^2)	25.9 ± 1.0	26.9 ± 1.0	25.4 ± 1.2

Values are means ± SEM.

LCn-3PUFA supplementation had no effect on the basal muscle protein fractional FSR; however, the increase (above basal values) in the muscle protein FSR during insulin/amino acid infusion was greater after than before LCn-3PUFA supplementation (Figure 1), most likely because of greater activation of the mTOR signaling pathway (Figure 1). In addition, the muscle protein concentration and the protein-to-DNA ratio (an index of muscle cell size) were both greater after LCn-3PUFA supplementation (Figure 2).

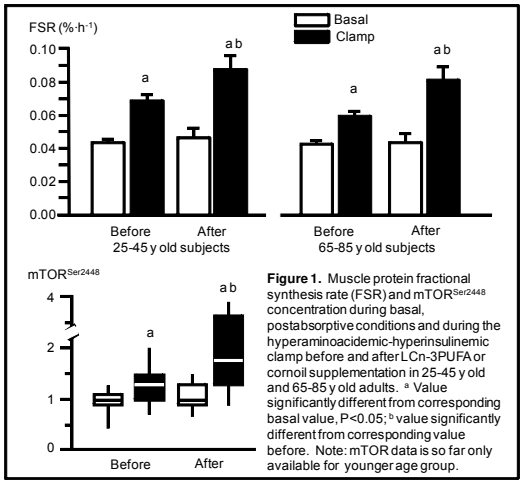

Figure 1. Muscle protein fractional synthesis rate (FSR) and mTOR^{Ser2448} concentration during basal, postabsorptive conditions and during the hyperaminoacidemic-hyperinsulinemic clamp before and after LCn-3PUFA or cornoil supplementation in 25-45 y old and 65-85 y old adults. [a] Value significantly different from corresponding basal value, $P<0.05$; [b] value significantly different from corresponding value before. Note: mTOR data is so far only available for younger age group.

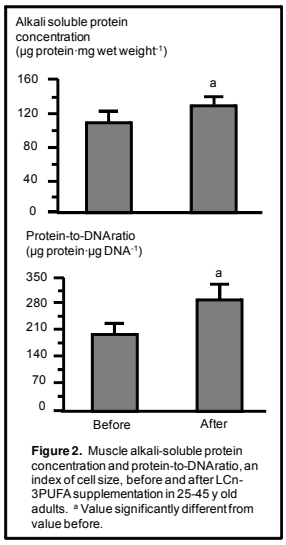

Figure 2. Muscle alkali-soluble protein concentration and protein-to-DNA ratio, an index of cell size, before and after LCn-3PUFA supplementation in 25-45 y old adults. [a] Value significantly different from value before.

Discussion, including implications and potential long-term extensions

Our pilot data provide evidence that LCn-3PUFA supplementation causes a considerable increase in the muscle protein anabolic response to hyperinsulinemia-hyperaminoacidemia in healthy young, middle-aged and older adults. This was probably mediated by the stimulatory effect of LCn-3PUFA on mTOR^{Ser2448} phosphorylation (an integral control point for muscle cell growth[19-21]) and consequent downstream signaling during hyperinsulinemia-hyperaminoacidemia.

Our findings are consistent with the reported effects of LCn-3PUFA supplementation on protein metabolism and muscle mass assessed *in vivo* in animals and extend those observations. Low-dose LCn-3PUFA supplementation (i.e., 1 - 2% of total daily energy intake - as in our study), alone or in combination with amino acid supplementation, has been reported to help maintain whole-body protein synthesis, whole-body protein net balance, and muscle mass in burned rats and tumor-bearing mice[2, 22]. Moreover, growing steers eating feed enriched in LCn-3PUFA doubled their non-oxidative whole-body disposal of amino acids (a marker of increased whole-body protein synthesis) during a hyperinsulinemic-euglycemic-euaminoacidemic clamp while simultaneously inducing greater activation of the Akt-mTOR-p70s6k signaling pathway in muscle[3]. Somewhat puzzlingly, large doses of LCn-3PUFA (>20% of total daily energy intake) reportedly have no effect on rat muscle protein synthesis[23-25] or even decreased it[26]. This, however, may have been because of an unsuspected subclinical toxicity of LCn-3PUFA themselves or possibly accompanying impurities[1, 27, 28]. Based on the evidence we provide, we contend that LCn-3PUFA, along with their other health benefits, are good candidates to be considered as intervention agents in a variety of conditions of muscle wasting [1]. In addition, our study provides compelling evidence for an interaction of fatty acid and protein metabolism in human muscle. This research area and the significance of the interaction between fatty acid and muscle protein metabolism remain to be explored.

Future plans including planned grant submissions

Future studies will be designed to: i) investigate the effect of LCn-3PUFA on muscle mass and function, physical performance and quality of life in a larger, population based randomized, placebo-controlled trial, ii) further evaluate the mechanisms responsible for the beneficial effect of LCn-3PUFA on muscle protein metabolism (e.g. measure the rates of muscle protein synthesis and breakdown; evaluate the specific signalling pathways involved in mediating the effect of LCn-3PUFA on muscle protein metabolism), and iii) evaluate whether the red blood cell membrane and/or muscle phospholipid fatty acid composition can be used as biomarkers for the age-associated loss of muscle mass and thus the risk of frailty and early mortality.

To achieve these goals we have submitted an R01 application to the NIH, which is currently undergoing review.

Literature Cited

1. Fetterman JW, Jr., Zdanowicz MM. Therapeutic potential of n-3 polyunsaturated fatty acids in disease. *Am J Health Syst Pharm.* Jul 1 2009;66(13):1169-1179.
2. Hayashi N, Tashiro T, Yamamori H, et al. Effect of intravenous omega-6 and omega-3 fat emulsions on nitrogen retention and protein kinetics in burned rats. *Nutrition.* Feb 1999;15(2):135-139.
3. Gingras AA, White PJ, Chouinard PY, et al. Long-chain omega-3 fatty acids regulate bovine whole-body protein metabolism by promoting muscle insulin signalling to the Akt-mTOR-S6K1 pathway and insulin sensitivity. *J Physiol.* Feb 15 2007;579(Pt 1):269-284.
4. Symons TB, Schutzler SE, Cocke TL, Chinkes DL, Wolfe RR, Paddon-Jones D. Aging does not impair the anabolic response to a protein-rich meal. *Am J Clin Nutr.* Aug 2007;86(2):451-456.
5. Boirie Y, Dangin M, Gachon P, Vasson MP, Maubois JL, Beaufrere B. Slow and fast dietary proteins differently modulate postprandial protein accretion. *Proc Natl Acad Sci U S A.* Dec 23 1997;94(26):14930-14935.
6. Vary TC, Jefferson LS, Kimball SR. Amino acid-induced stimulation of translation initiation in rat skeletal muscle. *Am J Physiol.* Dec 1999;277(6 Pt 1):E1077-1086.
7. Millward DJ, Garlick PJ, James WP, Nnanyelugo DO, Ryatt JS. Relationship between protein synthesis and RNA content in skeletal muscle. *Nature.* Jan 19 1973;241(5386):204-205.
8. Forsberg AM, Nilsson E, Werneman J, Bergstrom J, Hultman E. Muscle composition in relation to age and sex. *Clin Sci (Lond).* Aug 1991;81(2):249-256.
9. Drummond MJ, Dreyer HC, Fry CS, Glynn EL, Rasmussen BB. Nutritional and contractile regulation of human skeletal muscle protein synthesis and mTORC1 signaling. *J Appl Physiol.* Apr 2009;106(4):1374-1384.
10. Rennie MJ, Wackerhage H, Spangenburg EE, Booth FW. Control of the size of the human muscle mass. *Annu Rev Physiol.* 2004;66:799-828.
11. Kumar V, Selby A, Rankin D, et al. Age-related differences in the dose-response relationship of muscle protein synthesis to resistance exercise in young and old men. *J Physiol.* Jan 15 2009;587(Pt 1):211-217.
12. Volpi E, Mittendorfer B, Wolf SE, Wolfe RR. Oral amino acids stimulate muscle protein anabolism in the elderly despite higher first-pass splanchnic extraction. *Am J Physiol.* Sep 1999;277(3 Pt 1):E513-520.
13. Volpi E, Mittendorfer B, Rasmussen BB, Wolfe RR. The response of muscle protein anabolism to combined hyperaminoacidemia and glucose-induced hyperinsulinemia is impaired in the elderly. *J Clin Endocrinol Metab.* Dec 2000;85(12):4481-4490.
14. Rasmussen BB, Fujita S, Wolfe RR, et al. Insulin resistance of muscle protein metabolism in aging. *Faseb J.* Apr 2006;20(6):768-769.
15. Volpi E, Sheffield-Moore M, Rasmussen BB, Wolfe RR. Basal muscle amino acid kinetics and protein synthesis in healthy young and older men. *Jama.* Sep 12 2001;286(10):1206-1212.
16. Guillet C, Prod'homme M, Balage M, et al. Impaired anabolic response of muscle protein synthesis is associated with S6K1 dysregulation in elderly humans. *Faseb J.* Oct 2004;18(13):1586-1587.
17. Cuthbertson D, Smith K, Babraj J, et al. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *Faseb J.* Mar 2005;19(3):422-424.
18. Katsanos CS, Kobayashi H, Sheffield-Moore M, Aarsland A, Wolfe RR. Aging is associated with diminished accretion of muscle proteins after the ingestion of a small bolus of essential amino acids. *Am J Clin Nutr.* Nov 2005;82(5):1065-1073.
19. Baar K, Esser K. Phosphorylation of p70(S6k) correlates with increased skeletal muscle mass following resistance exercise. *Am J Physiol.* Jan 1999;276(1 Pt 1):C120-127.

20. O'Neil TK, Duffy LR, Frey JW, Hornberger TA. The role of phosphoinositide 3-kinase and phosphatidic acid in the regulation of mammalian target of rapamycin following eccentric contractions. *J Physiol.* Jul 15 2009;587(Pt 14):3691-3701.

21. Bodine SC, Stitt TN, Gonzalez M, et al. Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nat Cell Biol.* Nov 2001;3(11):1014-1019.

22. van Norren K, Kegler D, Argiles JM, et al. Dietary supplementation with a specific combination of high protein, leucine, and fish oil improves muscle function and daily activity in tumour-bearing cachectic mice. *Br J Cancer.* Mar 10 2009;100(5):713-722.

23. Wan JM, Istfan NW, Chu CC, Blackburn GL, Bistrian BR. Comparative effects of omega-3 and omega-6 polyunsaturated fatty acids on protein metabolism in rats bearing the mammary adenocarcinoma. *Metabolism.* Jun 1991;40(6):577-584.

24. Smith HJ, Greenberg NA, Tisdale MJ. Effect of eicosapentaenoic acid, protein and amino acids on protein synthesis and degradation in skeletal muscle of cachectic mice. *Br J Cancer.* Jul 19 2004;91(2):408-412.

25. Hirschberg Y, Pomposelli JJ, Blackburn GL, Istfan NW, Babayan V, Bistrian BR. The effects of chronic fish oil feeding in rats on protein catabolism induced by recombinant mediators. *Metabolism.* Apr 1990;39(4):397-402.

26. Sohal PS, Baracos VE, Clandinin MT. Dietary omega 3 fatty acid alters prostaglandin synthesis, glucose transport and protein turnover in skeletal muscle of healthy and diabetic rats. *Biochem J.* Sep 1 1992;286 (Pt 2):405-411.

27. Stern N, Korotkova M, Strandvik B, et al. Subchronic toxicity of baltic herring oil and its fractions in the rat (III) bone tissue composition and dimension, and ratio of n-6/n-3 fatty acids in serum phospholipids. *Basic Clin Pharmacol Toxicol.* Jun 2005;96(6):453-464.

28. Rabbani PI, Alam HZ, Chirtel SJ, Duvall RE, Jackson RC, Ruffin G. Subchronic toxicity of fish oil concentrates in male and female rats. *J Nutr Sci Vitaminol (Tokyo).* Jun 2001;47(3):201-212.