The effect of Leucine and Branched Chain Amino Acid supplementation on recovery following simulated football match play

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ABSTRACT

Both BCAA and leucine have been found to enhance recovery following both endurance and resistance training. The purpose of the study was to determine whether ingestion of BCAA or leucine was capable of accelerating markers of recovery following simulated football match play in academy footballers. 10 academy soccer players (mean ± s 17 ± 0.5 years, height 1.7 ± 0.08 m and body mass 74.56 ± 8.88 kg) were divided into three groups (leucine, BCAA or placebo) based on prior Multistage Fitness Test (MSFT) scores. All groups consumed 0.087g/kg/BW of their specific supplement 30 minutes prior to the commencement of the Loughborough Intermittent Shuttle Test (LIST) and had their recovery monitored post, 24, 48 and 72 hours after the test. The markers of recovery included an Adductor Squeeze Test (AST), a countermovement jump (CMJ) and a visual analogue scale (VAS). Data was analysed using a repeated measures ANOVA. Results found no significant differences between groups for all three markers of fatigue ($p \geq 0.05$). However significant time effects ($p \leq 0.05$) were found for the CMJ and VAS indicating that the LIST induced neuromuscular fatigue and perceived soreness. Perceived soreness peaked at 597%, 3933% and 365% immediately post-test for leucine, BCAA and placebo respectively. Whilst peak neuromuscular fatigue occurred at the 48h, 24h and 72h period for leucine (91.5%), BCAA (90%) and placebo (93%) respectively. Furthermore, AST peak decrements occurred straight after the LIST for both leucine (94%) and BCAA (98%) and at the 24h period for placebo (91%). In conclusion, both leucine and BCAA supplementation did not accelerate the time course of recovery following simulated match play using a small cost effective dosage. .

Muscle Damage, Function, Soreness, Fatigue

INTRODUCTION

Soccer is a global team sport played over a 9 month season. It incorporates periods of high intensity movements, interspersed by low intensity activity (69). Depending on position, players cover on average 8.5-13 km over a 90 minute period (75). Of that distance, high intensity running (21-24 km/h) and sprinting (>24 km/h) account for 3.9% & 5.3% respectively (30), with players also performing upwards of 700 turns (12), 27 tackles and 35 jumps throughout the game (55). The combination of the aforementioned factors culminate to stress multiple neuromuscular and metabolic parameters and thus create fatigue (5, 58).

The fatigue produced as a bi product of the match demands has been shown to produce performance decrements, both during and post-game (58). It takes on average 72 hours before pre match performance values return to baseline (58), with worst case scenarios highlighting muscle soreness and impaired force producing capacity for up to 7 days following competitive match play (58). Unfortunately, with the congested cycle of training and competition (65), this fatigue can lead to the inability to perform repeated exercise sessions, whilst also hindering the adaptations needed for performance enhancement (43). As such, a multitude of recovery strategies have been implemented to try and accelerate the time course of recovery including; cold water immersion (34), contrast therapy (33), compression garments (36), and nutritional interventions (7).

A caveat to recovery is the fact that it cannot be measured through one single variable and as such, assessing whether accelerative recovery protocols such as nutritional interventions actually work is difficult (52). That being said, because high intensity exercise causes protein breakdown (43) and the role of protein is growth and repair of muscle tissue (52). It makes intuitive sense that additional dietary protein could aid with the recovery process and attenuate

any catabolic and proteolytic mechanisms that may have occured (39). Although there are various facets to protein supplementation, research is now starting to quantify what the optimum dosages and timings are (52).

In terms of timing, a recent review by Aragon & Schoenfeld (2) highlighted that it is the total daily consumption of protein rather than the post training "anabolic window" that is more important. It appears that rates of Muscle Protein Synthesis (MPS) are maximised when 20g of protein are consumed every 2-3 hours, with a "muscle full" effect occurring with additional doses (4). In addition, the types of protein also effect rates of MPS (52). Both whey and soy are rapidly digested, resulting in transient aminoacidemia; whereas, Casein coagulates in the stomach, resulting in a slower rate of aminoacidemia over a longer duration (31). Whey has been found to be superior to both Soy and Casein at stimulating MPS (15), with this attributed to Whey's high amino acid content (20).

Amino Acids (AA) are required to produce various proteins including those responsible for tissue repair. As such, by increasing the amount of AA within the body, skeletal muscle metabolism can be more effectively regulated (14). This could have a positive impact on recovery rates, by enhancing the anabolic post exercise period (47), resulting in a decrease in muscle cell damage and an amelioration of muscle soreness (70). This positive effect is dependent on the form of supplementation (28), with whole proteins potentially not creating a large enough extra cellular environment of AA to alleviate soreness and muscle damage (85). As such research has begun to progress from providing whole protein supplements to more free form AA (43). Of the 20 AA, it is the Essential Amino Acids (EAA) primarily responsible for the stimulatory impact of protein on MPS (78), whilst non-essential amino acids play little to no role (78).

The Branch Chain Amino Acids (BCAA) are part of the EAA and possess the most complex metabolic roles (47). BCAA have been found to augment MPS rates in sub optimal whey

protein doses (21). This is potentially due to BCAA being the only AA up taken specifically by skeletal muscle (78). BCAA comprise 3 of the 9 EAA (19). Of said three, both isoleucine and valine are said to be glucogenic, whereas leucine is ketogenic (38). Around 50% of BCAA are catabolised in the muscle, where as in Liver the capacity is small (38). Because muscle is not a gluconeogenic tissue, it cannot completely oxidise isoleucine and valine (83). However, leucine unlike valine and isoleucine is able to regulate the skeletal muscle oxidation of glucose and produce a protein sparing environment whilst also indirectly stimulating insulin secretion (81, 19).

When all three BCAA are given concurrently, MPS is stimulated (11). However, when leucine is removed, MPS is decreased, suggesting that leucine is the rate limiting BCAA which determines MPS levels (46). In addition, leucine possess higher oxidation rates, stimulates myogenic pathways and influences immune function (39); indicating that leucine ingested in free form may be just as beneficial as ingestion of a complete mixture of BCAA (20). That being said, when leucine is sole ingested, a reduction in circulatory levels of isoleucine and valine can occur (20); leading to increased competition for transduction into the cell and potentially decreased rates of protein synthesis (20).

The importance of leucine is further highlighted by Moberg et al. (54) when they found that removal of leucine from an EAA solution caused an attenuation in post exercise anabolic processes and a decrease in mTORC1 activation. One proposed mechanism for this is because leucine has been found to have a stimulatory effect on leucyl-Trna synthetase (54). This leads to the regulation of both the innate and acquired immune systems which synthesize proteins, polypeptides and activate the mammalian target of rapamcyin complex 1 (mTOR1) signalling pathway (39). MTOR1 is a central Protein and its activation results in downstream target phosphorylation, ultimately creating an MPS response (47). Although leucine is required to stimulate mTOR1 maximally, it appears that said signalling is enhanced when the remaining

BCAA are added (54). As such, it is difficult to determine whether leucine alone or as part of a mixture can maintain the MPS response after exercise and thus accelerate the time course of recovery.

It appears that both leucine and BCAA can maximise MPS. However, their effect on recovery markers is equivocal. Positive findings have been illustrated following laboratory based endurance training protocols (51, 37) and resistance training protocols (39, 60, 72), whilst others (43, 60, 74, 46) have found no effect. The aforementioned differences in research findings can largely be attributed to the essentially limitless variations in research methodologies. Both endurance and resistance training protocols can manipulate exercise modes, durations, intensities, volumes and protocols as well as the size and frequency of the dosage, which can be given to different sample sizes with different experience levels (39, 43).

Unfortunately, the specific modality of exercise results in different molecular and skeletal adaptation in line with demands imposed upon them (52). Therefore, prior findings cannot be applied to a football setting. In addition, team sports are often controlled by financial and time constraints, as such practitioners rely on research which can provide a positive response from the smallest possible dosage. To the author's knowledge, the smallest dosage which has been shown to elicit a positive recovery effect was found by Shimomura et al. (72). Their study provided a single 100mg/kg/BW dose (around 5g on average) of BCAA or Dextrin (placebo) before a squat protocol consisting of 7 sets of 20 reps with 3 minute rest between sets. Their results illustrated that the BCAA group had significantly lower perceived soreness compared to the placebo group. Whilst also finding a larger suppression in muscle force decreases, serum myoglobin and elastase levels following BCAA supplementation, indicating a reduction in muscle damage (72).

Therefore, the aim of this study was to determine whether a small bolus of BCAA or leucine supplementation is sufficient to accelerate markers of recovery within an academy football

setting. The study has novel implications due to the fact it is the first to use both BCAA and leucine within a team sport recovery setting. This study hypothesised that both BCAA and leucine would improve recovery rates, with no differences found between the supplements.

METHOD

Experimental approach to the problem

The study used a non-randomised placebo-controlled, matched-pairs groups design to measure the effects of leucine and BCAA supplementation on the time course of recovery in football players. Baseline testing allowed participants to be familiarised with both the markers of fatigue and the Loughborough Intermittent Shuttle Test (LIST). The fatigues measures as outlined below were chosen due to their reliability and because they would not induce pre-test fatigue. Tests were performed in an order so as the ones requiring the greatest power and freshness were performed first (58).

Subjects

To detect a large effect size (d=1.5), with a power of 80%, assuming an alpha-level of 0.05, the minimum number of participants required for the study was 9. The investigation was carried out in 10 academy soccer players (mean ± s 17 ± 0.5 years, height 1.7 ± 0.08m and body mass 74.56 ± 8.88kg). All players had been within a full time training environment for at least 1 year, completed written informed consent (both parental and participant) and PARQ medical screening forms before participation. Ethical Approval was granted by the St Marys University Ethics subcommittee.

Procedures

The first test assessed neuromuscular fatigue by measuring Jump Height using the Just Jump System (SKU: PB182P). Participants were required to perform Counter Movement Jumps (CMJ) with hands held firmly on their hips, with the instruction only to jump as high as possible from a self-selected depth (24). Participants were given 3 attempts, with the highest jump taken as the actual measurement. Jumping performance is essential for success in soccer, thus

highlighting the validity of the CMJ (3). CMJ are both a quick and easy measure of anaerobic qualities (58), whilst also possessing a high test-retest reliability (ICC 0.89-0.95) (56). In addition, the use of the stretch shortening cycle during the CMJ allows for the interaction between neural, metabolic and mechanical elements (58). The SSC cycle is a major cause of exercise fatigue, thus making the CMJ useful in detecting fatigue in team sports (53).

The next fatigue measurements included an isolated muscle function test in the form of an Adductor Squeeze Test (AST). Although Maximal Voluntary Contraction (MVC) torque is considered the best method of determining muscle damage (84), the AST has been described as a low cost reliable and valid measure of adductor strength (29). Unfortunately, by isolating individual muscle groups, there is a reduced validity as it does not replicate the multi joint nature of soccer (58). However, it does lead to an increase in reliability (58), as shown by (29) who found the highest intrarater reliability at 0.92 when the test was performed in a supine position at 45º of hip flexion using a standard blood pressure sphygmomanometer. In addition, the biomechanical nature of the adductors, means they act as stabilisers of the thigh during running based tasks (29) and their high injury occurrence during soccer (5% of all injuries in soccer occur at the groin) validates the test for soccer players (Delahunt et al., 2011). Participants placed their across their chest and were instructed to squeeze the Sphygmomanometer (Sapphire Aneroid) as hard as possible, with the glutes remained firmly on the ground (29).

The third and final test determined subjective soreness levels in the form of Visual Analogue Scale (VAS). Participants used the VAS to point at a 10cm line with no pain at one end and most severe pain possible at the other (22). Pain was recorded by measuring the distance from the left anchor point (43). The VAS has been described as the best pen and paper measure of pain due to its reliability and sensitivity to pain/soreness (88). It has been found to have an intra class correlation coefficient of 0.97 with 90% of the pain ratings reproducible within 9mm (9).

The final part of the preparation day required participants to complete a 15 minute block of the LIST. The LIST requires the participants to run at speeds corresponding to 55% and 95% of $V0_2$ max (59) and is discussed in detail later. To calculate Vo2 max the Multistage Shuttle Run (MSFT) was utilised (64), which is present within the LIST literature (59). The MSFT has a large test retest reliability (r=0.975) and is a valid measure of $V0_2$ max with a correlation of 0.92 (Ramsbottom et al., 1988). Participants completed the MSFT in the morning and after a break were then familiarised with the LIST for 1 block (58).

The results from the MSFT test were used to create 3 groups with those of a similar fitness paired and then split (See table 1). This is because athletes who possess a higher fitness have been shown to recover quicker following match play (44). The three groups were as follows: Group 1 who consumed Leucine (L) at 0.087g/kg/BW; group 2 who consumed Branched Chain Amino Acids (BCAA's) at 0.087g/kg/BW (2:1:1 ratio of leucine, isoleucine and valine) and Placebo (P) which was Maltodextrin at 0.087g/kg/BW. Due to the similar calories per gram of the macronutrients utilised, the samples are classed as isocaloric with no energy differences (39). All supplements were under the recommended safe limit (14) and at a level found to maximise rates of protein synthesis (57). All samples were made during the morning of the study to account for daily fluctuations in body mass and in solution form for ease of ingestion. Supplements were sourced from the same company (MyProtein).

Table 1 the descriptive characteristics of the participants separated into the three supplement groups.

Group	MSFT	Mean MASS (kg)	Mean Supplement dosage (g)	Mean Height (cm)
Leucine	14±1.58	72.275±5.39	6.28±0.46	173.5±3.47
BCAA	13.5±1.11	75.425±11.12	6.56±0.96	180.1±9.35
Placebo	13.75±1.25	75.675±10.16	6.58±0.88	174.8±8.55

The solutions were taken prior to the commencement of the test as it is proposed that this would increase delivery of AA to the muscle (79). This is supported by Tipton et al. (79) who found significantly greater rates of MPS in pre ingestion compared to post ingestion of EAA. To conflict this, research has shown that AA taken prior to exercise have no effect on markers of recovery including CK, maximal voluntary contraction and Range of Motion (60, 85). However, said studies required their sample to participate in a fasted state and could account for the lack of effect. In addition, the literature is heavily biased towards resistance training, with the effect of timing on differing modes of exercise yet to be determined (77). Furthermore, participants consumed the same meals for breakfast, lunch and tea, with players encouraged not to snack in between meals. However, the quantity was not strictly controlled. Participants were also encouraged to sleep for at least 8 hours a night during the study.

Following a 15 minute standardised warm up incorporating 5 minutes of pulse raising activities, 5 minutes of dynamic stretching and 5 minutes of potentiation activities, all three groups performed the LIST. The LIST has a high test- retest ability (reproducible within 95% limit) and has been shown to closely mimic the physiological and metabolic demands of football match play (59). The simulation type study is preferred within the literature as it is said to reduce the external/intrinsic variability of normal match play (58); whilst properly assessing the benefits of an intervention (59). Although simulation tests do not account for any psychological, technical or tactical stressors (59), they have actually been found to produce greater muscle damage than actual match play, potentially due to the greater number of eccentric actions (50).

Because physical performance is both highly varied and unreliable, there is a distinct lack of control in the ecological validity (58). For example, research has depicted large differences in high intensity running distance during soccer matches (32). As such players covering more distance will potentially create more fatigue. This leads to difficulty in determining whether

the intervention is actually successful (59). In addition, external demands such as the quality of opponent, match result, location, playing surface, the nature of the game, pattern of movements and internal demands such as age, gender, muscle fibre type, culminate to create differing match induced muscular strain and may account for individual differences in recovery (58).

The LIST follows a protocol as outlined by Nicholas et al. (59) involving individualised, walking and sprinting as well as jogging and cruising movements at 55% and 95% of $V0_2$ max over a 15 minute block period. The movements are dictated by audio beeps at various velocities, following a sequence of 3x20m walk, 1x20m sprint, 4s recovery, 3x20m @55% $V0_2$ max and 3x20m @95% Vo2 max (59). The Block was repeated 6 times with 3 minutes recovery between blocks for a total of 90 minutes activity on an indoor 3g, 30m pitch (59). Post-test, each participant completed the three fatigue measures outlined above within 15 minutes of test completion. Follow up tests occurred at 24, 48 and 72 hour periods, with data collected before 10.00am every morning by the same researcher, using the same equipment at the training ground. At each testing point a standardised warm up was completed, which included no static stretching as this has been shown to reduce force producing capacity (58).

Statistical Analysis

Statistical Analysis Statistical analysis was performed using SPSS software (version 16; SPSS Inc., Chicago, IL), with significance set at $P \leq 0.05$. Prior to analysis all data sets were checked for normality through the Shapiro-Wilk test. The test revealed normal distributions for all groups for each recovery measure and as such parametric tests were utilised. To ensure no significant differences occurred between groups a one way ANOVA was utilised with the Turkey post hoc test applied. For changes in VAS, neuromuscular fatigue, and muscle function for each group, over time, a two way repeated measures ANOVA (group 3, time, 5) was

utilised. Results were analysed for sphericity, with the Greenhouse-Geisser correction used if sphericity was violated. Post hoc bonferonni test was used to determine group differences.

RESULTS

The match paired design required participants to be split based upon MSFT results. Post-test analysis revealed no significant differences between groups $F(2,7) = .364$, $p = .707$) for MSFT as a whole and as a multiple comparison between each other ($p \geq 0.05$). Both the CMJ ($F(4, 8) = 7.421$, $p = .008$) and VAS ($F(1.21, 2.41) = 18.716$, $p = .035$) highlighted significant time effects stipulating that the LIST induced both neuromuscular fatigue and perceived soreness. However, no significant time effects were observed for adductor strength $F(4, 8) = 2.116$, $p = .170$. VAS showed no significant group $F(2,4) = 3.390$, $p = .138$ or interaction effect $F(2.729, 9.550) = 0.921$, $p = .459$ with a small effect size of 0.41. Leucine, BCAA and placebo scores peaked immediately post-test for all groups (5.08, 5.77 & 5.57cm, respectively). Which when expressed as a percentage of baseline illustrates a 597%, 3933% and 365% increase for leucine, BCAA and placebo respectively. VAS returned to baseline at the 48h period for leucine and BCAA only (See figures 1a and 1b).

Figure 1a - VAS before and up to 72 hours following the LIST displayed as percentage change from baseline

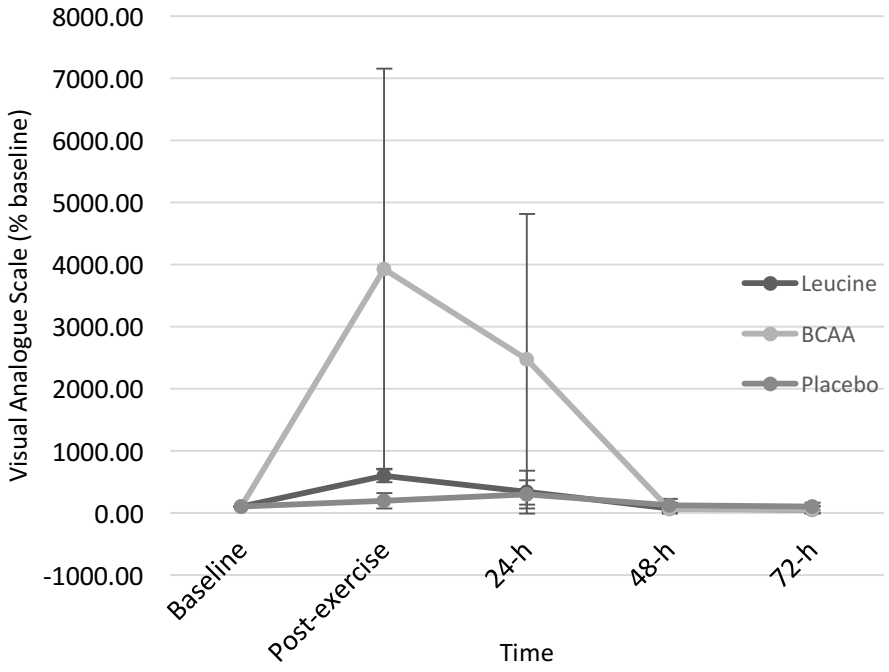
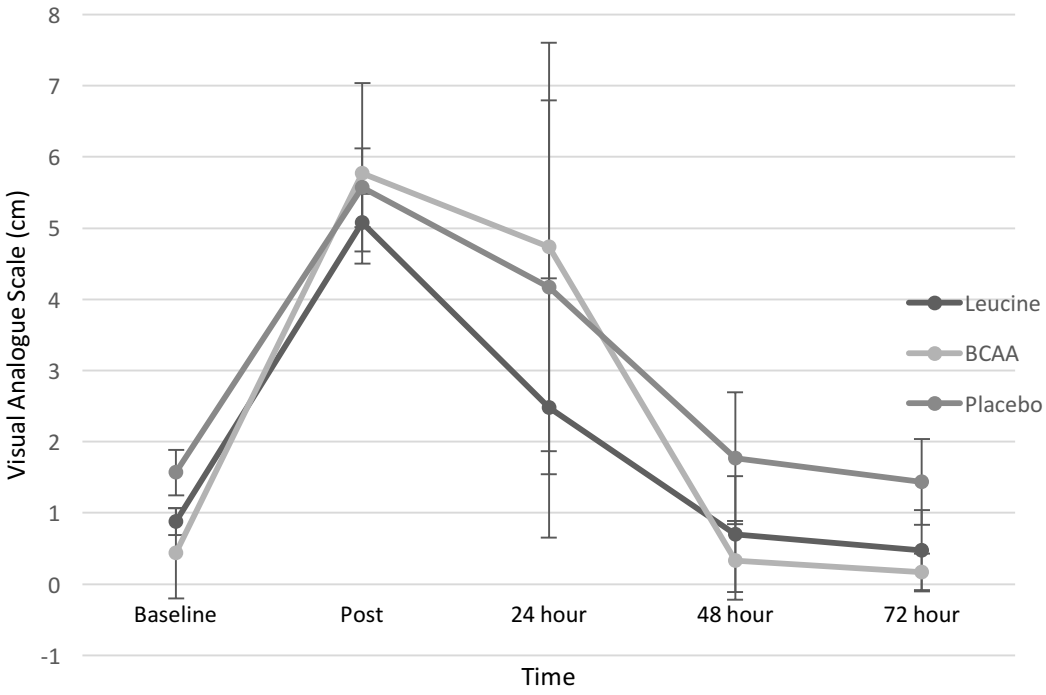


Figure 1b - VAS before and up to 72 hours following the LIST displayed as actual values



CMJ showed no significant group F(2,4) = .969, p = .454 or interaction effect F(8, 28) = 0.774, p = .629 with only a small effect size of 0.215. Peak height decrements occurred at the 48h, 24h and 72h period for leucine (91.5%, 43.5±1.81cm), BCAA (90%, 47.8±4.56) and placebo (93%, 47.5±5.43) respectively, with neither group returning to baseline (See figures 3a and 3b). Adductor strength also showed no significant group F(2, 4) = .480, p = .650 or interaction effect F(4.231, 14.808) =0.548, p = -.712 with only a small effect size of 0.17. Peak soreness occurred straight after the LIST for both leucine (94%) and BCAA (98%) and at the 24h period for placebo (91%) (see figures 2a and 2b). Further analysis revealed no significant differences for the BCAA and placebo groups at each time point for all dependant variables. However leucine, showed significant differences between baseline and post-test (p=0.00), post-test and 48 hours (p=0.011) and post-test and 72 hours (p=0.003) following Leucine supplementation for the VAS, but not for the CMJ or AST.

Figure 3a - Vertical jump before and up to 72 hours following the LIST displayed as percentage change from baseline

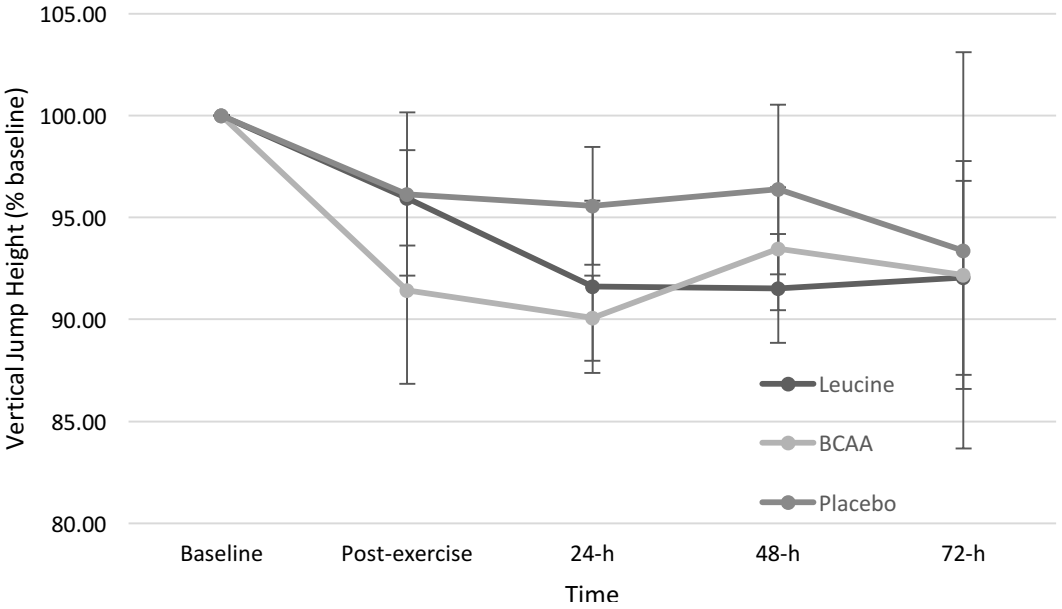


Figure 2b - Vertical Jump before and up to 72 hours following the LIST displayed as actual values

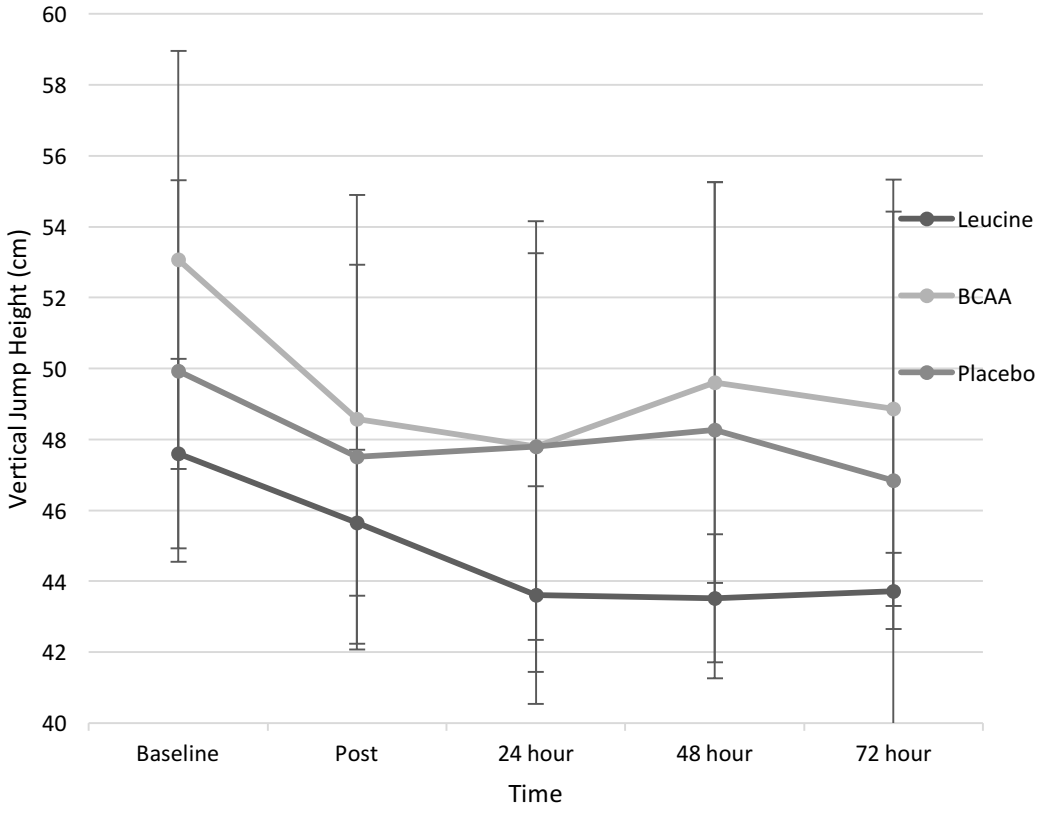


Figure 2a - Adductor squeeze before and up to 72 hours following the LIST displayed as percentage change from baseline

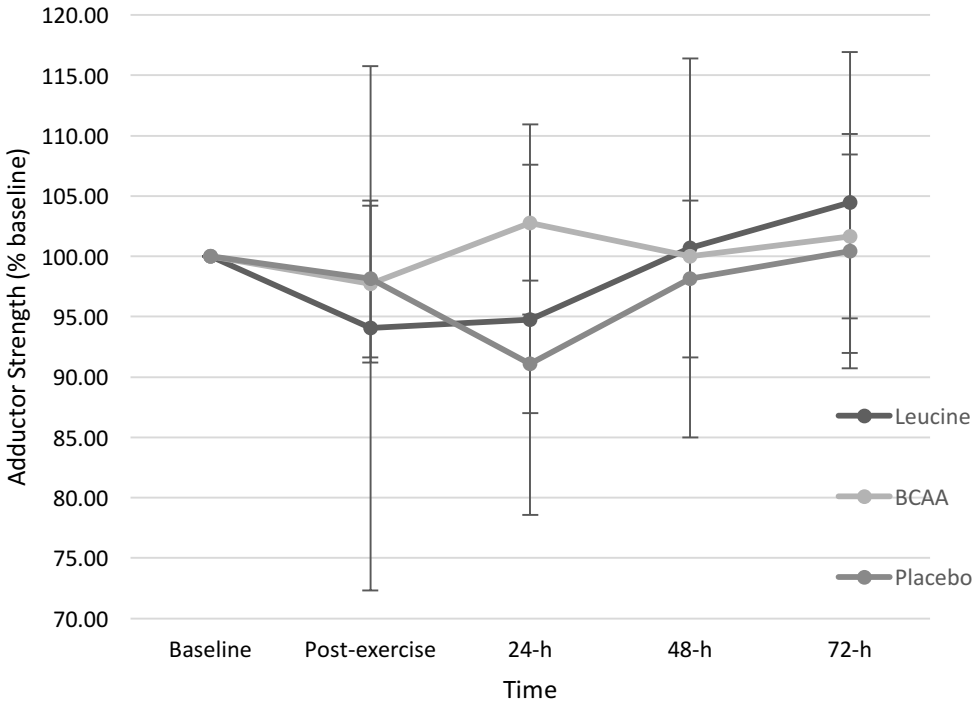
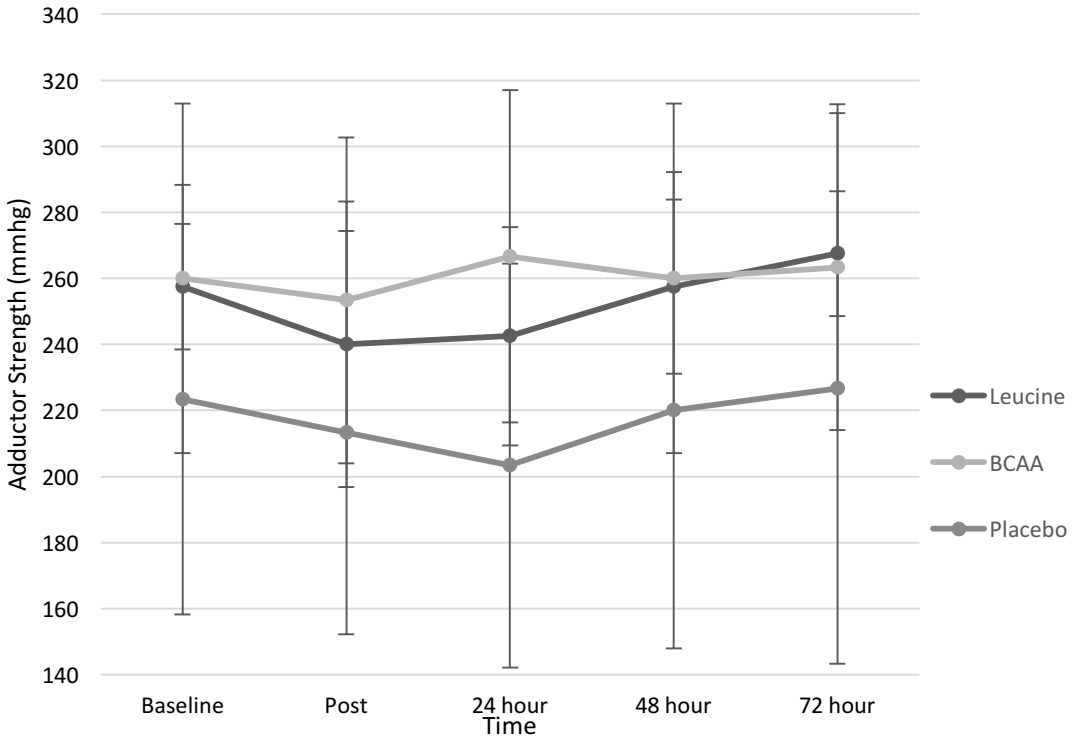


Figure 2b - Adductor squeeze before and up to 72 hours following the LIST displayed as actual values



DISCUSSION

The aim of the study was to determine whether BCAA or leucine supplementation accelerated markers of recovery following simulated football match play in academy football players. The principle findings highlight that there were no significant differences between groups throughout the recovery process for all dependant variables. However, although not significant, both BCAA and leucine did return to baseline quicker than placebo for the adductor strength and VAS tests. In addition, the study illustrated that the LIST is capable of causing significant reductions in neuromuscular power, and perceived soreness.

The LIST caused reductions in CMJ performance between 2 & 10 %. This is similar to previous research that found 12% reductions in CMJ height following the LIST (50), 12 and 4.4 % following competitive match play (50, 1) and 8 % following match play modelling (66). However, where earlier research found CMJ performance to be recovered by 48 (42) or 92 hours following actual match play (50,1) this study illustrated CMJ decrements to still be present at the end of data collection. Even though the LIST does not incorporate fatigue producing movement such as back pedalling, turning and jumping (59); it demands players to spend more time performing high intensity actions such as sprinting and may account for the slower recovery rates (58).

Neither leucine nor BCAA supplementation augmented the recovery of CMJ performance following the LIST. This finding is in accordance with Howatson et al. (39) who found no group differences between the BCAA and Placebo interventions following a drop jump protocol at all time points. In addition, Kirby et al. (46) provided 250mg/kg of sole leucine

before, during and immediately after 100 drop jumps and every morning after in the 3 consecutive days. Their results highlighted an attenuated reduction in peak force across all time points compared to placebo. However, like this study and Howatson et al. (39), no interaction and group effect was found for jump height, even though significant time effects were witnessed. The reductions in jump height suggest that tissue damage must have occurred, this damage hinders the calcium release per action potential and negatively effects the excitation-contraction coupling (73).

Following the AST, no significant differences were found between groups for the time course of recovery. Jackman et al. (43) also found no significant differences at all time points, with the maximal loss in force occurring at the 48 hour period for both groups (BCAA = -51% ± 3%, PLA = -48% ± 7%). Conflictingly, Howatson et al. (39) found a significant group time effect for MVC, where the decrement in force was 18 and 27% lower and the recovery of force was greater in the BCAA and placebo groups respectively. Potential differences between findings maybe that Jackman et al. (43) utilised a cohort of untrained participants, whereas Howatson et al. (39) participants were that of resistance trained volunteers. Johnston et al. (44) found that those who possess greater strength levels as measured through the Back Squat recover quicker. As such, it is no surprise that the resistance trained group were 90% recovered at the 72 hour period, whilst the non-trained group were still only 60% recovered.

Shimomura et al. (71) and Sugita et al. (76) like that of Howatson et al. (39) found improvements in muscle function following either BCAA or EAA supplementation. Sugita et al. (76) provided 12 AA over the duration of the study, suggesting that EAA not just BCAA are required to prevent losses in muscle function. However, White et al. (85) and Nosaka et al. (60) supplemented a mixture of EAA and found no effect, suggesting further research is required. In addition, both Shimomura et al. (71) and Sugita et al (76) utilised a cross over design which may have caused a repeated bout effect (40). The repeated bout effect can cause

a protective effect and a subsequent decrease in the amount of damage caused, thus limiting the strength of the study (39).

Peak soreness occurred within 30 minutes after the 90 minute simulation game for all three groups. This is in agreement with Magalhães et al. (50) who found no significant differences between match play and LIST ($p>0.05$), with peak soreness occurring at the 30 minute period post-test for both groups. To conflict this, Nedelec et al. (58) states that peak soreness generally occurs at the 48 hour period following both match play and laboratory based muscle damaging protocols (39). Unfortunately, methodological differences including protocols, samples, dosages and timings produce different levels of peak soreness, which are often measured by different subject fatigue measures (58).

Muscle soreness is said to occur due to inflammation of connective tissue, this increases the sensitivity of nociceptors and stimulates the subsequent increase in pain (62). There are multiple theories as to how BCAA can reduce muscle soreness. According to Howatson and van Someren (40) following muscle damaging protocols there is a bi-phasic response. The first response being due to the mechanical stress of exercise causing an increase in intracellular calcium levels and z-line disruption (85) and the second being an inflammatory response over the next few days. This secondary response increases protein uptake in order to stimulate cell signalling and the remodelling of damaged muscle (27). The supplementation of BCAA potentially promotes greater bioavailability and could account for the decrease in the secondary inflammatory response (39).

Another theory is depicted by Croisier et al. (25) who states that the attenuation of intramuscular inflammation occurs by inhibiting acute inflammation in response to the release of pro inflammatory cytokines after exercise. It appears that actions that cause muscle damage lead to the activation of polymorphonuclear leukocytes (63). Once cessation of exercise has

occurred, neutrophils enter the damaged muscle, which are shortly replaced by macrophages which produce the pro inflammatory cytokines (51). BCAA have been shown to reduce levels of inflammatory cytokines, whilst an increase in supplementation may also reduce the presence of myofiber proteins in the blood (43) which may account for their positive effect on soreness (6).

BCAA have been shown to accelerate recovery by reducing muscle soreness following heavy endurance activity. Matsumoto et al. (51) found a 32% significant ($p<0.05$) decrease in muscle soreness (measured through a VAS) compared to placebo following an intensified 3 day training period in elite endurance athletes. Whereas, Greer et al (37) reported marked decreases in creatine kinase and perceived soreness at the 4, 24 and 48 hour period following three 90 minute cycling bouts at 55% of $V0_2$ peak . However, although the movement demands of football are largely aerobic, it is the large eccentric component of high intensity activity such as resistance training and field sports that culminates to produce greater muscle damage (27).

This study found leucine and BCAA to have no effect in reducing muscle soreness compared to placebo. Nosaka et al (60) provided acute AA supplementation 30 minutes pre and post workout and also found no significant reductions in muscle soreness. Quite surprisingly, Kirby et al (46) who provided sole leucine, actually found higher perceived soreness in the leucine group compared to placebo, suggesting supplementation has no effect on such biomechanically complex movements. To conflict this, Jackman et al. (43) highlighted significant reduction in muscle soreness in the BCAA group at the 48 and 72 hour period when the knee was flexed following a 12x10 unilateral knee extensor training programme. Whilst the second experimental protocol used by Nosaka et al (60) found that AA supplementation (60% BCAA) in the days following a bout of elbow flexor training also resulted in a decrease in muscle soreness (30%).

Unfortunately the aforementioned studies utilised untrained participated and isolated muscle groups. This is unlikely to reflect athletic populations who routinely undergo large volumes of eccentric muscle damage (39). To overcome this, Howatson et al. (39) measured the time course of recovery following 100 consecutive drop jumps between a placebo group and a BCAA group. The BCAA group completed a 7 day loading period where supplements were consumed both morning and night. Their results highlighted that peak muscle soreness occurred at the 48h period for both groups, but were significantly lower in the BCAA compared to the placebo groups ($p<0.05$). Thus suggesting that the membrane integrity was more sufficiently maintained in the BCAA compared to the placebo group (39).

The potential discrepancies within the muscle soreness literature may be attributed to the course of BCAA dosage (43). Although small dosages (10g EAA, 2.1g L) have been found to maximise MPS (26). It appears that this is mainly an acute response lasting around 2 hours, with sustained rates of MPS potentially requiring repeated dosages (21). Studies that have provided repeated dosages (43, 60) were able to ameliorate soreness much more than those who provided one or two doses (60). Of interest, one study did manage to reduce soreness following a small 5g bolus after a squat protocol (72). However this was in a female cohort, meaning they consumed more BCAA per body mass (72).

To analyse this further, Jackman et al. (43) provided 88g of BCAA split up into different quantities during the recovery period, whilst Howatson et al. (39) provided a total of 280g with a 7 day loading period of 20g per day and 20g per day given during the subsequent recovery period. Both studies achieved positive effects more than just decreasing muscle soreness following larger BCAA supplementation dosages, thus highlighting the importance of quantity (39). In addition, Cockburn et al. (23) found the rise of BCAA in plasma to peak at 30 minutes post exercise. Jackman et al. (43) waited an hour, whilst Howatson et al. (39) administered

their BCAA straight away, thus creating an earlier bioavailability of BCAA. Although still unequivocal, this may account for their more positive effect at improving recovery rates.

Similar findings have been witnessed within the carbohydrate protein/BCAA literature. White et al. (85) found little effect with a bolus of 23g of protein containing only 5g of BCAA. Whereas Coombes and McNaughton (89) administered 12g of BCAA every day for two weeks and found significant reductions in CK. Conversely, studies that have administered small dosages (5.6g of BCAA), have still managed to reduce markers of muscle damage (61) and as such true clarification cannot be given. That being said it appears that the larger dose potentially allows for BCAA to exert both direct markers and indirect markers on muscular damage (85), whilst smaller doses are potentially up taken by skeletal muscle or reduced in bioavailability due to increased splanchnic clearance during exercise (60).

As well as dosage, the pre exercise supplement timing may have also caused the lack of effect. Even though BCAA oxidation is increased during endurance activity (48), the contribution of said oxidation of BCAA to total energy expenditure still remains small, with carbohydrates being the predominant substrate (18). Although equivocal, research suggests increased BCAA supplementation causes the activation of the BC complex which may increase their use as substrates during endurance activity (18). Meaning that pre experiment supplementation may have caused the lack of effect.

The placebo group was Maltodextrin which is a carbohydrate, this means that the groups were iso-caloric and ultimately possessed the same energy intake. However, carbohydrates have been shown to increase protein uptake (14), therefore if any extra protein was consumed outside of the study by the placebo group it would have had a synergistic recovery effect (43). This effect could be extrapolated if the BCAA and leucine supplements were up taken and used as a substrate during the LIST, thus placing the placebo group in a better postprandial state (43).

However, due to the lack of dietary control it is difficult to state whether any of the participants in the intervention groups also consumed more protein outside of the study.

This study did not control diet both before and after the exercise protocol. Even though participants live in catered accommodation at the training ground and are provided set meals daily, there was no control over the quantity the players consumed or further dietary behaviours when not at training. This will have undoubtedly created differences in protein intake which could affect both protein turnover, bioavailability and account for the lack of effect between groups (39). Although difficult in an applied setting, future research should employ a rigorously controlled diet (39).

A further hypothesis as to the cause of the potential decreased bioavailability of BCAA and leucine in this study is central fatigue theory. It suggests that prolonged exercise can be hindered by impaired central nervous system functioning due to an increases in 5-hydroxytryptamine (5-HT) concentrations (18). Studies that have administered 5-HT antagonists have managed to actually delay fatigue (90) which has culminated an increased focus on nutritional interventions to reduce 5-HT availability to the brain (18). BCAA supplementation has produced equivocal results with only few studies finding positive effects. For example, Blomstrand et al. (11) found marked improvements in endurance performance. However, a recent review by Burke (16) has dismissed earlier positive findings. None the less, in an attempt to maintain the bioavailability of BCAA and therefor improve net protein balance by preventing contractile protein loss, research provided BCAA in combination with carbohydrates (23).

Cockburn et al. (23) administered a milk based protein-carbohydrate supplement either before, straight after, 24 hours or not at all to 4 independent groups following a bout of 6 sets of 10 unilateral knee extensions. The studies main findings were that post supplement consumption

was superior to that of pre consumption at reducing muscle soreness and preventing decreases in muscle performance. Furthermore consumption of the supplement at any point was beneficial at reducing CK activity. Similar results were found by Saunders et al. (68) who found that after two bouts of riding a cycle ergometer to exhaustion, the group who consumed the carbohydrate-protein supplement had 83% lower levels of CK compared to the carbohydrate only group. However, the groups were not isocaloric with the protein group consuming 20% more calories.

To conflict this White et al. (85) found no significant differences between groups for both markers of muscle damage and soreness following eccentric quadriceps contractions, whilst Wojcik et al. (87) only found significant decreases in CK following protein-carbohydrate consumption. Furthermore, a 3 day intensive weight training programme where participants were given Powerade (42g) or a BCAA-carbohydrate blend also found no between group differences for markers of muscle damage and perceived soreness (45). However a large difference in energy consumption between the two groups in an attempt to replicate an applied setting may acoount for the lack of effect. Stock et al. (74) also found that a leucine-carbohydrate mixture given after 6 sets of back squats did not affect CK or muscle soreness throughout the time course of recovery compared to placebo. However only a single dose of leucine was provided (22.5mg/kg). As such it appears that the majority or research agrees that the addition of BCAA or to carbohydrate mixtures does not prevent muscular damage before during and after exercise (45).

The small sample size used in this study could have caused a lack of power in the analysis. This combined with the large inter-individual variability of most fatigue measures makes it difficult to generalise to further populations (43). Furthermore, the study found both small effect sizes and no significant differences between groups at any point for all dependant variables. Thus implying that the intervention groups were not capable of accelerating

recovery. However, the lack of dietary control mentioned previously, which could have caused discrepancies in total protein intake may have accounted for the lack of effect.

Practical Applications

This study was designed to be both cost effective and applicable to the applied setting, as such the dosage given was designed to be the least amount to gain the desired recovery effect based on prior evidence; thus saving time and money. Unfortunately the dosage given as a single bolus prior to exercise at 0.087g/kg/BW was not sufficient to provide a positive effect and accelerate the time course of recovery following simulated football match play. Practitioners should continue to accelerate the time course of recovery through implementation of recovery agents. For example, by providing repeated dosages of BCAA in the days following match play and thus creating continual bioavailability of BCAA, accelerated recovery may occur.

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Appendix

Ethical Approval

St Mary's University

Ethics Sub-Committee

Application for Ethical Approval (Research)

This form must be completed by any undergraduate or postgraduate student, or member of staff at St Mary's University, who is undertaking research involving contact with, or observation of, human participants.

Undergraduate and postgraduate students should have the form signed by their supervisor, and forwarded to the School Ethics Sub-Committee representative. Staff applications should be forwarded directly to the School Ethics Sub-Committee representative. All supporting documents should be merged into one PDF (in order of the checklist) and clearly entitled with your **Full Name, School, Supervisor.**

Please note that for all undergraduate research projects the supervisor is considered to be the Principal Investigator for the study.

If the proposal has been submitted for approval to an external, properly constituted ethics committee (e.g. NHS Ethics), then please submit a copy of the application and approval letter to the Secretary of the Ethics Sub-Committee. Please note that you will also be required to complete the St Mary's Application for Ethical Approval.

Before completing this form:

- Please refer to the **University's Ethical Guidelines**. As the researcher/ supervisor, you are responsible for exercising appropriate professional judgment in this review.
- Please refer to the Ethical Application System (Three Tiers) information sheet.
- Please refer to the Frequently Asked Questions and Commonly Made Mistakes sheet.
- If you are conducting research with children or young people, please ensure that you read the **Guidelines for Conducting Research with Children or Young People**, and answer the below questions with reference to the guidelines.

Please note:

In line with University Academic Regulations the signed completed Ethics Form must be included as an appendix to the final research project.

If you have any queries when completing this document, please consult your supervisor (for students) or School Ethics Sub-Committee representative (for staff).

St Mary's University Twickenham London

St Mary's Ethics Application Checklist

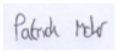
The checklist below will help you to ensure that all the supporting documents are submitted with your ethics application form. The supporting documents are necessary for the Ethics Sub-Committee to be able to review and approve your application.

Please note, if the appropriate documents are not submitted with the application form then the application will be returned directly to the applicant and may need to be re-submitted at a later date.

	Enclosed? (delete as appropriate)		Version No
Document	Yes	Not applicable	

1. Application Form	Mandatory		
2. Risk Assessment Form	Yes		
3. Participant Invitation Letter			
4. Participant Information Sheet	Mandatory		
5. Participant Consent Form	Mandatory		
6. Parental Consent Form	Yes		
7. Participant Recruitment Material - e.g. copies of Posters, newspaper adverts, website, emails		N/A	
8. Letter from host organisation (granting permission to conduct the study on the premises)	Yes		
9. Research instrument, e.g. validated questionnaire, survey, interview schedule		N/A	
10. DBS (to be sent separately)	Yes		
11. Other Research Ethics Committee application (e.g. NHS REC form)		N/A	
12. Certificates of training (required if storing human tissue)		N/A	

I can confirm that all relevant documents are included in order of the list and in one PDF document (any DBS check to be sent separately) named in the following format: ***Full Name, School, Supervisor.***

Signature of Applicant: 

Signature of Supervisor: 

St Mary's University Twickenham London

Ethics Application Form

1) Name of proposer(s)	Patrick David MAHER
2) St Mary's email address	145252@live.stmarys.ac.uk
3) Name of supervisor	Mark Waldron

4) Title of project The effect of Leucine and Branched Chain Amino Acid supplementation on recovery following simulated Football match play.

5) School or service	Sport, Health and Science
6) Programme (whether undergraduate, postgraduate taught or postgraduate research)	Strength & Conditioning – Postgraduate

7) Type of activity/research (staff/undergraduate student/postgraduate student)	Postgraduate student

8) Confidentiality	
Will all information remain confidential in line with the Data Protection Act 1998?	YES

9) Consent	
Will written informed consent be obtained from all participants/participants' representatives?	YES

10) Pre-approved protocol	
Has the protocol been approved by the Ethics Sub-Committee under a generic application?	NO

11) Approval from another Ethics Committee	
a) Will the research require approval by an ethics committee external to St Mary's University?	NO
b) Are you working with persons under 18 years of age or vulnerable adults?	YES

12) Identifiable risks	
	YES

a) Is there significant potential for physical or psychological discomfort, harm, stress or burden to participants?	
b) Are participants over 65 years of age?	NO
c) Do participants have limited ability to give voluntary consent? This could include cognitively impaired persons, prisoners, persons with a chronic physical or mental condition, or those who live in or are connected to an institutional environment.	NO
d) Are any invasive techniques involved? And/or the collection of body fluids or tissue?	NO
e) Is an extensive degree of exercise or physical exertion involved?	YES
f) Is there manipulation of cognitive or affective human responses which could cause stress or anxiety?	NO
g) Are drugs or other substances (including liquid and food additives) to be administered?	YES
h) Will deception of participants be used in a way which might cause distress, or might reasonably affect their willingness to participate in the research? For example, misleading participants on the purpose of the research, by giving them false information.	NO
i) Will highly personal, intimate or other private and confidential information be sought? For example sexual preferences.	NO
j) Will payment be made to participants? This can include costs for expenses or time.	NO
k) Could the relationship between the researcher/ supervisor and the participant be such that a participant might feel pressurised to take part?	NO

l) Are you working under the remit of the Human Tissue Act 2004?	NO

13) Proposed start and completion date

Please indicate:

- When the study is due to commence.
- Timetable for data collection.
- The expected date of completion.

Please ensure that your start date is at least 3 weeks after the submission deadline for the Ethics Sub-Committee meeting.

The study is due to commence during March, with one familiarisation and one data collection day needed.

14)Sponsors/Collaborators

Please give names and details of sponsors or collaborators on the project. This does not include your supervisor(s) or St Mary's University.

- Sponsor: An individual or organisation who provides financial resources or some other support for a project.

- Collaborator: An individual or organisation who works on the project as a recognised contributor by providing advice, data or another form of support.

N/A

15. Other Research Ethics Committee Approval

- Please indicate whether additional approval is required or has already been obtained (e.g. the NHS Research Ethics Committee).
- Please also note which code of practice / professional body you have consulted for your project.
- Whether approval has previously been given for any element of this research by the University Ethics Sub-Committee.

N/A

16. Purpose of the study

In lay language, please provide a brief introduction to the background and rationale for your study.

- Be clear about the concepts / factors / performances you will measure / assess/ observe and (if applicable), the context within which this will be done.
- Please state if there are likely to be any direct benefits, e.g. to participants, other groups or organisations.

It is widely recognised that a negative nitrogen balance, as a result of an insufficient protein intake, can result in impaired recovery following exercise (Kreider et al., 2004). In addition, those who regularly undertake physical exercise will be exposed to both greater intramuscular protein oxidation (Rodriguez et al., 2007) and protein breakdown (Phillips et al., 2002). Therefore require more dietary protein to

attenuate any proteolytic mechanisms that occur as a result of training (Campbell et al., 2007). Despite regularly producing inconsistent results resistance training has dominated protein literature, leaving other modes of exercise requiring further study (Campbell et al., 2007).

Football or Soccer involves physically demanding activities such as sprinting, accelerating, decelerating, changing direction and jumping, in combination with psychological and technical factors (Nedelec et al., 2012). The combination of these factors has been shown to produce performance decrements, otherwise known as fatigue (Allen et al., 2008). This fatigue can lead to an increase in muscle soreness lasting up to 7 days and a reduction in the muscle force producing capacity, which ultimately combines to create impaired muscle function (Jackman et al., 2010). This impairment may prevent repeated exercise sessions and hinder the adaptation to follow the training process required for performance enhancement (Jackman et al., 2010).

To try and enhance recovery the Branched Chain Amino Acids (BCAA's) Leucine, Isoleucine and Valine, which constitute one third of total skeletal muscle protein (Mero, 1999), will be utilized. BCAA's are preferred to other amino acids due to their uptake by skeletal muscle and enhancement of net protein synthesis rates (Ahlborg et al., 1974; Tipton et al., 1999; Blomstrand et al., 2006), resulting in accelerated recovery (Campbell et al., 2007). Leucine plays the most significant role of all three BCAA in stimulating protein synthesis due to its higher oxidation rates (Kimball & Jefferson, 2006) and its role in "triggering" myogenic pathways and influence on immune function (Wilkinson et al., 2013). Metabolites of amino acids, particularly Leucine regulate both the innate and acquired immune systems to synthesize proteins and polypeptides and activate the mTOR signaling pathway (Li, Yin, Li, Kim, Wu, 2007).

The majority of research has investigated Leucine as part of a mixture with BCAA. This is due to the fact that sole ingestion of Leucine may result in reduced circulatory levels of isoleucine and valine (Churchward-Venne et al., 2013). An increased competition for transduction into the cell may also result in decreased rates of protein synthesis (Churchward-Venne et al., 2013). However, if Leucine is removed form an amino acid mixture an attenuation in the anabolic effect may occur, whilst mTORC1 signaling may also be inhibited (Mohberg et al., 2013). Thus clearly stipulating that the study of Leucine in combination with the remaining BCAA's warrants further validation.

Amino Acids may also be used as an energetic substrate during predominantly aerobic activity. However, if large amounts are ingested prior to exercise, there will be a greater utilisation during exercise which results in less availability for recovery purposes (Phillips et al., 2013). In addition, pre exercise BCAA supplementation has been shown to produce few ergogenic effects (Burke, 2001; Nelson et al., 2012). Therefor to promote adaptive remodeling and attenuate skeletal muscle damage (Nelson et al., 2012), BCAA should be used post exercise to enhance the recovery profile (Blomstrand et al., 2006). This study will determine the effect of sole ingestion of Leucine and BCAA on recovery following simulation Football match play.

17. Study Design/Methodology

In lay language, please provide details of:

a) The design of the study (qualitative/quantitative questionnaires etc.)
b) The proposed methods of data collection (what you will do, how you will do this and the nature of tests).
c) You should also include details regarding the requirement of the participant i.e. the extent of their commitment and the length of time they will be required to attend testing.
d) Please include details of where the testing will take place.
e) Please state whether the materials/procedures you are using are original, or the intellectual property of a third party. If the materials/procedures are original, please describe any pre-testing you have done or will do to ensure that they are effective.

Study Design

The study uses a non-randomised placebo-controlled, matched-pairs groups design to measure the effects of Lecuine and BCAA supplementation on the time course of recovery in football players. Initial testing will allow participants to be familiarised with both the fatigue measures and LIST. With baseline measurements also taken in a fatigue free state. The participants will also need to complete a Multistage Fitness TEST (MSFT) in order to determine Vo2 max scores needed for the LIST. The results from the MSFT will also be used to create the 3 groups, with those of a similar fitness paired and then split. This is because fitter athletes have been shown to recover quicker, as such there can be no significant differences between the groups. Samples will be made up on the morning of the study based on the participant's body mass for that day. Samples will be made under the supervision of Mark Waldron and a lab technician from St Mary's University over skype.

Upon the day of collection, participants will be split into three groups of minimum 6 participants, one of which taking Leucine, one the BCAA and the other taking a placebo sample. All three groups will then perform the LIST, which has a high test-retest ability and has been shown to closely mimic the physiological and metabolic demands of football match play (Nicholas, Nuttall, & Williams, 1999). The simulation type study is preferred as the external demands are more controlled. Following the LIST

each group will take their specific supplement and their fatigue markers will be recorded over the next 72 hours.

Supplements will be sourced from the same company (My Protein) and provided to the participants in fluid form. The fluid will contain one of the following: Leucine (L) at 0.087g/kg/BW, Branched Chain Amino Acids (BCAA's) at 0.087g/kg/BW and Placebo (P) which will be sugar and water, all of which are under the recommended safe limit (Borsheim, Tipton, Wolf, & Wolfe, 2002). The fluid will be made up so as at no point do either of the groups know which sample they have taken.

The LIST requires participants to run at 55% and 90% Vo2 max as such a shuttle test will be utilised (Ramsbottom et al., 1988) to determine the speeds the participants need to run at. Once the speeds are determined and the participants have completed a 15 minute standardised warm up the test will begin (Nicholas et al., 2010). The test will be completed on an indoor 3g pitch at the training ground which is 30m in length and consists of two parts, part A and part B. Part A requires the participants to complete 5x 15 minute blocks separated by 3 minutes of recovery involving individualised sprinting, running and walking. Part B involves an exhaustion test, where the participants perform shuttles at 55% and 90% Vo2 max until failure and their score is recorded (Nicholas et al., 2010).

Post-test the time course of recovery will be analysed between the three groups and compared to their baseline measures taken prior to the test when the players are fresh. All tests will be completed at the training ground. The fatigue measurements will include an isolated muscle function test in the form of an adductor squeeze using a blood pressure cuff. The biomechanical nature of the adductors means they are recruited during running based tasks to stabilise the thigh (Roe et al., 2016). The squeeze test has been described as a low cost reliable and valid measure of adductor strength (Delahunt et al., 2009). Jump Height from the performance of a CMJ on an OPTA jump mat will determine neuromuscular fatigue. The use of the stretch shortening cycle during the CMJ allows for the interaction between neural, metabolic and mechanical elements (Nicol, Avela & Komi, 2006). Whilst a Visual Analogue Scale will be utilised to determine soreness levels following match play. VAS allows participants to point a line with markers such as intense pain at one end and no pain at the other, it has been described as the best pen and paper measure of pain due to its reliability and sensitivity to pain/soreness (Zusman, 1986).

Statistical Analysis Statistical analysis will be performed using SPSS software (version 16; SPSS Inc., Chicago, IL), with significance set at $P \leq 0.05$. For changes in VAS, neuromuscular fatigue, and muscle function for each group compared to baseline a repeated measures analysis of variance will be utilised. Whilst to compare the effects of the three groups on the time course of recovery a repeated measures Anova will be used.

18. Participants

Please mention:

a) The number of participants you are recruiting and why. For example, because of their specific age or sex.
b) How they will be recruited and chosen.
c) The inclusion/exclusion criteria.
d) For internet studies please clarify how you will verify the age of the participants.
e) If the research is taking place in a school or organisation then please include their written agreement for the research to be undertaken.

Moderate effect size?

A) The sample will contain players from the Carlisle United Football Club Academy. All participants will be between the ages of 16 and 18 and Male. Sample size was calculated as follows. To detect a large effect size (d = 1.5), with a power of 80%, assuming an alpha-level of 0.05, the minimum number of participants required for the study is 9. Therefore, we aim to recruit a minimum of 9 participants and a maximum of 18 depending on availability an injury status. Sample size estimations were performed using G*Power (version 3.0.10).
B) Participants recruited through convenience as a homogenous sample from my club. They will be recruited through formal invitation to the club directors and given full information of what the study entails.
C) They must be free from injury, illness and willing to complete the study. Both ascent and consent forms will be completed before the study and anyone wishing to withdraw from the study at any time will be acquiesced.
D) N/A
E) N/A

19. Consent

If you have any exclusion criteria, please ensure that your Consent Form and Participant Information Sheet clearly makes participants aware that their data may or may not be used.

a) Are there any incentives/pressures which may make it difficult for participants to refuse to take part? If so, explain and clarify why this needs to be done

b) Will any of the participants be from any of the following groups?

- Children under 18
- Participants with learning disabilities
- Participants suffering from dementia
- Other vulnerable groups.

c) If any of the above apply, does the researcher/investigator hold a current DBS certificate? A copy of the DBS must be supplied **separately from** the application.

d) How will consent be obtained? This includes consent from all necessary persons i.e. participants and parents.

A) As players are part of the squad they will potentially feel obliged to take part in the study. However, it will be strongly stated that it is not compulsory and they can withdraw from the study at any time. A Presentation will be delivered to the players illustrating the potential benefits of the study and they can decide post presentation.
B) Yes U18'S
C) the researcher and all other coaches at the club hold a current DBS certificate
D) As the sample will consist of under 18s, both ascent and consent forms will need to be completed following the dissemination of information sheets.

20. Risks and benefits of research/ activity

a) Are there any potential risks or adverse effects (e.g. injury, pain, discomfort, distress, changes to lifestyle) associated with this study? If so please provide details, including information on how these will be minimised.

b) Please explain where the risks / effects may arise from (and why), so that it is clear why the risks / effects will be difficult to completely eliminate or minimise.

c) Does the study involve any invasive procedures? If so, please confirm that the researchers or collaborators have appropriate training and are competent to deliver these procedures. Please note that invasive procedures also include the use of deceptive procedures in order to obtain information.

d) Will individual/group interviews/questionnaires include anything that may be sensitive or upsetting? If so, please clarify why this information is necessary (and if applicable, any prior use of the questionnaire/interview).

e) Please describe how you would deal with any adverse reactions participants might experience. Discuss any adverse reaction that might occur and the actions that will be taken in response by you, your supervisor or some third party (explain why a third party is being used for this purpose).

f) Are there any benefits to the participant or for the organisation taking part in the research (e.g. gain knowledge of their fitness)?

A) As the study replicates that of competitive match play, there are potential injury risks. These will be minimized by the inclusion of a suitable warm up, screening forms to determine previous and current injuries (athletes with a current injury will be excluded from participation) and the exercise is transient with sufficient recovery provided before and after the exercise protocols.

B) As above there are no real risks to the participants. The exercise is transient and supervised by physiotherapists with advanced first aid qualifications.

C) The fatigue measures used are non-invasive in the form of CMJ, muscle function and wellness.

D) The athletes already use the wellness questionnaire within their regular training regime and as such are well accustomed to it. The questionnaires contain no sensitive/ upsetting data.

E) Adverse reactions to the supplement could occur, if so, the participant will be able to consult with club doctor or personal GP and removed from the study. In addition, all staff members are first aid trained to the appropriate level, with the clubs physiotherapists possessing further first aid qualifications.

F) Benefits include a greater knowledge of the time course of recovery for said cohort and whether substance A has any effect on the recovery process.

21. Confidentiality, privacy and data protection

a) What steps will be taken to ensure participants' confidentiality?

- Please describe how data, particularly personal information, will be stored (all electronic data must be stored on St Mary's University servers).
- Consider how you will identify participants who request their data be withdrawn, such that you can still maintain the confidentiality of theirs and others' data.

b) *Describe how you will manage data using a data a management plan.*

- *You should show how you plan to store the data securely and select the data that will be made publically available once the project has ended.*
- *You should also show how you will take account of the relevant legislation including that relating data protection, freedom of information and intellectual property.*

c) Who will have access to the data? Please identify all persons who will have access to the data (normally yourself and your supervisor).

d) Will the data results include information which may identify people or places?

- Explain what information will be identifiable.
- Whether the persons or places (e.g. organisations) are aware of this.
- Consent forms should state what information will be identifiable and any likely outputs which will use the information e.g. dissertations, theses and any future publications/presentations.

a) All participant information will remain confidential throughout the duration of the project in line with the data protection act 1998. All data will be collected and stored electronically on St Mary's University servers, with all paper documents locked away securely. Participant's names will be replaced by a code known only to the research group. All data will be disposed securely after 5 years. Anyone who withdraws from the research project will have all information and data collected destroyed.

b) As above participants will have a number code attached to their name so as only the code will be made public if the study is published. All data will be collected and stored on a password-protected computer known only by the research group on St Marys University servers. Data will be presented as group averages thus no allowing identification of individuals.

c) Patrick Maher & Dr Mark Waldron
d) The participant names will be coded and the name of the club will never be stated. The results of the study will only identify the effect of the supplements on recovery and no traceability to the particpant.

22. Feedback to participants

Please give details of how feedback will be given to participants:

- As a minimum, it would normally be expected for feedback to be offered to participants in an acceptable to format, e.g. a summary of findings appropriately written.
- Please state whether you intend to provide feedback to any other individual(s) or organisation(s) and what form this would take.

A summary of key findings will be disseminated to the athletes. Any individual data required will only be in terms of pre and post findings.

The proposer recognises their responsibility in carrying out the project in accordance with the University's Ethical Guidelines and will ensure that any person(s) assisting in the research/ teaching are also bound by these. The Ethics Sub-Committee must be notified of, and approve, any deviation from the information provided on this form.

Signature of Proposer(s) Patrick Maher	Date: 31/01/2017
Signature of Supervisor (for student research projects)	Date: 31/01/2017

Information Sheet

Section A: The Research Project

A research project investigating whether Leucine supplementation may accelerate recovery following football match play.

I would like to invite you to participate in this project, which aims to reduce your recovery time following simulated football play and thus allowing you to begin training earlier at a lesser risk of injury. This project is part of my final year of my MSc degree in strength and conditioning. It is hoped that the results of this study could provide useful information to football clubs including staff and players as to the effects of leucine on recovery.

The research is organised by myself Patrick Maher under the supervision of my supervisor Dr Mark Waldron. With the results of this study if published presented in a way such that your anonymity is protected. Your name will be replaced by a code only known to the research group.

My contact details are as follows: Patrick Maher, 07896968771, patrick.maher@carlsileunited.co.uk

Section B: Your Participation in the Research Project

You have been invited to take part in the study because you're are a full time professional football player whose need for accelerated recovery times is pivotal. It is not compulsory to take part in the study and you can drop out at any time by simply speaking to myself at training. Your participation in this research study is entirely voluntary.

If you are injured or ill during the course of the study, you will not be required to participate. This is because your results will not be a true reflection of your normal time course of recovery. Furthermore, as you're a player at the football club, you will have already been heart screened and we have your complete medical history. However, if you have developed a recent medical condition, you must inform us before participation

If you agree to take part in the study you will be required for two sessions. The first session will be a familiarisation and Vo2 max session, it involves the baseline testing of all markers of fatigue (adductor squeeze tests, vertical jump and Visual Analogue Scale) in combination with a 15 minute familiarisation to a simulation football test (Loughborough Intermittent Shuttle Test). We also require your current Vo2 max status to complete the LIST, as such a Multistage Fitness Test (MSFT)

will also need to be completed. In total this will take around 2 hours so as to provide adequate recovery between the MSFT and the LIST familiarisation. The second session is the actual data collection. It involves the completion of the same LIST followed by consumption of an amino acid which purports to accelerate your recovery (Leucine, BCAA or a placebo). This will take around 2 and a half hours, with the LIST test taking 90 minutes plus a 20 minute warm up. With the consumption of supplementation and testing taking around 45 minutes. I will then record you fatigue status every day before training using the above fatigue markers, which will take around 30 minutes each day for 72 hours.

The test is designed to be maximal and similar to that of a football game, if you are in discomfort you can drop out at any time. In addition, the club physiotherapist who is IFAS accredited will be at each session.

All substances involved in the study will have been batch tested and possess the informed sport label. The requirement will be below the recommended dose and your legal rights will not be affected if anything goes wrong. However, adverse reactions to the supplement could occur, if so, you the participant will be able to consult with club doctor or personal GP and removed from the study. In addition, all staff members are first aid trained to the appropriate level, with the clubs physiotherapists possessing further first aid qualifications. These can also be sought prior to seeing the club doctor if you so require.

There are no special precautions you need to take before participation as you have successfully passed your heart checks during preseason and you will complete medical questionnaires prior to participation in the study.

All information collected from you will be number coded to protect anonymity, with said information only privy to the research group. It will be stored on a password protected computer on St Marys University servers. It will be analysed by myself and compared to both baseline testing results and the two other groups. At no point will anybody know your results, even at the point of publishing.

The benefits of taking part include adding to the research field in an area that directly effects yourself. If either supplement is shown to enhance recovery it may be beneficial to yourself in the future.

Finally, as a player at the club, you will no doubt feel obliged to take part in the study. However, I cannot emphaise enough that participation is entirely voluntary and you can withdraw at any time. This withdrawal will have no repercussions on your time at the football club.

YOU WILL BE GIVEN A COPY OF THIS FORM TO KEEP TOGETHER WITH A COPY OF YOUR CONSENT FORM

St Mary's University Twickenham London

Consent Forms

Name of Participant: _____

Title of the project: The effect of leucine or BCAA on recovery following simulated football match play

Main investigator and contact details: Patrick Maher 07896968771, Patrick.maher@carlsileunited.co.uk

Members of the research team: Dr Mark Waldron (supervisor)

1. I agree to take part in the above research. I have read the Participant Information Sheet which is attached to this form. I understand what my role will be in this research, and all my questions have been answered to my satisfaction.

2. I understand that I am free to withdraw from the research at any time, for any reason and without prejudice.

3. I have been informed that the confidentiality of the information I provide will be safeguarded.

4. I am free to ask any questions at any time before and during the study.

5. I have been provided with a copy of this form and the Participant Information Sheet.

Data Protection: I agree to the University processing personal data which I have supplied. I agree to the processing of such data for any purposes connected with the Research Project as outlined to me.

Name of participant (print)……..

Signed…….…… Date…….....

If you wish to withdraw from the research, please complete the form below and return to the main investigator named above.

Title of Project: _____

I WISH TO WITHDRAW FROM THIS STUDY

Name: _____

Signed: _____ Date: _____

St Mary's University Twickenham London

Name of Participant: _____

Title of the project: The effect of leucine or BCAA on recovery following simulated football match play

Main investigator and contact details: Patrick Maher 07896968771, Patrick.maher@carlsileunited.co.uk

Members of the research team:

1. I agree to my child taking part in the above research. I have read the Participant Information Sheet which is attached to this form. I understand what my child's role will be in this research, and all my questions have been answered to my satisfaction.
2. I understand that I am free to withdraw my child from the research at any time, for any reason and without prejudice.
3. I have been informed that the confidentiality of the information I and my child provides will be safeguarded.
4. I am free to ask any questions at any time before and during the study.
5. I have been provided with a copy of this form and the Participant Information Sheet.

Data Protection: I agree to the University processing personal data which I and my child have supplied. I agree to the processing of such data for any purposes connected with the Research Project as outlined to me.

Name of parent (print)……..

Signed…….…… Date…….....

--If you wish to withdraw your child from the research, please complete the form below and return to the main investigator named above.

Title of Project: _____

I WISH TO WITHDRAW MY CHILD FROM THIS STUDY

Name of Participant: _____

Name of Parent _____

Signed: _____ Date: _____

Risk Assessment

Section 1 – Activity and Coordinator details:

Activity coordinator name:	Patrick Maher	Tutor / supervisor:	Dr Mark Waldron
Phone number:	07896968771	Email address:	paddymaher@hotmail.co.uk
Activity title:	The effect of sole ingestion of Leucine and BCAA on recovery following simulation football match play		
Activity location(s) full details:	St Aiden's football pitches Carlisle		

Outline of activity (please specify the type of activity being undertaken):	No	Yes	If yes, please provide details:
1. Use of Human Subjects: demographic type, requirements, age/young persons?		Y	Youth athletes from a professional football club, u18s.
2. Use of an intervention (either solely or in combination) including dosage or application: E.g. ingestion of food, liquids or supplement, diet, massage, occlusion, environmental exposure, physical activity or other. Outline of specific dosage or application where relevant E.g. mg per kilo of body weight		y	Players complete Loughborough Intermittent Shuttle Test, then consume 0.087g/kg/Bw of either Leucine or BCAA or Placebo. Record fatigue over the next 72 hours.
3. Use of data and/or sample collection (solely or in combination): E.g. questionnaire/survey, human tissue sampling (blood / urine / saliva / sweat or other), respiratory analysis, body composition, performance tests or other.		y	Record initial LIST results to split groups. Use jump mat, adductor squeeze and Visual Analogue Scale to measure fatigue
4. Use of chemicals/gas cylinders: Type(s), hazardous or not, MSDS available?	N		
5. Equipment to be used:			
Laptop with LIST instructions, timing gates, adductor squeeze blood pressure cuff, vertical jump mate, analogue scale.			

Outcome due to Hazard description (Substance / equipment / procedure)		Initial Risk Level High(13-25) Med (5-12) Low (0-4)	Necessary controls to eliminate or adequately reduce the **Initial Risk Level** of an associated hazard to a suitable **Remaining risk level**.	g Risk Level High(13-25) Med (5-12) Low (0-4)
1	Access and usage of designated facility, site or location, including private or public.	High	Written approval to access and make use of the designated facility, site or location must be sought from the relevant owner, keeper or manager, where appropriate. Terms & conditions, qualifications, notification, booking requests, reporting, statements of intent or other formal agreement must be confirmed in advance of access and use. A separate assessment of local hazards should be undertaken to ensure safe working practice. Gauge which person(s) will be responsible for dealing with any potential emergency incident, including use of First Aiders, Security or other individual.	Low
2	Fire management and evacuation	High	Ensure appropriate responsibilities of relevant individuals or parties are established ensuring fire prevention, evacuation and individual roles are established prior to activity commencing. Please refer to University policy.	Low
3	Environmental exposure (internal and external) including temperature, humidity, lighting ventilation or relative weather conditions	High	Ensure suitable local working conditions including regulating temperature, lighting, and humidity where possible. Prepare relevant individuals to manage uncontrollable/unavoidable conditions including instruction on use of personal protective equipment, clothing, shelter, rehydration, rest periods/breaks or other relevant means to maintain suitable comfort and function. Brief individuals on managing experience of deliberate exposure to stressful conditions, including adequate avoidance of visual/physical exposure to sources of high level lighting and associated heat. Ensure low lighting allows participant to still work safely including avoiding injury or subsequent strain/pathology.	Low
4	Risks relating to layout , storage, space, obstructions including fall of objects, spillages, slips, trips & falls	High	Ensure all equipment layouts maintain a safe working environment. Ensure measures are in place to manage trip hazards including suitable form(s) of cable covers, excess cabling, objects or equipment do not affect walkway routes. Highlight visually and by instruction unavoidable trip hazards. Manage liquids to avoid spillages. Establish equipment available to manage spillages and who is responsible to address an incident. Ensure the avoidance of any falling object, or safe management processes for any item likely to or intended fall.	Low
5	Manual handling, repetitive movements and working at heights	High	Ensure users have received adequate training, adhering to the HSE and University manual handling guideline found on the staff H&S portal page. Identify and avoid or adequately reduce repetitive	Low

			movements that may cause harm. Any individual working with ladders must receive appropriate training and qualification.	
6	Use of Equipment including electrical	High	Ensure users have received adequate competency training as specified on Section E) of the **Student Research Approval Form** to use the equipment. Check electrical items are Pat tested annually, no faults are present. No fluids near the equipment. Check wires for damage during setup and prior to use. Switch off equipment when not in use or in the event of an incident.	Low
7	Mechanical (machinery) and use of portable tools / equipment	High	Staff and students may only use equipment that is permitted and training has been provided for.	Low

SECTION 2: Risk Controls - For each hazard identified in Section 1, complete Section 2. Please refer to the Risk Assessment Guidance notes on simmsCAPital folder for Risk Matrix. **Please note that L refers to Likelihood; S refers to Severity and RS refers to Risk Score (L times S equals RS)**

SECTION 2: Risk Controls (continued) - For each hazard identified in Section 1, complete Section 2. Please refer to the Risk Assessment Guidance notes on simmsCAPital folder for Risk Matrix.

Please note that L refers to Likelihood; S refers to Severity and RS refers to Risk Score (L times S equals RS)

Hazard No.	Outcome due to Hazard description (Substance / equipment / procedure)	Level High (13-25) Med (5-12) Low (0-4)	Controls needed to eliminate or adequately reduce risks	Risk Level High(13-25) Med (5-12) Low (0-4)
8	Exposure to sharps – use of razors, lancets or other sharp object	High	Ensure users wear separate PPE per human subject. Ensure different (disposable only) razor, lancet or other invasive equipment used per human subject. Do not reuse disposable item more than once. Discard in suitable biohazard sharps container.	Low
9	Human subject physical activity, manipulation, treatment or other including use of equipment where relevant.	High	Prior to commencing any activity, ensure the practical activity coordinator(s) has completed all necessary competency training as specified on Section E) of the **Student Research Approval Form**. Adhere to the University Ethics procedures ensuring all human subjects have received a relevant information sheet, completed an Informed consent form and (Physical Activity Readiness Questionnaire (PARQ)/Medical History Form under the relevant Health and Safety procedures. Ensure documents are countersigned and dated at the same time (witness process) by the relevant research coordinator. Ensure documents are retained during the testing period, and then returned to the University for data protected archiving when testing has been completed This may vary where members of the public are approached on an 'at the time' basis under typical survey work. All ingestion interventions must address maximum safe dosage fit for the relevant human participant (s).	Low
10	Exposure and reaction to use of food, drinks or supplements	High		Low
11	Biological hazards	High	Ensure Practical Activity Coordinators (PACs) students have received Human Tissue sampling training and the relevant competency forms signed off by the trainer, considering all aspects of the School's use of Human Tissue (HT) Guideline (available in MyModule student information folder) for the management of biological hazards.	Low

12	Working at heights	High	Students and Human subjects not to work at heights. Only suitably qualified staff may assist.	Low
13	Lone working, including out of hours	High	Students not to undertake unsupervised out of hours activity within University facilities. Field testing locations should be assessed for safe exposure and co-worker or check in systems should be adopted where deemed appropriate. Ensure University Security is notified of any supervised work taking place outside of normal weekday hours (Monday-Friday 9am-5pm)	Low
14	Use of chemicals	High	Ensure suppliers are appropriately registered and provide adequate Material Safety Data Sheet (MSDS). Refer and adhere to all instructed practice as detailed by the relevant MSDS for all chemicals used. **See HSE CHIP and REACH regulations.**	Low
15	Other (Please specify and attach a copy of the relevant methodology with associated safety notes):	High	Ensure participants understand that they can drop out at any time and it will be acquiesced. Risk of over exertion reduced as trained professionals are in close proximity (physio, club doctor). Supplements used will be within recommended safe limits and batch tested from my protein bearing the informed sport logo.	Low

SECTION 3: Arrangement for supervision and/or monitoring effectiveness of control

Monitoring achieved through pre and post checks, continual test supervision and/or a separately recruited individual where further supervision or monitoring is required. Even where students demonstrate high levels of competency, regular checks should be made by supervising staff that should also be

readily available to assist with any questions or problems students might have. Any practice should be amended or stopped if an emerging hazard dictates such a response. This option should be adopted where any uncertainty occurs, seeking advice from suitable staff.

SECTION 4: Referral guidelines relevant to the intended activity (scanned PDF of hard copy, listed web link or other source):

Please ensure that all relevant reliable sources of information can be easily referred to at any time both during the preparation phase and period of activity. Please note some of the relevant University links will include:

Information source	Location	Areas of information
Student Information Folder	MyModules	Student Research Approval Form Laboratory specific guidelines and consumable costs School of SHAS Use of Human Tissue Guideline (new)
University Ethics Committee	Student portal	Ethics Application process and associated forms Example Human Subject Consent Form
University Health and Safety portal	Student portal	Health and Safety Policy guidelines including Risk Management, Loan working, Manual lifting, Display Screen Equipment and COSHH
The Health and Safety Executive	Website found through any web search engine	Well-presented sources of legally approved regulation and legislation covering COSSH, CHIP, RIDDOR, DSE and many other areas of health and safety at work
Further discipline specific sources of information may be relevant to the area of activity including accreditation bodies such as BASES, BASRAT, SENR, AfN, BPS, UKSCA, REPS, HTA, ITEC etc		

SECTION 5: Emergency response procedures

In the event of an emerging incident, engage the individual(s) who have been previously agreed as responsible for addressing an emergency incident. Assess and eliminate (where safe) hazards that might place the individual(s) needing care or carer(s) at risk. Apply up to date first aid and/or seek medical assistance where appropriate. Contact the University security team for assistance with any incident on or off campus. Contact relevant staff (tutor, Technical team or other) where relevant. Complete relevant reporting form (accident, medical emergency or near miss) available to staff on the University H&S portal page, passing to the Technical Services team for processing. Complete HSE RIDDOR form where relevant, which can be found on the HSE website.

Important contact details (including where activities are undertaken off campus):

- **St Mary's University Security – 0208 240 4335 or 4060** (advise in the event of calling the emergency services)
- St Marys University main reception – 0208 240 4000
- **Health and Safety Executive (HSE)** Information line – 0845 345 0055 / www.HSE.gov.uk

Please make note of any other relevant contacts here:SECTION 7: Period of cover – *If a more complex assessment is required, continue below:*

PERIOD OF COVER FOR TASK/EVENT		PRINT NAME OF TASK/EVENT LEADER(S)	SIGNATURE	DATE SIGNED	HAZARDS IDENTIFIED (mark with a tick or a cross)
FROM	TO				
Feb 2017	April 2017		Patrick Maher	31/01/2017	y

SECTION 8: Student liability declaration:

By signing this risk assessment I confirm that I have read and understood the above information that is relevant to my activity, and will ensure adherence to appropriate practice at all times, based on completing formal competency training relevant to the activity I am planning to undertake. I understand that the above statements are intended to be generalised, being applicable to all forms of activity. Not all parts may apply to a specific activity, but it is my responsibility to outline any possible/further detail of necessary hazard management procedures as safety notes within the relevant activity methodology, as statements of intent within the associated Ethics Application Form and as associated Human Subject Consent Form and Information sheet.

SIGNATURE:	Patrick Maher	PRINT NAME:	Patrick Maher	DATE COMPLETED:	31/01/17