1   **Abstract**

2   **Aims/ Objectives**

3   To evaluate current literature via systematic review to ascertain whether amino acids/

4   vitamins provide any influence on musculotendinous healing, and by which physiological

5   mechanisms.

6   **Methods**

7   EBSCO, PUBMED, Science Direct, Embase Classic/ Embase, and MEDLINE were searched

8   using terms including "vitamins", "amino acids", "healing", "muscle" and "tendon". The

9   primary search had 479 citations, 466 of which were excluded predominantly due to non-

10   randomised design. Randomised human and animal studies investigating all supplement

11   types/ forms of administration were included. Critical appraisal of internal validity was

12   assessed using the Cochrane risk of bias tool or the Systematic Review Centre for Laboratory

13   Animal Experimentation (SYRCLE) risk of bias tool for human and animal studies,

14   respectively. 2 reviewers performed duel data extraction.

15   **Results**

16   Twelve studies met criteria for inclusion: 8 examined tendon healing, 4 examined muscle

17   healing. All studies used animal models, except 2 human trials using a combined integrator.

18   Narrative synthesis was performed via content analysis of demonstrated statistically

19   significant effects, and thematic analysis of proposed physiological mechanisms of

20   intervention. Vitamin C/ taurine demonstrated indirect effects on tendon healing through

21   anti-oxidant activity. Vitamin A/ glycine showed direct effects on extra-cellular matrix tissue

22   synthesis. Vitamin E shows an anti-proliferative influence on collagen deposition. Leucine

23   directly influences signalling pathways to promote muscle protein synthesis.

24   **Discussion**

25   Preliminary evidence exists demonstrating vitamins and amino acids may facilitate multi-

26   level changes in musculotendinous healing; however recommendations on clinical utility

27   should be made with caution. All animal studies and one human study show high risk of bias

28   with moderate inter-observer agreement (k=0.46).

29   Currently, there is limited evidence to support the use of vitamins and amino acids for

30   musculotendinous injury. Both high quality animal experimentation of the proposed

31   mechanisms confirming the physiological influence of supplementation; and human studies

32   evaluating effects on tissue morphology and biochemistry are required before practical

33   application.

34   [299 words]

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Introduction

Dietary supplements are defined as any concentrated source of a nutritional compound demonstrating a physiological effect (Department of Health, 2011). A meta-analysis (n=159) shows greater usage of supplements in athletes compared to non-athletes with increasing frequency in elite performers (Knapik et al., 2016). Despite heterogeneity between studies, a pooled prevalence estimate of 60% indicates high usage in the athletic population. Furthermore, supplement use guidelines are inadequate; athletes may misunderstand supplement effects; and display insufficient knowledge to independently plan their diet (Molinero & Márquez, 2009; Petróczi et al., 2007; Torres-McGehee et al., 2012). This may lead to disordered eating behaviours and in extreme cases may leave them at heightened risk of musculoskeletal injury such as fracture (Bonci et al., 2008). Dietary management strategy can also be misinterpreted by coaches who discourage supplement use due to the potential risks of contamination and inadvertent doping (Judkins & Prock, 2012), but who have inadequate knowledge in nutritional practices to plan effectively during injury recovery (Tipton, 2011). Thus, leaving athletes reticent to supplement their diet during rehabilitation (Tack, 2016). This is despite evidence from randomised controlled trials that supplements can provide therapeutic effects on muscle morphometry and strength following injury (Hespel et al., 2001; Holm et al., 2006); and muscular metabolic efficiency following immobility (Eijnde et al., 2001). A survey of 145 athletes demonstrated only 34% considered supplementation to improve musculoskeletal tissue repair (e.g. chondroitin, glucosamine, methyl-sulfonyl-methane and omega-3 fatty acids) (Malinauskas et al., 2007). In similar surveys, maintaining strength/endurance and avoiding sickness were more commonly cited reasons for supplementation (Petróczi et al., 2007a/b; Petróczi et al., 2008).

67 Animal experiments investigating skin wound healing indicate supplementation can elicit

68 positive effects on collagen synthesis (Ejaz et al., 2009; Uzgare et al., 2009) and tensile

69 breaking-strength (Shukla et al., 1999). More specifically, vitamins and vitamin-related-

70 compounds can increase growth factor release (retinoids) (Wicke et al., 2000); as well as

71 enhancing tensile breaking-strength (vitamin E-like antioxidant, Raxofelast) (Galeano et al.,

72 2001). Other supplements, such as olive oil, can reduce oxidative damage during healing

73 (Rosa et al., 2014); whilst the amino acid arginine can improve wound angiogenesis

74 (Raynaud-Simon et al., 2012). The choice of supplement is therefore critical. Additionally,

75 there is evidence of negative effects through excessive facilitation of pro-inflammatory

76 pathways by omega-3 fatty acids (McDaniel et al., 2008); and in the case linseed and fish

77 oils, of reduced tissue angiogenesis (Otranto et al., 2010). Human trials with arginine

78 demonstrate improvements in blastogenic response to injury (Barbul et al., 1990; Sax,

79 1994); and improved collagen deposition seen following supplementation with amino acid

80 mixture containing arginine, beta-hydroxy beta-methylbutyrate and glutamine (Williams et

81 al., 2002). Additionally, time to wound healing is reduced following supplementation of a

82 mixture containing protein, zinc, iron and vitamin C (Collins et al., 2005).

83 This review elaborates on a multi-topic feasibility search which incorporated all supplement

84 types and the influence on musculotendinous tissue healing. Subsequently a PICO

85 (population, intervention, comparator, outcome) question was devised: "what is the effect

86 of dietary supplements on musculoskeletal tissue (e.g. cartilage, tendon, muscle, ligament)

87 healing compared to placebo or other control?", and was used to formulate a search of

88 Google Scholar and PUBMED to evaluate the quality/ volume of existing literature.   This

89 search produced 95 papers which when assessed for eligibility was reduced to 24 studies.

90   Amino acids and vitamins demonstrated sufficient literature to perform a more specific

91   systematic review and consequently these classifications of supplements encapsulated all

92   literature found to evaluate the effect of supplements in muscle and tendon.

93   The specific research question "what is the effect of vitamins and/ or amino acids on

94   musculoskeletal tissue (e.g. cartilage, tendon, muscle, ligament) healing parameters

95   compared to placebo or other control?" was then devised. This methodology (systematic

96   review via content and thematic analysis) was chosen to synthesise findings of animal and

97   human model experiments, previously undertaken in other areas of medicine (Sallis, 2000;

98   Virmani et al., 2003), and in response to the call for methodologically sound reviews to

99   evolve the research base (Reagan-Shaw et al., 2008).

100   As such this review aims to identify whether these selected dietary supplements (amino

101   acids and vitamins) provide any influence on muscle and tendon tissue healing in animal and

102   human models of injury (traumatic, degenerative or exercise induced); and if so, what

103   mechanisms underpin this influence.

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Methods

This review followed specific methodology guidelines (Centre for Reviews and

Dissemination, 2009), and reported in accordance with the Preferred reporting items for

systematic review and meta-analysis (PRISMA) statement for reporting systematic reviews

(Moher et al. 2015). The review was based upon an *a priori* protocol which described

essential procedures to be followed (e.g. the PICO question of issue, comprehensive search

strategy and a piloted data extraction pro forma).

Eligibility Criteria

This review followed the following inclusion criteria:

- Randomised controlled trials of human and animal models of tendon and muscle

  injury. Although it has been reported that randomisation and blinding in animal

  studies is often not stringently adhered to (Hess, 2011); the choice was made to

  include these trials but be explicit in critique of these processes. Despite continuing

  debate around the poor predictive value of animal experiments for humans

  (McGonigle & Ruggeri, 2014); this review includes animal models to support findings

  from human trials. Methodological flaws (e.g. insufficient, allocation concealment/

  blinding) (Kilkenny et al., 2009) can limit prediction from animals to humans (Hackam

138   & Redelmeier, 2006; Henderson et al., 2013); leading to problems such as outcome

139   reporting bias (Tsilidis et al., 2013). To attenuate these risks (Sena et al., 2010)

140   explicit application of randomisation and control group use is specified for inclusion.

141   • Trials examining the influence of vitamins and/ or amino acid compounds applied

142   orally, by injection or topically (either alone or in combination).

143   • The studies must use outcomes indicative of physiological changes in healing (e.g.

144   ultimate tensile strain, cellular tissue proliferation, and vascularisation).

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146   Non-randomised trials were excluded to reduce selection bias (Hahn et al. 2005). Only

147   English language papers were included due to a lack of translation services. Papers were

148   initially screened by title/ abstract. Potentially relevant abstracts were sourced in full text

149   and assessed independently against distinct inclusion criteria by two reviewers (CT/ FS) for

150   eligibility. A third reviewer was available for consultation and consensus (LK).

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152   Non-randomised experimental or observational studies (including *in vitro* studies), and

153   those investigating pharmaceutical drugs were excluded from the review.

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155   **Search**

156   EBSCO, PUBMED, Science Direct and- via the OVID platform- Embase Classic/ Embase,

157   MEDLINE and Global Health databases were searched from inception (1947) to 11th June

158   2017.

159   The following search field was used: (("muscle"[All Fields] OR "musculoskeletal"[All Fields]

160   OR "tendon"[All Fields] NOT "cartilage"[All Fields] NOT "skin"[All Fields] NOT

161   "cutaneous"[All Fields] NOT "bone"[All Fields]) AND ("Amino acid"[All Fields] OR

162   "vitamin"[All Fields] OR "arginine"[All Fields] OR "leucine"[All Fields] OR "phenol"[All Fields]

163   OR "beta-Hydroxy beta-methylbutyric acid"[All Fields] OR "retinol"[All Fields] OR

164   "ascorbic"[All Fields] OR "taurine"[All Fields])) AND ("healing"[All Fields] OR "repair"[All

165   Fields] OR "collagen"[All Fields]) AND dietsuppl[sb].

166   Search terms were informed by a feasibility search, and in conjunction with a health

167   information librarian. Citations of key articles found in the feasibility search were evaluated

168   as a grey literature search.

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170   **Data Extraction**

171   Data extraction was undertaken by two reviewers using a piloted form. Reasons for

172   exclusion were noted. Data extraction included: date of study, animal or human model, type

173   of tissue, subject details (animal type, age, sex), dosage and type of supplement (compound,

174   administration or consumption), dosage and type of control or comparator, duration of

175   supplementation, follow up times and losses to follow up, outcomes.

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177   Primary outcomes were pre-determined to be:

178   -   Histological changes to tissue collagen content or rate of synthesis

179   -   Biochemical changes in serum composition

180   -   Biomechanical alterations in force tolerance of healing tissue (e.g. ultimate tensile

181   strength)

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183   Secondary outcomes were examined for clinical relevance and collected as appropriate (e.g.

184   radiographic evidence of morphological change).

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186 **Data analysis**

187 Although the optimal synthesis method to strengthen findings of treatment effect from

188 randomised trials is considered to be meta-analysis (DerSimonian & Laird, 1986) in this case

189 it was deemed inappropriate as there was insufficient homogeneity of data across tissue

190 types, interventions and outcomes to conform to data pooling. Instead a narrative synthesis

191 was undertaken as per the method described by Popay et al. (2006) combining content

192 analysis of the frequency of outcomes demonstrating statistically significant treatment

193 effects, and thematic analysis of the mechanisms of identified effects. This method provides

194 a framework appropriate for the analysis of intervention effectiveness when statistical

195 means are not applicable (Rodgers et al., 2009); providing flexibility in structure to develop a

196 theory of intervention effect by exploring relationships in the evidence base. This method

197 has been applied previously with success (Arai et al., 2007) with conclusions similar to meta-

198 analysis on the same group of randomised trials (DiGuiseppi & Higgins, 2001). The

199 comparison of narrative synthesis to meta-analysis on the same topic demonstrates the

200 importance of using structure to maintain transparency of the process and establishes

201 trustworthiness of the synthesis product by reducing selection bias (Rodgers et al., 2009).

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203 **Critical Appraisal**

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205 Evaluation of risk of bias was undertaken using the Cochrane risk of bias tool (Higgins et al.,

206 2011) for human studies or the Systematic Review Centre for Laboratory Animal

207 Experimentation (SYRCLE) risk of bias tool for animal studies (Hooijmans et al., 2014). Two

208 reviewers examined the studies for risk of bias and internal validity. Specifically the

209   appraisers evaluated the studies relative to sources of bias (e.g. allocation concealment,

210   blinding, selective outcome reporting) and classified to low, high or unclear risk of bias. In

211   order to assess inter-observer agreement of the risk of bias, the Kappa Statistic was used

212   (Cohen, 1960).

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Results

Search

Figure 1 presents the study selection process. Primary search results found 479 papers. 416

articles were excluded after comparison against the research question, on the basis of title

and abstract, with 63 remaining for evaluation of full text. 44 of these were excluded due to

methodology inclusion criteria (non-randomised studies, narrative and systematic reviews);

or due to examining incorrect tissue type (bone, cartilage, ligament, skin wounds). 7 were

excluded on the basis of examining a supplement type different to amino acids/ vitamins

(e.g. fatty acids), however these studies additionally had non-randomised designs. 12

articles fulfilled the inclusion criteria.

ADD FIGURE 1: PRISMA Flow diagram with the following:

Figure 1. PRISMA flow diagram showing study selection process (Moher et al. 2009)

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Data Extraction

Tables 1 and 2 provide a summary of the characteristics and findings of included studies. 8

studies examined tendon healing (Table 1): 1 assessing vitamin A and E (Greenwald et al.,

1990); 2 assessing vitamin C (Hung et al., 2013; Ömeroğlu et al., 2009); 1 assessing taurine

246   (Akdemir et al., 2015); 2 assessing glycine (Vieira, Guerra et al., 2015; Vieira, De Oliveira et

247   al., 2015), and 2 assessing nutrient complexes which included various supplements, (e.g.

248   arginine, methylsulfonylmethane and vitamin C) (Gumina et al., 2012; Notarnicola et al.,

249   2012). Only the integrator articles used human models. The animal experiments examined

250   models of various injury types (e.g. incision/ repair, collagenase-induced, partial tear).

251   Human studies examined surgical repair and tendinopathy models.

252   4 studies examined the effect of leucine supplementation of muscle healing in animal

253   models (Table 2). 2 studies examined healing following cryolesion, with the other studies

254   examining exercise-induced muscle exhaustion and electrically-stimulated contraction-

255   induced injury.

256   Tissue healing outcomes included biomechanical measures of tissue integrity;

257   histopathological assessment of tissue via various procedures (e.g. immunostaining,

258   biochemical assay, birefringence analysis); blood serum testing; and morphometric analysis

259   (e.g. gross analysis of limb, microscopic examination of tissue).

260   **Quality Assessment**

261   All animal model studies demonstrated a high risk of bias. This was due to a number of

262   common deficiencies in the methodological quality of these studies. For example, allocation

263   concealment was not described in any of the papers, and as per the review decision rules

264   lack of reporting was assumed to demonstrate failure to complete. Blinding was only

265   undertaken in 1 paper (Kato et al., 2016), however this study did not randomise at the point

266   of allocation and only prior to histochemical analysis. 2 studies (Vieira, De Oliveira et al.,

267   2015; Vieira, Guerra et al., 2015) randomised only prior to light microscopy analysis.

268   Of the two human studies, one was measured as a high risk of bias (Gumina et al., 2012) due

269   to failure to blind assessors and complete intention-to-treat analysis. However, this study

270   did demonstrate excellent randomisation and allocation concealment and undertook a

271   power calculation to reduce likelihood of type II error. The other human study (Notarnicola

272   et al., 2012) showed low risk of bias, due to computer-assisted randomisation/ allocation

273   concealment, double-blinding, group matching of homogeneity and intention-to-treat

274   analysis.

275   Inter-observer agreement regarding the risk of bias was considered 'moderate' (k=0.46)

276   according to the Viera and Garrett (2005) Kappa interpretation model.   Moderate interrater

277   reliability may suggest an incorrect representation of the studies' risk of bias (McHugh,

278   2012). However, with most studies demonstrating high risk of bias, caution is already

279   suggested when generalising the results.

		Outcomes				Summary	
		Biomechanical	**Histopathological**	**Biochemical**	**Morphological**	**FINDINGS**	**RISK OF BIAS**
				ANIMAL STUDIES			
Akdemir et al., (2015) Model: Rodent [Achilles tendon incision/repair] N= 16 Supplement: Taurine	Dosage: 200mg Taurine Route: Injection (x 1 post repair) Control: Saline Follow up: 42 days	↑Mean max load (p=0.025) ↑ Mean max stress (p=0.025) ↑ Mean energy uptake (p <0.05)	(Verhodstd- modified score) ↓ mean fibrosis, fibroblast proliferation (p <0.05), oedema. ↑neutrophil infiltration (p <0.05)			Efficiency of tendon gliding improved over 6 weeks with a reduction in tissue adhesion due to fibrosis, and increased biomechanical integrity.	R1- High R2- High
Greenwald et al., (1990) Model: Chicken [flexor profundus tendon incision/repair] N= 96 Supplement: Vitamin A, E and beta-carotene	Dosage: Vitamin A (150,000 IU/kg) (once daily for 45 days) Route: Oral Control: Standard chow (vit. A = 22,000 IU/kg; vit. E = 35 IU/kg; betacarotene = 1 mg/kg) Follow up: 7/ 45 days	↑ UTS at 7 days (p=0.007), 30 days (p=0.06), 45 days (p<0.001)				Vitamin A elevates UTS during early healing, but perhaps at the risk of preventing true internal tendon strength. Conversely, vitamin E reduces UTS perhaps due to the reduction in peri-tendon adhesions by anti-oxidant activity. Beta-carotene shows margin elevation of UTS.	R1- High R2- High
	Dosage: Vitamin E (1000 IU/kg) [Otherwise as above]	↓ UTS at 7 days (p=0.004), 45 days (p<0.001)					
	Dosage: Beta-carotene (90mg/kg) [Otherwise as above]	Margin ↑ UTS (p>0.05)					
Ömeroğlu et al., (2009) Model: Rodent [experimental Achilles rupture] N= 42 Supplement: Vitamin C	Dosage: 150mg vitamin C Route: Injection (post rupture and then every 2 days for 3- 21 days Control: 1.5cc saline Follow up: 3/10/21 days		↑ revascularisation at day 3 (p=0.01) ↑ collagen production at day 10 (p=0.021), day 21 (p=0.103) ↑ collagen diameter at day 3/10/21 (p<0.001) ↑ fibroblast proliferation at day 3 (p=0.042)			Vitamin C facilitates histological changes in increased collagen proliferation and collagen fibre diameter, as well as increased numbers of fibroblasts.	R1- High R2- High
Vieira, De Oliveira et al. (2015) Model: Rodent [collagenase induced Achilles tendon injury]	Dosage: 5% glycine diet, daily for 7-21 days Route: Oral Control: Standard chow and water	↑ UTS and maximum displacement load at 21 days (p<0.05)	↑ birefringence of collagen at 7 days ↑ epitenon thickness at 7 days (p<0.05)	↑ GAGs at 7 (p<0.01) and 21 days (p<0.05) ↑ hydroxyproline at 7 and 21 days (p<0.05)		Glycine enhances tissue levels of GAGs, hydroxyproline and NCPs during early healing, leading to greater collagen	R1- High R2- High

N= 50 Supplement: Glycine	Follow up: 7/21 days			↑ NCPs at 7 days (reduced by 21 days) (p<0.01)		synthesis and enhanced extracellular matrix re-modelling. Thus leading to greater biomechanical strength.	
Vieira, Guerra et al., (2015) Model: Rodent [collagenase induced Achilles tendon injury] N= 35 Supplement: Glycine +/- green tea	Dosage: *Camellia sinensis* (green tea/ GT) [700 mg/kg/day] +/- 5% glycine diet (daily for 7-21 days) Route: Oral, daily for 7-21 days Control: Standard chow and water Follow up: 7/21 days	↑ UTS at 21 days (GT and glycine) (p<0.05)	↑ birefringence of collagen at 7 days (GT and glycine) (p<0.05)	↑ GAGs at 21 days (GT and glycine) (p<0.05) ↑ hydroxyproline at 7 days (GT and glycine) and 21 days (GT only) (p<0.05) ↓ NCP at 7 and 21 days (GT and glycine) (p<0.05)		The combination of glycine and GT shows greater benefits to early collagen organisation at 7 days, leading to enhanced fibre stability and load tolerance.	R1- High R2- High
Hung et al., (2013) Model: Chicken [incisional injury to the flexor digitorum profundus tendon] N= 57 Supplement: Vitamin C	Dosage: Vitamin C (5mg/ml or 50mg/ml) Route: Injection immediately post injury Control: Saline Follow up: 14/ 42 days	↓ tendon gliding resistance at 6 weeks (both dosages) (p=0.010)	↓ swelling at 2 weeks (50ml) ↓ tendon adhesions and fibrosis at 2 weeks (5ml)	↑ glutathione at 2 weeks (p=0.05)	↓ fibrotic size at 6 weeks (5mg) (p=0.013	Vitamin C injection enhances anti-oxidant action, leading to reduced tendon adhesion and restoration of glutathione levels. There is a greater response for gliding resistance and fibrotic size with a lower dose (5mg).	R1- High R2- High
HUMAN STUDIES							
Gumina et al., (2012) Model: Human [Arthroscopic repair of a large postero-superior rotator cuff tear] N= 90 Supplement: Combined integrator (Tenosan: arginine L-alpha-ketoglutarate, methylsulfonylmethane, hydrolyzed type I collagen and bromelain)	Dosage not given. Route: Oral (2 sachets per day for 12 weeks) Control: No supplement Follow up: 84 days				↓ incidence of post repair classification type I-III (Sugaya's classification) (p=0.045) ↓ percentage re-rupture (p=0.111) ↑ percentage of type I-II repair integrity (p<0.05)	There is a higher incidence of type I-II classification of repair intensity and a reduced rate of re-rupture in the intervention group.	R1- High R2- Unclear

Notarnicola et al., (2012) Model: Human [Insertional Achilles tendinopathy] N= 64 Combined integrator (Tenosan: arginine-L-alpha-ketoglutarate (500 mg), MSM (550 mg), hydrolyzed collagen type I (300 mg), Vinitrox (125 mg), bromelain (50 mg), and vitamin C (60 mg).	Dosage: arginine-L-alpha-ketoglutarate (500 mg), MSM (550 mg), hydrolyzed collagen type I (300 mg), Vinitrox (125 mg), bromelain (50 mg), and vitamin C (60 mg [+ extra-corporeal shockwave therapy] Route: Oral (2 sachets per day for 60 days) Control: Placebo [+ extra-corporeal shockwave therapy] Follow up: 6 months	↓ mean oximetry value at 6 months (p<0.0001)	Despite small effect sizes, the findings suggest ECSWT and a combined integrator can reduce tissue perfusion at 6 months in Achilles tendinopathy.	R1- Low R2- Unclear

Table 1

Summary of study characteristics and findings of selected supplements on tendon healing

[UTS- Ultimate tensile strength   GAGs- Glycosaminoglycans   NCP- Non-collagenous proteins   MSM- Methylsulfonylmethane   R1- Reviewer 1 [CT]   R2- Reviewer 2 [FS]
ECSWT- extra-corporeal shockwave therapy

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		Outcomes				Summary	
		Biomechanical	Histopathological	Biochemical	Morphological	FINDINGS	RISK OF BIAS
				ANIMAL STUDIES			
Anthony et al., (1999) Model: Rodent [exercise induced exhaustion of gastrocnemius/ plantaris] N= 30 Supplement: Leucine	Dosage: a) Leucine + exercise (54g/L), b) Leucine (54g/L) + exercise + carbohydrate (235.5g glucose/ 235.5g sucrose) Route: Oral diet provided immediately post exercise. Control: c) Sedentary control (no supplement), d) Exercise + dietary deprivation, e) exercise + carbohydrates (262.5g glucose/ 262.5g sucrose) Follow up: (outcomes taken 1 hour post eating)		↑ fractional rate of protein synthesis [Protein synthesis elevated to the same as non-exercise controls 1 hour after exercise] (leucine +/- carbohydrates) (p<0.05)	↑ recovery of skeletal muscle glycogen 1 hour post exercise (carbohydrate +/- leucine only) (p<0.05) ↑ plasma insulin and muscle glycogen (carbohydrate + leucine) vs carbohydrate only (p<0.05)		Leucine may stimulate protein synthesis by enhancing muscle sensitivity to available insulin immediately after ingestion. Leucine works independently to stimulate protein synthesis (almost as effectively as leucine combined with carbohydrate) despite not raising insulin levels.	R1- High R2- High
Kato et al., (2016) Model: Rodent [electrically stimulated tibialis anterior contractions] N= 49 Supplement: Leucine	Dosage: Essential amino acids: leucine (1g/kg) Route: Oral (2 days pre-exercise and 1-14 days post) Control: Oral saline gavage Follow up: 1-14 days	↑ speed of return to stronger muscle function (3 days in leucine group) (p<0.05) Return of isometric dorsiflexion torque to the level of controls in 14 days (leucine only)	↓ numbers of damaged fibres (leucine) (p<0.001)	↓ interleukin-6 expression 1 day post exercise (leucine only) (p<0.001)		Provision of leucine enhances muscle healing by suppressing inflammation (indicated by reduced IL-6). Thus leading to attenuation of muscle damage and earlier return of muscle function following exercise.	R1- High R2- High
Pereira, Baptista et al., (2014) Model: Rodent [soleus muscle cryolesion] N=48 Supplement: Leucine	Dosage: Leucine (1.35g/kg) Route: Oral gavage (13 days pre-injury, 10 days post) Control: Oral saline gavage	↓ reduction in strength production between pre and post fatigue tetanic stimulus (compared to the reduction shown in control animals)		↑ myofiber cross sectional area at day 10 (p<0.05) ↓ inflammatory cell infiltration at day 10 (p<0.05)		Leucine improves recovery of muscle fibre size and function (resistance to fatigue) post injury. The mechanism of these changes involves	R1- High R2- High

	Follow up: 1-10 days		↓ area density of collagen type III at day 10 (p<0.05) ↓ macrophage infiltration on day 3 (p<0.05) ↓ expression of mTOR/ p-mTOR at day 10 (p<0.05) ↓ activation of FOXO3a at day 3 and 10 (p<0.05) Cryolesion-induced increase in ubiquinated protein was attenuated (p0.05)			the protein ubiquination pathway. Changes include a slight reduction in mTOR expression (but no other alteration to the PI3K/Akt/ mTOR pathway); and reduced FOXO3a activation/ ubiquinated protein content. This would indicate reduced post exercise proteolysis.	
Pereira, Silva et al., (2014) Model: Rodent [tibialis anterior cryolesion] N= 22 Supplement: Leucine	Dosage: Leucine (1.35g/kg) Route: Oral gavage (13 days pre-injury, 10 days post) Control: Oral saline gavage Follow up: 10 days	Prevention of reduction in pre-fatigued tetanic strength (however not sustained during fatigue protocol)	↓ expression of phosphorylated TGF-beta receptor type-1 at day 10 (p<0.05) ↓ Smad2/3-positive nuclei at day 10 (p<0.05) ↓ MyHC-n positive regenerating myofibers at day 10 (p<0.05) ↑ MyHC-II at day 10 (p<0.05) ↓ hydroxyproline to levels of control (p<0.05) ↑ fractional rate of protein synthesis (p<0.05)		↓ procollagen/ thin collagen fibers. ↓ inflammatory area (p<0.05)	Leucine supplementation improves muscle contractile performance, without change in myofiber size. There is an accelerated shift from neonatal MyHC to adult MyHC and attenuation of proteins which promote excessive collagen synthesis.	R1- High R2- High

Table 2
Summary of study characteristics and findings of selected supplements on muscle healing

CSA- cross sectional area   FOXO3- Forkhead box O3   GAGs- glycosaminoglycans   IL-6- interleukin 6   MDA- malondialdehyde mTOR- Mechanistic target of rapamycin   myHC-II- myosin heavy chain II (adult)
NCP- non-collagenous proteins TGF-β- transforming growth factor beta]

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Data Analysis

288   Table 3 illustrates the results of the content analysis showing the common tissue healing

289   outcomes and mechanisms of supplementation effect across studies. Outcomes with

290   statistically significant effect only are displayed (p values <0.05). Whilst there is debate

291   around the appropriate use of p-values as a measure of significance (Wasserstein & Lazar,

292   2016), these tables aim to represent the key findings of the studies examined, rather than

293   making any judgements as to the degree of intervention effect each supplement provides.

294   This allows presentation of the large variability of heterogenous outcomes upon which the

295   reviewer can base the theoretical examination of underlying mechanisms whilst reducing

296   the probability of chance and providing evidence against the null hypothesis. Interestingly,

297   inclusion of outcomes with no significant effect or negative effect did not influence the final

298   conclusions in this review and were removed to focus the analysis.

299   Thematic analysis indicates that various characteristics of tissue healing effect are shared

300   across studies. Tables 1 and 2 delineate themes of the treatment effects by the outcomes

301   presented in the studies (e.g. biomechanical, biochemical, etc.). However, as the content

302   analysis displays only the outcomes chosen by the original researchers they may be open to

303   publication bias, and other mechanisms may be possible.

304   Themes were also extracted relative to when mechanisms were observed within normal

305   healing phases (Watson, 2006) and are displayed in figures 2 and 3. Figure 2 illustrates that

306   the changes in the extra-cellular matrix in response to tendon injury (beyond those seen in

307   controls) occur predominantly throughout the inflammatory and proliferation phases of

308   healing (within the first 21 days), but that changes are sustained into the remodelling phase

309   (beyond 42 days). Morphological benefits remain evident for 6 months. Figure 3

310 demonstrates that the mechanisms of effect in muscle tissue occur earlier and are

311 commenced almost immediately (1 hour) post injury during the bleeding phase.

312 Modification of muscle protein synthesis balance is then sustained during the inflammatory

313 phases, where inflammation and oxidation is attenuated by supplementation.

314 Further themes are apparent relative to how healing is influenced by supplementation, and

315 patterns are observed relative to whether there is evidence of direct effects on cellular

316 proliferation, or indirect physiological processes which precede healing. Of course, this

317 observation is dependent upon which outcomes are used, but allows exploration of the

318 effects of supplements on the response to injury. Within tendon tissues, mechanisms

319 include enhanced anti-oxidant action; control of immune response; and modification of

320 elements of the extra-cellular matrix (ECM) content. The former effects (anti-oxidant action

321 and immune response control) could be themed as indirect actions to cellular proliferation

322 as they show changes which facilitate the tissues response to allow improved cellular

323 regeneration, but do not directly cause cell synthesis. The latter mechanism (proliferation of

324 ECM constituent components) includes increases in cellular components of collagen

325 (hydroxyproline); balance of fibroblastic response; increases in glycosaminoglycan levels;

326 reduction in non-collagenous proteins; and increases in the size and amount of collagen

327 produced. These could be categorised as direct changes which facilitate proliferation and

328 remodelling of regenerating tissue. In muscle healing, indirect mechanisms are also

329 apparent (e.g. increased anti-oxidant or inflammatory action). However, rather than

330 alterations to the extra-cellular matrix (as direct actions), biomechanical and muscular

331 efficiency changes occur alongside promotion of plasma insulin sensitivity and a reduction in

332 proteolysis. Following injury this would mean the number of damaged muscle fibres is

333   reduced, and there is improved functional rate of protein synthesis, leading to improved

334   muscle tissue integrity, function and cross-sectional area.

335   A final theme is apparent relative to the physiological level at which supplements act. The

336   variety of outcomes show effects on tissue at a molecular biological level (e.g. biochemical

337   or genetic change); a cellular biological level (e.g. protein signalling pathways); a

338   histopathological level (e.g. tissue biopsy and microscopic evaluation); and a morphological

339   level (e.g. radiological or clinical examination). The themes show the supplements

340   contribute to healing at various cellular levels, at various times alongside the healing phases,

341   with both indirect and direct effects on the proliferation of tissue. As such there are a

342   variety of outcomes which can be used to demonstrate their effect on healing and the

343   structural integrity of the healing tissue.

344

TENDON

		↓ NCP	↑ hydroxyproline concentration	↑ GAGs	↑ anti-oxidant action (reduced MDA/ elevated glutathione)	↑ fibroblast differentiation	↓ immune cell response (macrophages, lymphocytes)	↓ fibroblast proliferation	↑ collagen diameter	↑ collagen proliferation	↓ swelling	↑ tissue perfusion/ oximetry	↓ UTS	↑ UTS or mean maximum stress	Morphologiical evidence of enhanced healing
ANIMAL STUDIES															
Akdemir et al. (2015)	Taurine					✓	✓	✓				✓		✓	
Vieira et al (2015a)	Glycine		✓	✓						✓				✓	✓
Vieira et al. (2015b)	Glycine, green tea	✓	✓	✓						✓				✓	
Greenwald et al. (1990)	Vitamin A													✓	
Greenwald et al. (1990)	Vitamin E												✓		
Ömeroğlu et al. (2009)	Vitamin C								✓	✓		✓			
Hung et al. (2013)	Vitamin C				✓			✓				✓			
HUMAN STUDIES															
Gumina et al. (2012)	Combined integrator 1														✓
Notarnicola et al. (2012)	Combined integrator 2											✓			

Table 3

Effects of supplementation on physiological parameters of healing in tendon and muscle tissue (statistically significant changes in response to intervention over control presented)

345

346

MUSCLE

		↑ plasma changes to insulin/ glycogen	↓ interleukin 6	↑ MyHC-II gene expression	↓ transcription factor protein encoding relative to cell proliferation (mTOR, FOXO3)	↓ Smad2/3 + nucleus	↓ expression of phosphorylated TGF-β	↓ hydroxyproline	↓ ubiquinated protein	↓ number of damaged fibres	↑ muscle strength or function	↑ functional rate of protein synthesis	↑ muscle CSA
ANIMAL STUDIES													
Anthony et al. (1999)	Leucine	✓										✓	
Kato et al. (2016)	Leucine		✓							✓	✓		
Pereira, Baptista et al. (2014)	Leucine		✓		✓				✓				✓
Pereira, Silva et al. (2014)	Leucine		✓	✓		✓	✓		✓			✓	

Table 3 continued

Effects of supplementation on physiological parameters of healing in tendon and muscle tissue (statistically significant changes in response to intervention over control presented)

[Combined integrator 1- Arginine L-α- ketoglutarate, MSM, hydrolysed collagen I, bromelain   Combined integrator 2- Arginine L-α- ketoglutarate, MSM, hydrolysed collagen I, bromelain, Vinitrox, vitamin C

CSA- cross sectional area   FOXO3- Forkhead box O3   GAGs- glycosaminoglycans   IL-6- interleukin 6   MDA- malondialdehyde   mTOR- Mechanistic target of rapamycin   myHC-II- myosin heavy chain II (adult)   NCP- non-collagenous proteins TGF-β- transforming growth factor beta]

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ADD FIGURE 2: Mechanisms of tendon issue change with the following:

Figure 2. Graphical representation of mechanisms of supplement effect on tendon tissue relative to musculoskeletal healing phases

(modified as described by Watson, 2006)

ADD FIGURE 3: Mechanisms of muscle tissue change with the following:

Figure 3. Graphical representation of mechanisms of leucine effect on muscle tissue relative to musculoskeletal healing phases (modified as described by Watson, 2006)

353

354

Discussion

355

356 This review shows that specific amino acids (leucine, glycine, taurine) and vitamins (A, E and

357 C) provide particular effects on the physiology of musculoskeletal tissue healing. These

358 mechanisms occur at multiple levels (from molecular physiology to morphological tissue

359 structure) and influence cell regeneration directly (cell proliferation) and indirectly (cellular

360 environment enhancement). These changes occur alongside improvements in

361 biomechanical integrity. The findings are predominantly guided by animal experiments, due

362 to the ethical difficulties of performing histological examination of human tissue. The

363 mechanisms described are indicative of the outcomes which demonstrated a degree of

364 statistical significance, and perhaps do not outline all potential mechanisms. Similar effects

365 in skin and experimental models are provided as comparative effects, with awareness that

366 specific regenerative effects are dependent upon the host tissue cells.

367

368 A number of studies describe effects on anti-oxidant capacity of the supplements as an

369 indirect influence on tendon healing. Two studies (Hung et al., 2013; Ömeroğlu et al., 2009)

370 examined vitamin C and its role as a free radical scavenger (Buettner & Moseley, 1993). In

371 the presence of reactive oxygen species, vitamin C donates two electrons to reduce the free

372 radical, and a less reactive ascorbyl radical is released. Ömeroğlu et al. (2009) examined a

373 rodent model of Achilles tendon rupture and provided injections of 150mg vitamin C every

374 other day for 21 days. In comparison to saline injections the study found that vitamin C

375 increased tissue perfusion in the early proliferative phase of healing alongside increased

376 fibroblast proliferation (3 days post). At 10 and 21 days, collagen proliferation and diameter

377 is elevated, and at 43 days tendon swelling is less. This may indicate an early healing

378 response, leading to elevated collagen development and longer term benefits. Hung et al.

379 (2013) demonstrated reductions in fibroblast proliferation at 10 and 43 days (corresponding

380 with the findings of greater balance between collagen formation and fibrosis) and increased

381 glutathione at 14 days, indicating enhanced anti-oxidant action. These changes contributed

382 to reduced fibrotic size and resistance to tendon gliding at 43 days. The influence of a mixed

383 anti-oxidant supplementation (including vitamin C) was measured in a double-blind,

384 randomised, placebo controlled trial of trauma patients with non-musculoskeletal wounds

385 (Blass et al., 2012). The results indicated that anti-oxidants (alongside glutamine) increased

386 rate of healing by faster wound closure time in the supplement group versus placebo (35

387 days ± 22 vs. 70 days ± 35, p<0.01). Examination of topical vitamin C showed similar results,

388 alongside increased collagen fiber density (p<0.05) (Lima et al., 2009). These results,

389 alongside the findings of this review, demonstrate an apparent anti-oxidant mechanism to

390 promote similar enhancement of healing in tendon.

391

392 Another anti-oxidant is taurine; an amino acid which has been found to regulate collagen

393 production and inhibit fibrosis (Gordon et al., 1986). A controlled animal study examined

394 taurine's effect on skin wound tensile strength and malondialdehyde (MDA) content- a

395 measure of lipid peroxidation and oxidative stress following injury (Abuja and Albertini,

396 2001; Dinçer et al., 1996). The results demonstrate taurine enhanced wound content and

397 strength relative to histological evidence of enhanced collagen production and greater force

398 displacement (p<0.01), and that topical application reduced MDA content more than

399 injection. It could be suggested that increases in wound strength are secondary to increased

400 anti-oxidant capacity and collagen synthesis, and assisted by taurine's ability to aid vitamin C

401 metabolism (Kaplan et al., 2004). Akdemir et al. (2015) examined the effect of 200mg

402 taurine injections post rodent Achilles tendon repair, measuring UTS and histopathological

403 assessment after 42 days. Their results found reductions in immune cell activity, reduced

404 fibroblast proliferation/ increased fibroblast differentiation, and increased UTS compared to

405 saline control. The authors suggest taurine may reduce fibrosis and elevate repair integrity,

406 and postulate this is due to both anti-oxidant and anti-inflammatory effects, and inhibition

407 of fibronectin expression and fibrin formation during healing. This suggests that the

408 influence of taurine on tendon wounds, is at least in part, in concurrence to the findings in

409 skin wounds.

410 Other supplements included in this review demonstrate more direct influences on tendon

411 healing. Previous work by Ehrlich et al. (1973) examined the effect of vitamin A on the

412 restoration of hydroxyproline content and histological tissue grading after provision of

413 gluticorticoid which inhibits collagen synthesis and connective tissue repair. A rodent model

414 of implanted polyvinyl sponge granuloma was used with sub-grouping into control, corticoid

415 alone, vitamin A alone and combined corticoid and vitamin A. Injection was the route of

416 administration for all groups. The results indicated vitamin A was able to partial reverse

417 declines in fibroblast, collagen and hydroxyproline content diminished by gluticorticoid

418 injection. Previous work by the same authors show that healing rate of skin wounds, not

419 inhibited by cortisone, is not enhanced by vitamin A provided topically or via intramuscular

420 systemic administration (Hunt et al., 1969). There is therefore some confusion as to whether

421 vitamin A can influence collagen accumulation. One study in this review examined the

422 effects of vitamins A (150,00 IU/kg) and E (1000 IU/kg) in a model of tendon repair healing

423 in chickens (Greenwald et al., 1990). The animals were given daily oral doses and ultimate

424 tensile stress (UTS) was evaluated at 7 and 45 days post repair. The results show an increase

425 in UTS at both 7 days (897g +/- 164 vs. 356g +/- 68, p<0.007) and 45 days (1972g +/- 255 vs.

426   915g +/- 113, p<0.001) which supports the findings of Ehrlich et al. (1973), however without

427   concurrent histopathological evidence of collagen change, this is speculative.

428   A systematic review examining the effectiveness of vitamin E on measures on cell

429   proliferation, infection and wound healing found a dearth of robust studies in this area

430   (Hobson, 2016). This is despite the search finding a high number of primary research studies

431   (n=31) and representing a reasonable level of quality (level 2b). Greenwald et al. (1990)

432   found a reduction in UTS compared to control at 7 (163g +/- 81 vs. 356 +/- 68, P<0.004) and

433   45 days (445g +/- 125 vs. 915g +/- 113, p<0.001) indicating vitamin E has a deleterious

434   influence on healing tendon. Vitamin E has been shown to act as a lysosomal membrane

435   stabiliser (Ehrlich et al., 1972). Lysosomes- organelles responsible for the enzymatic

436   responses to injury to balance matrix regeneration (Stromberg et al., 1977)- have been

437   found to inhibit tendon healing (Ehrlich et al., 1972) leading to a reduction in collagen cell

438   number and decreased UTS. It is possible that similar mechanisms may explain these

439   results.

440   Dietary arginine above recommended daily allowance has been shown to increase collagen

441   accumulation in healthy animals (Barbul et al., 1990; Kirk et al., 1993). This review found no

442   studies investigating the effect of this amino acid in animal models. Two human studies

443   examined the effects of arginine L-α-ketglutarate within a combined integrator. The studies

444   examined the effect of the supplement on rotator cuff repair integrity (Gumina et al., 2012);

445   and tissue oximetry in Achilles tendinopathy (Notarnicola et al., 2012). Gumina et al. (2012)

446   combined arginine with methylsulfonylmethane, hydrolysed collagen I and bromelain, for a

447   dosing period of 12 weeks. Subsequently, they evaluated repair integrity in the intervention

448   group against a no-supplement control and found a higher incidence of more favourable

449   Sugaya classification type I-II repair intensity (Sugaya et al., 2007); and a lower rate of re-

450   rupture. Unfortunately, whilst the trial randomised and concealed allocation, they failed to

451   report the dosage of supplements provided in the integrator. Additionally, the trial did not

452   incorporate drop outs to the final analysis and failed to utilise a placebo control, and

453   subsequently demonstrates a high risk of bias.

454

455   Polytetrafluoroethylene implants allow researchers to evaluate deposition of fibroblasts and

456   extracellular matrix tissue. A double-blind trial (Williams et al., 2002) randomised healthy

457   adults (n=18) to receive daily amino acids (14g arginine, 3g HMB, 14g glutamine) or an

458   isonitrogenous, isocaloric control supplement of non-essential amino acids. Implants

459   removed at 7 or 14 days were analysed for hydroxyproline and amino-nitrogen. Whilst no

460   significant differences in collagen accumulation (measured by hydroxyproline) were evident

461   at 7 days, at 14 days there were significant increases in hydroxyproline content accumulated

462   (+67%, $p<0.03$); occurring independent of an increase in total protein deposition. Evidence

463   from elderly humans (Kirk, 1993) supports these results, demonstrating a 52% collagen

464   increase, via a pro-inflammatory mechanism of action and enhancement of fibroblastic

465   synthesis. During healing fibroblasts stimulate collagen synthesis deposition within the ECM

466   (Stechmiller et al., 2005); and the production of growth factors (e.g. insulin-like growth

467   factor 1, transforming growth factor β) promotes proliferation, angiogenesis and protein

468   synthesis (Schultz & Mast, 1998). During the remodelling phase fibroblasts produce ECM

469   components (collagen, gelatin and proteoglycans), and release metalloproteinases and

470   tissue inhibitors of metalloproteinases to orchestrate tissue remodelling (Bryant, 2000;

471   Schultz & Mast, 1998; Tarnuzzer & Schultz, 1996).

472 The other human study in this review (Notarnicola et al., 2012) used the same combined

473 integrator, however also added 60mg vitamin C. Subjects (n=64) were randomised and

474 matched for age and gender and the integrator (or placebo) was provided orally for 60 days

475 with or without extra-corporeal shockwave therapy (ESWT). The findings show that the

476 supplement/ ESWT group had a reduced oximetry value at 6 months indicating a reduction

477 in tendon micro-circulation and neovessel development; a component of the

478 pathophysiology of tendinopathy (Knobloch, 2008). This study demonstrates a low risk of

479 bias as it appropriately used randomisation, blinding, allocation concealment, intention-to-

480 treat analysis and group matching for homogeneity. However, for both studies using the

481 combined integrator, effects are confounded by the use of multiple ingredients and caution

482 is needed in extrapolating these results.

483

484 The final supplement which evaluated an effect on tendon tissue is glycine; an amino acid

485 synthesised by other amino acids, including hydroxyproline. Experiments demonstrate

486 glycine's capacity to prevent inflammatory cell infiltration and reduce joint oedema

487 following injuries of experimental arthritis (Li et al., 2001). It is proposed that this anti-

488 inflammatory effect involves glycine receptor activation in leukocytes and suppression of

489 immunocytes (Li et al., 2007). Two studies (Vieira, De Oliveira et al., 2015; Vieira, Guerra et

490 al., 2015) assessed the effect of glycine (with or without green tea) in a rodent collagenase-

491 induced Achilles' tendon injury model. Each provided a 5% glycine diet and showed

492 increases in collagen proliferation, alongside elevated hydroxyproline, non-collagenous

493 protein content and glycosaminoglycan content at 7 days. Hydroxyproline,

494 glycosaminoglycans (GAGs) and non-collagenous proteins (NCPs) are components of the

495 extracellular matrix. Hydroxyproline is a dominant protein of the ECM, comprising 20% of

496 fibrillary collagen structure; contributing to its molecular stability (Mouw et al., 2014). The

497 non-collagenous matrix consists of GAGs (and other molecules including glycoproteins,

498 proteoglycans) which surround collagen fibrils and bind water to assist mechanical tolerance

499 to stress (Kannus, 2000). Increased tendon content of these ECM constituents, through

500 glycine supplementation, may enhance collagen synthesis during the early proliferation

501 phase, augmenting tensile strength. The addition of green tea to the glycine diet seems to

502 enhance collagen organisation at 7 days leading to further fibre stability and load tolerance.

503

504 *In vivo* and *in vitro* experiments indicate that the branched chain amino acid leucine acts as

505 a signalling molecule to regulate protein synthesis in skeletal muscle (Anthony, Anthony et

506 al., 2000; Kimball & Jefferson, 2001; Norton & Layman, 2006). Leucine effects at a

507 posttranscriptional level as a critical regulator of mRNA translation initiation (Anthony et al.,

508 2001) which facilitates protein synthesis (Svanberg et al. 1997, Yoshizawa et al., 1998). The

509 role of mammalian target of rapamycin (mTOR) signalling is essential for translation

510 initiation (Anthony, Yoshizawa et al., 2000). Leucine facilitates an initial release of insulin

511 (or increased insulin sensitivity in muscle); alongside a signalling cascade independent of

512 phosphoinositol 3-kinase (PI3-K), protein kinase B (PKB/ Akt) or 3-phosphoinositide

513 dependent protein kinase 1 (PDK1) activation (Anthony et al. 2001). At mTOR these

514 processes facilitate optimal activation of translation initiation, and are fine-tuned by other

515 unidentified pathways (Anthony et al., 2001). Four studies found in this review evaluated

516 the effect of leucine on healing muscle tissue (Anthony et al., 1999; Kato et al., 2016;

517 Pereira, Baptista et al., 2014; Pereira, Silva et al., 2014). The studies outline a process of an

518 immediate response in the bleeding phase to attenuate inflammation and increase protein

519 synthesis, starting with increased serum insulin and glycogen within one hour and elevated

520   fractional rate of protein (FRP) synthesis (Anthony et al., 1999). Reduction of the pro-

521   inflammatory cytokine, interleukin-6, occurs alongside reduction in muscle fibre damage.

522   Subsequently, cross sectional muscle area is increased, with reduction in protein

523   ubiquination; reduction in hydroxyproline as a proxy for reduced collagen; and modification

524   of transcription signalling pathways to attenuate cell proliferation (e.g. expression of

525   phosphorylated TGF-beta/ reduced Smad2/3+ nucleus). Pereira, Baptista et al. (2014)

526   identify that the reduction in mTOR expression occurs without other changes to the PI3K/

527   Akt/ mTOR pathways and that the change in FOXO3a expression is indicative of reduced

528   post exercise proteolysis. Additionally, Pereira, Silva et al., (2014) also demonstrates a

529   genetic shift towards adult Myosin Heavy Chain which assists muscle action. Alongside the

530   leucine-assisted changes to collagen synthesis, this may suggest a fine tuning of healing

531   response to ensure muscle contractile action is balanced with collagen formation. In

532   summation leucine acts during bleeding/ early proliferation to enhance muscle

533   regeneration.

534

535   **Implications/ Limitations**

536

537   This review summarises potential mechanisms for how selected supplements can influence

538   tendon and muscle healing, the basis of which are limited to the outcomes used by the

539   original studies. As no consensus is clear across the literature, minimal recommendations

540   can be given as to the clinical utility of such supplements for musculoskeletal healing.

541   Clinicians should however remain cognisant of nutritional practices throughout

542   rehabilitation, particularly in respect to the regenerative influences of vitamins and amino

543   acids.

544

545   As the predominant source of this data is from animal models it is essential to reflect on

546   their use to guide human consumption. Animal models can approximate human

547   physiological response to injury (Woo & Buckwalter, 1988) and act as a test for wound

548   healing agents (Gottrup et al., 2000). However, all animal studies analysed showed a high

549   risk of bias, indicating common methodological shortcomings. Lack of blinding,

550   randomisation, or simply poor reporting would be assisted by adherence to the Animals in

551   Research: Reporting in vivo experiments (ARRIVE) guidelines (Kilkenny et al., 2009) and

552   support future developments in research. Additionally, two articles are likely influenced by

553   confounding variables; specifically extra-corporeal shockwave therapy (Notarnicola et al.

554   2012) and the addition of green tea (Vierra, Guerra et al. 2015), which must be considered

555   when evaluating supplement effects. An additional limitation of this research is that the

556   search results did not produce evidence for effects of supplements commonly associated

557   with recovery such as branched-chain amino acids (Negro et al., 2008; Sharp and Pearson,

558   2010). This is likely due to the strict inclusion criteria of randomised trial design. As such this

559   review may not sufficiently elucidate all mechanisms of tissue response to injury related to

560   amino acids supplementation.

561

562   **Future Research**

563

564   High quality animal experimentation studies investigating the effects of supplements on

565   molecular, cellular and whole tissue levels; utilising outcomes of histological evidence of

566   tissue synthesis and biomechanical integrity; and at time points throughout the healing

567   process, need to be conducted to elaborate further on these findings. Human studies should

568   concentrate on assessing the effectiveness of supplements compared to isocaloric/

569   isonitrogenous placebo and be investigated with clinical and radiological outcomes to

570   evaluate whether the proposed mechanisms translate to practice.

571

572

573   **Conclusions**

574   Amino acids and vitamins demonstrate both indirect (anti-oxidant) and direct (synthesis rate

575   modifying) mechanisms of action in healing of tendon and muscle in animal models. These

576   mechanisms act at various stages of the healing cycle, and work on all physiological levels

577   from molecular to morphological. The translation of these mechanisms in humans is

578   speculative, however there is potential that supplements may provide some clinical utility.

579   Further research is required to test these hypotheses.

580   [Words 4484]

581

582

583   **Authorship and Conflict of interest**

584   All below stated authors have made contributions to this thesis.

585   Mr Christopher Tack- Primary investigator and author

586   Mrs Faye Shorthouse- Secondary data extraction and critical appraiser

587   Ms Lindsy Kass- Dissertation supervisor

588   I declare that I am the primary author of this article and that I have not used any sources

589   other than those listed in the bibliography and identified as references. I further declare

590   that I have not submitted this article to any other publication.

591   I declare that I have no affiliations with or involvement with any entity or organisation with

592   any financial interest or non-financial interest in the subject matter of this manuscript.

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References

Abuja, P. M., & Albertini, R. (2001). Methods for monitoring oxidative stress, lipid peroxidation and oxidation resistance of lipoproteins. *Clinica ChimicaActa*, *306*(1), 1-17. doi: 10.1016/S0009-8981(01)00393-X

Akdemir, O., Lineaweaver, W. C., Cavusoglu, T., Binboga, E., Uyanikgil, Y., Zhang, F., ... Yagci, T. (2015). Effect of taurine on rat Achilles tendon healing. *Connective tissue research*, *56*(4), 300-306. doi: 10.3109/03008207.2015.1026437

Anthony, J. C., Anthony, T. G., Kimball, S. R., & Jefferson, L. S. (2001). Signaling pathways involved in translational control of protein synthesis in skeletal muscle by leucine. *The Journal of nutrition*, *131*(3), 856S-860S.

Anthony, J. C., Anthony, T. G., Kimball, S. R., Vary, T. C., & Jefferson, L. S. (2000). Orally administered leucine stimulates protein synthesis in skeletal muscle of postabsorptive rats in association with increased eIF4F formation. *The Journal of nutrition*, *130*(2), 139-145.

Anthony, J. C., Anthony, T. G., & Layman, D. K. (1999). Leucine supplementation enhances skeletal muscle recovery in rats following exercise. *The Journal of nutrition, 129(*6), 1102-1106.

Anthony, J. C., Yoshizawa, F., Anthony, T. G., Vary, T. C., Jefferson, L. S., & Kimball, S. R. (2000). Leucine stimulates translation initiation in skeletal muscle of postabsorptive rats via a rapamycin-sensitive pathway. *The Journal of nutrition*, *130*(10), 2413-2419.

Arai, L., Britten, N., Popay, J., Roberts, H., Petticrew, M., Rodgers, M., & Sowden, A. (2007). Testing methodological developments in the conduct of narrative synthesis: a demonstration review of research on the implementation of smoke alarm interventions. Evidence & Policy: A Journal of Research, Debate and Practice, 3(3), 361-383. doi: 10.1332/174426407781738029

617   Barbul, A., Lazarou, S. A., Efron, D. T., Wasserkrug, H. L., & Efron, G. (1990). Arginine enhances

618   wound healing and lymphocyte immune responses in humans. *Surgery, 108*(2), 331-

619   6. doi: 10.1177/0148607194018002194

620   Blass, S. C., Goost, H., Tolba, R. H., Stoffel-Wagner, B., Kabir, K., Burger, C., ... Ellinger, S. (2012). Time

621   to wound closure in trauma patients with disorders in wound healing is shortened by

622   supplements containing antioxidant micronutrients and glutamine: a PRCT. *Clinical*

623   *nutrition,31*(4), 469-475. doi: 10.1016/j.clnu.2012.01.002

624   Bryant, R. A. (2000). *Acute & chronic wounds: nursing management*. (2nd ed.). Michigan, USA:

625   Mosby.

626   Buettner, G. R., & Moseley, P. L. (1993). EPR spin trapping of free radicals produced by bleomycin

627   and ascorbate. *Free radical research communications*, *19*(sup1), s89-s93.

628   doi: 10.3109/10715769309056s89

629   Bonci, C. M., Bonci, L. J., Granger, L. R., Johnson, C. L., Malina, R. M., Milne, L. W., ... Vanderbunt, E.

630   M. (2008). National Athletic Trainers' Association position statement: preventing, detecting,

631   and managing disordered eating in athletes. *Journal of athletic training*, *43*(1), 80-108. doi:

632   10.4085/1062-6050-43.1.80

633   Centre for reviews, & dissemination (CRD). (2009). Systematic reviews: CRD's guidance for

634   undertaking reviews in health care. *Centre for Reviews and Dissemination.* Retrieved July 2,

635   2017 from https://www.york.ac.uk/media/crd/Systematic_Reviews.pdf

636   Cohen, J. (1960). A coefficient of agreement for nominal scales. *Educational and psychological*

637   *measurement*, *20*(1), 37-46. doi: 10.1177/001316446002000104

638   Collins, C. E., Kershaw, J., & Brockington, S. (2005). Effect of nutritional supplements on wound

639   healing in home-nursed elderly: a randomized trial. *Nutrition*, *21*(2), 147-

640   155. doi: 10.1016/j.nut.2004.10.006

641   Department of Health. (2011) Food Supplements: Summary information on legislation relating to the

642   sale of food supplements. *DOH*. Retrieved July, 2, 2017 from

643   https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/204303/S

644   upplements_Summary__Jan_2012__DH_FINAL.doc.pdf

645   DerSimonian, R., & Laird, N. (1986). Meta-analysis in clinical trials. Controlled clinical trials, 7(3), 177-

646   188. doi: 10.1016/0197-2456(86)90046-2

647   DiGuiseppi, C., Higgins, J. P. T. (2001). Interventions for promoting smoke alarm ownership and

648   function. The Cochrane Database of Systematic Reviews, 2. Retrieved July, 2, 2017 from

649   http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD002246/abstract;jsessionid=E8332

650   36A411F53A8B6D43106920424B7.f04t03?systemMessage=Wiley+Online+Library+%27Journ

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652   08.00+EDT+%2F+13.00+BST+%2F+17.30+IST+for+up+to+75+minutes+due+to+essential+mai

653   ntenance. DOI:10.1002/14651858

654   Dinçer, S., Babül, A., Erdoğan, D., Özoğul, C., & Dinçer, S. L. (1996). Effect of taurine on wound

655   healing. *Amino Acids*, *10*(1), 59-71. doi: 10.1007/BF00806093

656   Ehrlich, H. P., Tarver, H., & Hunt, T. K. (1972). Inhibitory effects of vitamin E on collagen synthesis

657   and wound repair. *Annals of surgery*, *175*(2), 235.

658   Ehrlich, H. P., Tarver, H., & Hunt, T. K. (1973). Effects of vitamin A and glucocorticoids upon

659   inflammation and collagen synthesis. *Annals of surgery, 177*(2), 222.

660   Eijnde, B., Ursø, B., Richter, E. A., Greenhaff, P. L., & Hespel, P. (2001). Effect of

661   oral creatinesupplementation on human muscle GLUT4 protein content after

662   immobilization. *Diabetes*, *50*(1), 18-23. doi: 10.2337/diabetes.50.1.18

663   Ejaz, S., Chekarova, I., Cho, J. W., Lee, S. Y., Ashraf, S., & Lim, C. W. (2009). Effect of aged garlic

664   extract on wound healing: a new frontier in wound management. *Drug and chemical*

665   *toxicology, 32*(3), 191-203.doi: 10.1080/01480540902862236

666   Galeano, M., Torre, V., Deodato, B., Campo, G. M., Colonna, M., Sturiale, A., ... Altavilla, D.

667   (2001). Raxofelast, a hydrophilic vitamin E-like antioxidant, stimulates wound healing in

668   genetically diabetic mice. *Surgery*, *129*(4), 467-477.doi: 10.1067/msy.2001.112072

669   Gottrup, F., Ågren, M. S., & Karlsmark, T. (2000). Models for use in wound healing research: a survey

670   focusing on in vitro and in vivo adult soft tissue. *Wound Repair and Regeneration, 8*(2), 83-

671   96. doi: 10.1046/j.1524-475x.2000.00083.x

672   Gordon, R. E., Shaked, A. A., & Solano, D. F. (1986). Taurine protects hamster bronchioles from acute

673   NO2-induced alterations. A histologic, ultrastructural, and freeze-fracture study. *The*

674   *American journal of pathology*, *125*(3), 585.

675   Greenwald, D. P., Sharzer, L. A., Padawer, J., Levenson, S. M., & Seifter, E. (1990). Zone II flexor

676   tendon repair: effects of vitamins A, E, β-carotene. *Journal of Surgical Research, 49*(1), 98-

677   102. doi: 10.1016/0022-4804(90)90117-K

678   Gumina, S., Passaretti, D., Gurzi, M. D., & Candela, V. (2012). Arginine L-alpha-

679   ketoglutarate, methylsulfonylmethane, hydrolyzed type I collagen and bromelain in rotator

680   cuff tear repair: a prospective randomized study. *Current medical research and opinion,*

681   *28*(11), 1767-1774. doi: 10.1185/03007995.2012.737772

682   Hackam, D. G., & Redelmeier, D. A. (2006). Translation of research evidence from animals to

683   humans. JAMA, 296(14), 1727-1732. doi: 10.1001/jama.296.14.1731

684   Hahn, S, Puffer, S., Torgerson, D. J., & Watson, J. (2005). Methodological bias in cluster randomised

685   trials. *BMC Medical Research Methodology*, *5*(1), 10. doi: 10.1186/1471-2288-5-10

686   Henderson, V. C., Kimmelman, J., Fergusson, D., Grimshaw, J. M., & Hackam, D. G. (2013). Threats to

687   validity in the design and conduct of preclinical efficacy studies: a systematic review of

688   guidelines for in vivo animal experiments. PLoS medicine, 10(7), e1001489. doi:

689   10.1371/journal.pmed.1001489

690   Hespel, P., Op't Eijnde, B., Leemputte, M. V., Ursø, B., Greenhaff, P. L., Labarque, V., ... Richter, E. A.

691   (2001). Oral creatine supplementation facilitates the rehabilitation of disuse atrophy and

692   alters the expression of muscle myogenic factors in humans. *The Journal of*

693   *physiology*,*536*(2), 625-633. doi: 10.1111/j.1469-7793.2001.0625c.xd

694   Hess, K. R. (2011). Statistical design considerations in animal studies published recently in cancer

695   research. *Cancer research, 71*(2), 625-625. doi: 10.1158/0008-5472.CAN-10-3296

696   Higgins, J. P., Altman, D. G., Gøtzsche, P. C., Jüni, P., Moher, D., Oxman, A. D., ... Sterne, J. A. (2011).

697   The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *British*

698   *Medical Journal*,*343*, d5928. doi: 0.1136/bmj.d5928

699   Hobson, R. (2016). Vitamin E and wound healing: an evidence-based review. *International wound*

700   *journal*, *13*(3), 331-335. doi: 10.1111/iwj.12295.

701   Hooijmans, C. R., Rovers, M. M., de Vies, R. B., Leenaars, M., Ritskes-Hoitinga, M., & Langendam, M.

702   W. (2014). SYRCLE's risk of bias tool for animal studies. *BMC medical research methodology,*

703   *14*(1), 43. 10.1186/1471-2288-14-43

704   Holm, L., Esmarck, B., Mizuno, M., Hansen, H., Suetta, C., Hølmich, P., ... Kjær, M. (2006). The effect

705   of protein and carbohydrate supplementation on strength training outcome of rehabilitation

706   in ACL patients. *Journal of orthopaedic research*, *24*(11), 2114-2123. doi: 10.1002/jor.20147

707   Hung, L. K., Fu, S. C., Lee, Y. W., Mok, T. Y., & Chan, K. M. (2013). Local vitamin-C injection

708   reduced tendon adhesion in a chicken model of flexor digitorum profundus tendon

709   injury. *JBJS*, *95*(7), e41. doi: 10.2106/JBJS.K.00988

710   Hunt, T. K., Ehrlich, H. P., Garcia, J. A., & Dunphy, J. E. (1969). Effect of vitamin A on

711   reversing the inhibitory effect of cortisone on healing of open wounds in animals and

712   man. *Annals of Surgery, 170(*4), 633.

713   Judkins, C., & Prock, P. (2012). Supplements and inadvertent doping–how big is the risk to

714   athletes. In M. Lamprecht (Ed.), *Acute topics in sport nutrition*. (Vol. 59). (pp. 143-

715   152). Karger Publishers.

716   Kannus, P. (2000). Structure of the tendon connective tissue. *Scandinavian journal of*

717   *medicine & science in sports*, *10*(6), 312-320. doi: 10.1034/j.1600-

718   0838.2000.010006312.x

719   Kato, H., Miura, K., Nakano, S., Suzuki, K., Bannai, M., & Inoue, Y. (2016). Leucine-enriched

720   essential amino acids attenuate inflammation in rat muscle and enhance muscle

721   repair after eccentric contraction. *Amino acids*, *48*(9), 2145-2155.

722   doi: 10.1007/s00726-016-2240-1.

723   Kilkenny, C., Parsons, N., Kadyszewski, E., Festing, M. F., Cuthill, I. C., Fry, D., ... Altman, D. G.

724   (2009). Survey of the quality of experimental design, statistical analysis and reporting

725   of research using animals. *PloS one*, *4*(11), e7824. doi: 10.1371/

726   journal.pone.0007824.

727   Kimball, S.R., Jefferson, L.S., & Leonard, S. (2001). Regulation of protein synthesis by

728   branched-chain amino acids. *Current Opinion in Clinical Nutrition and Metabolic*

729   *Care. 4*(1), 39–43.

730   Kirk, S.J., Hurson, M., Regan, M.C., Holt, D.R., Wasserkrug, H.L., & Barbul, A. (1993). Arginine

731   stimulates wound healing and immune function in elderly human beings. *Surgery,*

732   *114(*2), 155–160.

733   Knapik, J. J., Steelman, R. A., Hoedebecke, S. S., Austin, K. G., Farina, E. K., & Lieberman, H. R.

734   (2016). Prevalence of dietary supplement use by athletes: systematic review and

735   meta-analysis. *Sports Medicine (Auckland, NZ)*, *46*, 103. doi: 10.1007/s40279-015-

736   0387-7.

737   Knobloch, K. (2008). The role of tendon microcirculation in Achilles and patellar

738   tendinopathy. *Journal of orthopaedic surgery and research*, *3*(1), 18.

739   doi: 10.1186/1749-799X-3-18

740   Lima, C. C., Pereira, A. P. C., Silva, J. R. F., Oliveira, L. S., Resck, M. C. C., Grechi, C. O., … &

741   Garcia, J. A. D. (2009). Ascorbic acid for the healing of skin wounds in rats. *Brazilian*

742   *Journal of Biology*, *69*(4), 1195-1201. doi: 10.1590/S1519-69842009000500026

743   Li, P., Yin, Y. L., Li, D., Kim, S. W., & Wu, G. (2007). Amino acids and immune function. *British*

744   *Journal of Nutrition*, *98*(2), 237-252. doi: 10.1017/S000711450769936X

745   Li, X., Bradford, B. U., Wheeler, M. D., Stimpson, S. A., Pink, H. M., Brodie, T. A., … &

746   Thurman, R. G. (2001). Dietary glycine prevents peptidoglycan polysaccharide-

747   induced reactive arthritis in the rat: role for glycine-gated chloride channel. *Infection*

748   *and immunity*, *69*(9), 5883-5891. doi: 10.1128/IAI.69.9.5883-5891.2001

749   Malinauskas, B. M., Overton, R. F., Carraway, V. G., & Cash, B. C. (2007). Supplements of

750   interest for sport-related injury and sources of supplement information among

751   college athletes. *Adv Med Sci*, *52*, 50-54.

752   McDaniel, J. C., Belury, M., Ahijevych, K., & Blakely, W. (2008). Omega-3 fatty acids effect on

753   wound healing. *Wound Repair and Regeneration, 16*(3), 337-345.

754   doi: 10.1111/j.1524-475X.2008.00388.x.

755   McGonigle, P., & Ruggeri, B. (2014). Animal models of human disease: challenges in enabling

756   translation. Biochemical pharmacology, 87(1), 162-171. doi:

757   10.1016/j.bcp.2013.08.006

758   McHugh, M. L. (2012). Interrater reliability: the kappa statistic. *Biochemia medica*, *22*(3),

759   276-282.

760   Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., … Stewart, L. A.

761   (2015). Preferred reporting items for systematic review and meta-analysis protocols

762   (PRISMA-P) 2015 statement. *Systematic reviews*, *4*(1), 1. doi: 10.1186/2046-4053-4-1

763   Molinero, O., & Márquez, S. (2009). Use of nutritional supplements in sports: risks,

764   knowledge, and behavioural-related factors. *Nutrición Hospitalaria*, *24*(2).

765   Mouw, J. K., Ou, G., & Weaver, V. M. (2014). Extracellular matrix assembly: a multiscale

766   deconstruction. *Nature reviews Molecular cell biology, 15*(12), 771-785.

767   doi: 10.1038/nrm3902

768   Negro, M., Giardina, S., Marzani, B., & Marzatico, F. (2008). Branched-chain amino acid

769   supplementation does not enhance athletic performance but affects muscle

770   recovery and the immune system. The Journal of sports medicine and physical

771   fitness, 48(3), 347.

772 Norton, L. E., & Layman, D. K. (2006). Leucine regulates translation initiation of protein

773   synthesis in skeletal muscle after exercise. *The Journal of nutrition, 136*(2), 533S-

774   537S.

775 Notarnicola, A., Pesce, V., Vicenti, G., Tafuri, S., Forcignanò, M., & Moretti, B. (2012). SWAAT

776   study: extracorporeal shock wave therapy and arginine supplementation and other

777   nutraceuticals for insertional Achilles tendinopathy. *Advances in therapy*, 1-16. doi:

778   10.1007/s12325-012-0046-4.

779 Ömeroğlu, S., Peker, T., Türközkan, N. and Ömeroğlu, H., 2009. High-dose vitamin C

780   supplementation accelerates the Achilles tendon healing in healthy rats. *Archives of*

781   *orthopaedic and trauma surgery, 129*(2), 281-286. doi: 10.1007/s00402-008-0603-0

782 Otranto, M., Do Nascimento, A. P., & Monte-Alto-Costa, A. (2010). Effects of

783   supplementation with different edible oils on cutaneous wound healing. *Wound*

784   *repair and regeneration, 18*(6), 629-636. doi: 10.1111/j.1524-475X.2010.00617.x.

785 Pereira, M. G., Baptista, I. L., Carlassara, E. O., Moriscot, A. S., Aoki, M. S., & Miyabara, E. H.

786   (2014). Leucine supplementation improves skeletal muscle regeneration after

787   cryolesion in rats. *PLoS One*, *9*(1), e85283. doi: 10.1371/journal.pone.0085283

788 Pereira, M. G., Silva, M. T., Carlassara, E. O., Gonçalves, D. A., Abrahamsohn, P. A., Kettelhut,

789   I. C., ... & Miyabara, E. H. (2014). Leucine supplementation accelerates connective

790   tissue repair of injured tibialis anterior muscle. *Nutrients*, *6*(10), 3981-4001.

791   doi: 10.3390/nu6103981.

792 Petróczi, A., Naughton, D. P., Mazanov, J., Holloway, A., & Bingham, J. (2007a). Performance

793   enhancement with supplements: incongruence between rationale and

794   practice. *Journal of the international society of sports nutrition*, *4*(1), 19.

795   doi: 10.1186/1550-2783-4-19.

796   Petróczi, A., Naughton, D. P., Mazanov, J., Holloway, A., & Bingham, J. (2007b). Limited

797   agreement exists between rationale and practice in athletes' supplement use for

798   maintenance of health: a retrospective study. *Nutrition journal*, *6*(1), 34. doi:

799   10.1186/1475-2891-6-34.

800   Petróczi, A., Naughton, D. P., Pearce, G., Bailey, R., Bloodworth, A., & McNamee, M. (2008).

801   Nutritional supplement use by elite young UK athletes: fallacies of advice regarding

802   efficacy. *Journal of the International Society of Sports Nutrition*, *5*(1), 22.

803   doi: 10.1186/15502783-5-22

804   Popay, J., Roberts, H., Sowden, A., Petticrew, M., Arai, L., Rodgers, M., ... Duffy, S. (2006).

805   Guidance on the conduct of narrative synthesis in systematic reviews. *A product*

806   *from the ESRC methods programme Version, 1,* b92. doi: 10.13140/2.1.1018.4643

807   Raynaud-Simon, A., Belabed, L., Le Naour, G., Marc, J., Capron, F., Cynober, L., & Darquy, S.

808   (2012). Arginine plus proline supplementation elicits metabolic adaptation that

809   favors wound healing in diabetic rats. *American Journal of Physiology-Regulatory,*

810   *Integrative and Comparative Physiology, 303*(10), R1053-R1061.

811   doi: 10.1152/ajpregu.00003.2012

812   Reagan-Shaw, S., Nihal, M., & Ahmad, N. (2008). Dose translation from animal to human

813   studies revisited. The FASEB journal, 22(3), 659-661. doi: 10.1096/fj.07-9574LSF

814   Rodgers, M., Sowden, A., Petticrew, M., Arai, L., Roberts, H., Britten, N., & Popay, J. (2009).

815   Testing methodological guidance on the conduct of narrative synthesis in systematic

816   reviews: effectiveness of interventions to promote smoke alarm ownership and

817   function. Evaluation, 15(1), 49-73. doi: DOI: 10.1177/1356389008097871

818   Rosa, A. D. S., Bandeira, L. G., Monte-Alto-Costa, A., & Romana-Souza, B. (2014).

819   Supplementation with olive oil, but not fish oil, improves cutaneous wound healing

820   in stressed mice. *Wound Repair and Regeneration, 22*(4), 537-547.

821   doi: 10.1111/wrr.12191

822   Sallis, J. F. (2000). Age-related decline in physical activity: a synthesis of human and animal

823   studies. Medicine and science in sports and exercise, 32(9), 1598-1600. doi:

824   10.1097/00005768-200009000-00012

825   Sax, H. C. (1994). Arginine Stimulates Wound Healing and Immune Function in Elderly

826   Human Beings. *Journal of Parenteral and Enteral Nutrition, 18*(6), 559-560.

827   doi: 10.1177/0148607194018002194

828   Sena, E. S., Van Der Worp, H. B., Bath, P. M., Howells, D. W., & Macleod, M. R. (2010).

829   Publication bias in reports of animal stroke studies leads to major overstatement of

830   efficacy. PLoS biology, 8(3), e1000344. doi: 10.1371/journal.pbio.1000344

831   Sharp, C. P., & Pearson, D. R. (2010). Amino acid supplements and recovery from high-

832   intensity resistance training. The Journal of Strength & Conditioning Research, 24(4),

833   1125-1130

834   Shukla, A., Rasik, A. M., Jain, G. K., Shankar, R., Kulshrestha, D. K., & Dhawan, B. N. (1999). In

835   vitro and in vivo wound healing activity of asiaticoside isolated from Centella

836   asiatica. *Journal of ethnopharmacology, 65*(1), 1-11. doi: 10.1016/S0378-

837   8741(98)00141-X.

838   Schultz, G. S. (1998). Molecular analysis of the environment of healing and chronic wounds:

839   cytokines, proteases and growth factors. *Wounds*, *10*, 1F-9F.

840   Stechmiller, J. K., Childress, B., & Cowan, L. (2005). Arginine supplementation and wound

841   healing. *Nutrition in Clinical Practice, 20*(1), 52-61.

842   doi: 10.1177/011542650502000152

843   Stromberg, B. V., Wood, F. M., & Simmons, D. J. (1977). Enzymatic changes in the healing rat

844   Achilles tendon. *Journal of Surgical Research, 23*(2), 132-140. doi: 10.1016/0022-

845   4804(77)90201-3

846   Sugaya, H., Maeda, K., Matsuki, K., & Moriishi, J. (2007). Repair integrity and functional

847   outcome after arthroscopic double-row rotator cuff repair: a prospective outcome

848   study. *Journal of Bone & Joint Surgery*, *89*(5), 953-960. doi: 10.2106/JBJS.F.00512

849   Svanberg, E., Jefferson, L. S., Lundholm, K. & Kimball, S. R. (1997) Postprandial stimulation of

850   muscle protein synthesis is independent of changes in insulin. *American Journal of*

851   *Physiology-Endocrinology And Metabolism*, *272*(5)*,* E841–E847.

852   Tack, C. (2016). Dietary Supplementation During Musculoskeletal Injury: Protein and

853   Creatine. *Strength & Conditioning Journal*, *38*(1), 22-26.

854   doi: 10.1519/SSC.0000000000000180

855   Tarnuzzer, R. W., & Schultz, G. S. (1996). Biochemical analysis of acute and chronic wound

856   environments. *Wound Repair and Regeneration*, *4*(3), 321-325. doil: 10.1046/j.1524-

857   475X.1996.40307.x

858   Tipton, K. D. (2010). Nutrition for acute exercise-induced injuries. *Annals of Nutrition and*

859   *metabolism*, *57*(Suppl. 2), 43-53. doi: 10.1159/000322703

860   Torres-McGehee, T.M., Pritchett, K.L., Zippel, D., Minton, D.M., Cellamare, A., & Sibilia, M.

861   (2012). Sports nutrition knowledge among collegiate athletes, coaches, athletic

862   trainers, and strength and conditioning specialists. *Journal of Athletic Training, 47*(2),

863   205-211. doi: 10.4085/1062-6050-47.2.205

864   Tsilidis, K. K., Panagiotou, O. A., Sena, E. S., Aretouli, E., Evangelou, E., Howells, D. W., ... &

865   Ioannidis, J. P. (2013). Evaluation of excess significance bias in animal studies of

866   neurological diseases. PLoS biology, 11(7), e1001609. doi:

867   10.1371/journal.pbio.1001609

868   Uzgare, A., Sandra, K., Zhang, Q., Cynober, L., & Barbul, A. (2009). Ornithine alpha

869   ketoglutarate enhances wound healing. *Journal of the American College of Surgeons,*

870   *209*(3), S72. doi: 10.1016/j.jamcollsurg.2009.06.174

871   Vieira, C. P., Oliveira, L. P. D., Guerra, F. D. R., Almeida, M. D. S. D., Marcondes, M. C. C. G., &

872   Pimentel, E. R. (2015). Glycine improves biochemical and biomechanical properties

873   following inflammation of the achilles tendon. *The Anatomical Record*, *298*(3), 538-

874   545. doi: 10.1002/ar.23041

875   Vieira, C. P., Guerra, F. D. R., de Oliveira, L. P., Almeida, M. S., Marcondes, M. C. C., &

876   Pimento, E. R. (2015). Green tea and glycine aid in the recovery of tendinitis of the

877   Achilles tendon of rats. *Connective tissue research, 56*(1), 50-58. doi:

878   10.3109/03008207.2014.983270

879   Viera, A. J., & Garrett, J. M. (2005). Understanding interobserver agreement: the kappa

880   statistic. *Family Medicine*, *37*(5), 360-363.

881   Virmani, R., Kolodgie, F. D., Farb, A., & Lafont, A. (2003). Drug eluting stents: are human and

882   animal studies comparable?. *Heart*, *89*(2), 133-138. doi: 10.1136/heart.89.2.133

883   Wicke, C., Halliday, B., Allen, D., Roche, N. S., Scheuenstuhl, H., Spencer, M. M., ... Hunt, T. K.

884   (2000). Effects of steroids and retinoids on wound healing. *Archives of Surgery,*

885   *135*(11), 1265-1270. doi: 10.1001/archsurg.135.11.1265

886   Wasserstein, R. L., & Lazar, N. A. (2016). The ASA's statement on p-values: context, process,

887   and purpose. *The American Statistician, 70*(2), 129-133.

888   doi: 10.1080/00031305.2016.1154108

889   Watson, T. (2006). Tissue Repair: The Current State of the Art. *Sportex Medicine, 28* , 8-12.

890   Retrieved July, 2, 2017

891   from http://uhra.herts.ac.uk/bitstream/handle/2299/1085/103740.pdf;sequence=1

892   Woo, S. L. Y., & Buckwalter, J. A. (1988). Injury and repair of the musculoskeletal soft tissues:

893   workshop, Savannah, Georgia, June 18–20, 1987. *Journal of Orthopaedic*

894   *Research*, *6*(6), 907-931. doi: 10.1002/jor.1100060615

895   Williams, J. Z., Abumrad, N., & Barbul, A. (2002). Effect of a specialized amino acid mixture

896   on human collagen deposition. *Annals of surgery, 236*(3), 369-375. doi:

897   10.1097/01.SLA.0000027527.01984.00

898   Yoshizawa, F., Kimball, S. R., Vary, T. C., & Jefferson, L. S. (1998). Effect of dietary protein on

899   translation initiation in rat skeletal muscle and liver. *American Journal of Physiology-*

900   *Endocrinology And Metabolism*, *275*(5), E814-E820.

901

902