Title: Leucine supplementation increases muscle strength and volume, reduces inflammation and affects wellbeing in adults and adolescents with cerebral palsy.

Author names:

Nicola Theis[1], Meghan A. Brown[1], Paula Wood[2], Mark Waldron[3,4*]

Affiliations:

[1]School of Sport and Exercise, University of Gloucestershire, Gloucestershire, UK

[2]Treloar's School and College, Treloar's Trust, Alton, Hampshire, UK

[3]College of Engineering, Swansea University, Swansea, UK

[4]School of Science and Technology, University of New England, NSW, Australia.

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***Contact Details for the Corresponding Author:**

Dr Mark Waldron, College of Engineering, Swansea University, Bay Campus, Swansea, SA20RZ (mark.waldron@swansea.ac.uk).

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Abbreviations:

Brach-chain amino acid (BCAA)

Cerebral palsy (CP)

Coefficient of variation (CV)

Confidence interval (CI)

C-reactive protein (CRP)

Gross motor function classification system (GMFCS)

Trial registration:

NCT03668548 registered at www.clinicaltrials.gov

Abstract

Background: Spastic cerebral palsy (CP) is characterised by muscle weakness owing, in part, to a blunted muscle protein synthetic response. This might be normalized by long-term leucine supplementation.

Objectives: The study assessed the effects of 10-week leucine supplementation in adolescents and adults with CP.

Methods: The study was a single-centre randomised controlled trial. Twenty-four participants were randomised to a control group (n = 12) or a leucine group (n = 12). L-Leucine (192 mg/kg body mass) was dissolved in water and administered daily for 10 weeks. Primary outcome measures; elbow flexor muscle strength and muscle volume (measured by 3D ultrasound technique) and inflammation (C-reactive protein concentration) were assessed before and after 10 weeks, alongside secondary outcomes; body composition (measured by CP-specific skinfold assessment), metabolic rate (measured by indirect calorimetry) and wellbeing (self-reported daily questionnaire). Data were compared with a series of two-way mixed ANOVA's.

Results: Twenty-one participants completed the intervention (mean ± SD, control group: n = 11, age: 18.3 ± 2.8 y, body mass: 48.8 ± 11.9 kg, 45% male; leucine group: n = 10, age: 18.6 ± 1.7 y, body mass: 58.3 ± 20.2 kg, 70% male). After 10 weeks, there was a 25.4% increase in strength (p = 0.019) and a 3.6% increase in muscle volume (p = 0.001) in the leucine group with no changes in the control group. This was accompanied by a 59.1% reduction in CRP (p = 0.045) and improved perceptions of wellbeing (p = 0.006). No changes in metabolism or body composition were observed in either group ($p > 0.05$).

Conclusions: Improvements in muscle strength and volume with leucine supplementation might provide important functional changes for adults and adolescents with CP and could be partly explained by reduced inflammation. The improved wellbeing highlights its capacity to improve the quality of daily living.

Key words: Muscle, cerebral palsy, leucine, inflammation, wellbeing

Introduction

Cerebral palsy (CP) is caused by damage to the developing brain and descending pathways, leading to altered patterns of growth and development (1). Those with CP may encounter early symptoms of paresis and spasticity, leading to increased muscle atrophy (2) and abnormal growth of contractile and non-contractile tissue (3). This causes significant weakness of the muscle and compromises daily function (4). As such, interventions aimed at increasing muscle mass or preventing muscle atrophy for those with CP must be established.

For those with CP, several factors may contribute to reduced levels of protein synthesis and therefore, muscle atrophy or diminished growth capacity. For example, sub-optimal nutritional status (5) and oropharyngeal dysfunction (6) can hinder feeding. Furthermore, chronic low-grade inflammation has been linked to sustained neurological injury (7) and the observed reductions in physical activity and chronic inflammation have been shown to block protein synthesis pathways (8), thus promoting a negative net protein balance (9). Ingestion of the branched-chain amino acid (BCAA) leucine has been shown to augment anti-inflammatory networks (10), stimulate protein synthesis pathways, and potentially provide antiproteolytic effects, resulting in a positive protein balance and potential net muscle mass gain (11-12). The provision of high-quality amino acid solutions via beverages might circumvent the feeding issues that arise from oral motor dysfunction among those with CP, as well as assisting with energy and protein balance.

There are various other benefits to leucine supplementation among those with CP. For example, increases in resting metabolism and changes in substrate utilisation might help to offset the health risks of sedentary behaviour and muscle atrophy reported in this population (13). Administration of leucine-rich amino acid mixtures can increase energy expenditure (14) and

promote lean body mass (15). Furthermore, the health and wellbeing of those with CP can be challenged by various social, environmental and personal constraints (16), which can lead to emotional problems and low life satisfaction (17). Changes in plasma BCAA availability can have neurochemical and functional consequences in the brain and, while their effects on cerebral function are controversial (18), inadequate diet or under-nourishment is likely to disrupt mood state (19) which could be offset by oral BCAA administration in addition to a calorie controlled-diet. However, there has been no investigation of the effects of leucine supplementation on wellbeing in CP. Therefore, the purpose of this study was to assess the effects of 10-weeks leucine supplementation on muscle growth, metabolism, body composition, inflammation and wellbeing in adolescents and young adults with CP.

Methods

Study design and participants

The study was a single-centre randomised controlled trial comparing 10 weeks of leucine supplementation with a control. Adolescents and young adults with CP were recruited from a special educational needs school and college. Inclusion criteria were: 1) a diagnosis of spastic cerebral palsy 2) Gross Motor Function Classification System (GMFCS) II-V 3) aged 12-25 years. Exclusion criteria included: 1) orthopaedic surgery of the upper-limbs in the past 12 months 2) botulinum toxin type A injections in the past 6 months 3) serial casting in the past 6 months 4) insufficient cognitive understanding to comply with procedures. Parental/guardian consent was obtained from participants under 18 years. Those over 18 years gave their own written or verbal consent in the presence of a carer. Ethical approval was granted by an Institutional Ethics Committee. Trial registration number was NCT03668548.

Randomization

The randomization schedule with a 1:1 allocation ratio was generated by an individual independent to the study prior to the start of recruitment. The same individual placed allocation of participants in sequentially numbered opaque sealed envelopes. The trial manager revealed allocation, and informed participants and therapists, after participants completed the baseline assessment.

Procedures

Intervention

Participants completed testing at baseline and after 10 weeks at a similar time of day. All participants (with assistance from parents/guardians or carers where required) were asked to complete a daily food and fluid diary (including feeds and supplements aside from the intervention drink) and a daily wellbeing questionnaire throughout the trial period. Three days of food diaries within the first two weeks of the study were analysed using dietary analysis software (Nutritics Ltd, Swords, Ireland) to determine mean daily energy and macronutrient intake. Based on published upper tolerable limits of children (20), the intervention group were supplemented daily with 192 mg/kg body mass of L-leucine, up to 15 g (12.4 ± 2.2 g) (Bulk Powders, Sports Supplements Ltd., Essex, UK) dissolved with 300 ml of water and approximately 50 ml of fruit concentrate (Robinsons Orange Squash, Britvic Soft Drinks, Herefordshire, UK), to mask the taste of leucine, while the control group were provided with 300 ml of water and 50 ml of fruit concentrate drink. The drinks were prepared by people independent to the study and consumed by participants throughout the day for 10 weeks. In this time, all participants were asked to maintain their typical eating and activity routines.

Primary outcome measures

Muscle strength. Elbow flexor strength was assessed using hand-held dynamometry (FDIX, Wagner Instruments, Greenwich, CT). The dynamometer was fixed to a rigid custom-made device, which allowed participants to perform isometric elbow flexion contractions at approximately 90°. The dynamometer was placed perpendicular to the arm to be tested, and mid-way between the elbow and wrist, on the least affected arm. With all participants in a seated position, resistance was applied by the examiner to avoid movement of the limb being tested (Coefficient of variation (CV%) = 13.1). A rest period of 30 s was given between three consecutive trials. If trials differed by >10% an additional trial was performed. The trial with the highest recorded force was used for further analysis.

Muscle volume. Muscle volume of the elbow flexors from the least affected arm were measured using two-dimensional B-mode ultrasound images combined with 3D motion data (Stradwin v5.1 software, Mechanical Engineering, Cambridge University, Cambridge, UK) using previously established methods (21). Bicep brachii and brachialis muscle boundaries were identified and digitised, and volume reconstructions were computed. Muscle volumes of biceps brachii and brachialis were summed to give an overall elbow flexor muscle volume. Every third frame of the muscle sweep was segmented and reconstructed into a rendered 3D muscle (CV% = 1.2) along with the values of the reconstructed muscle volume.

C-reactive protein. The index fingertip or in the case of severe spasticity, the earlobe, of the participant was cleaned using a sterile alcohol swab and allowed to air dry. Capillary blood was drawn and a sample of whole blood (300 μL) was collected into a capillary tube and centrifuged at 3000 r/min for 5 min. The resultant plasma was removed and stored at -20°C. C-reactive protein was quantified using a commercially available, latex particle-enhanced immunoturbidimetric assay (CRPL3, Roche Diagnostics, Burgess Hill, UK), and monitored

spectrophotometrically using an automated system (Cobas 8000 c702 analyser, Roche Diagnostics, Burgess Hill, UK). The analytical characteristics were: limit of detection 0.3 mg/L; limit of quantitation 0.6 mg/L; mean laboratory inter-assay CV% during the study was 3.5% at a level of 26.5 mg/L and 10.6% at a level of 133.2 mg/L.

Wellbeing. The daily wellbeing questionnaire asked participants to rate their fatigue, sleep quality, general muscle soreness, stress levels and mood on a five-point scale (scores of 1 to 5) (22). Wellbeing was then determined by summing the five scores. The median rating for each variable across week 1 and week 10 were compared between groups.

Secondary outcome measures

Fat and carbohydrate oxidation and resting energy expenditure. Resting energy expenditure was calculated via indirect calorimetry collected using a portable metabolic system (K4 b2, Cosmed, Italy), which was calibrated before every use with one reference gas mixture (95% O_2, 5% CO_2). Indirect calorimetry was performed whilst participants were in a seated or supine position for approximately 10 min. The same position and rest period was maintained for pre and post measurements. All measures were taken in the morning <60 min after wakening and with each participant fasted for a minimum of 8 h. Mean data from the final 2 min of gas collection were utilised for analysis. Steady state was confirmed by inspection of the oxygen uptake values and fat and carbohydrate oxidation and resting energy expenditure were calculated based on previous equations (23).

Body composition. Percentage body fat was estimated based on CP-specific prediction equations (24), which incorporate GMFCS level, maturational status and two-site skinfold measures (CV% = 2.8). The mean of two measurements of subscapular and triceps skinfolds

from the least affected side was taken in all participants using standardized techniques (Harpenden calipers, CMS Weighing Equipment Ltd, London, UK). Participants GMFCS level was assessed by a Physiotherapist. For use in the equations, GMFCS was categorized into two groups: 'more severe' (GMFCS levels III, IV, V) and 'less severe' (GMFCS levels I, II)[24]. Maturational status was assessed by means of secondary sex characteristics (breasts in females; pubic hair in males) (25). Observations were self-reported in those over 18 years or performed by parents/guardians or carers in those under 18 years. A Tanner stage of 1 or 2 was defined as prepubescent, Tanner stage 3 was defined as pubescent, and Tanner stage 4 or 5 was defined as post-pubescent (25). Estimated body fat percentage was then calculated based on the prediction equations (24) and corrections for children with CP (26). This was subtracted from body mass at each time point to give lean body mass.

Data analysis

A sample of twenty-four participants with was required, based on an effect size of 0.30, statistical power of 0.80 and inclusive of a 30% dropout. Primary analyses were "per-protocol" from participants who completed >70% of supplementation and took part in both pre and post testing. Independent samples t-tests were conducted to assess any baseline differences in the dependent variables (muscle strength, muscle volume, C-reactive protein, fat and carbohydrate oxidation, resting energy expenditure, body fat percentage, sum of skinfolds, and perceptions of wellbeing). In addition, independent t-tests were also performed to assess baseline differences in mean daily total energy intake and macronutrient contributions (g, % of total energy intake) between groups.

To address the main purpose of the study, a series of two-way within and between analysis of variance were then performed to evaluate the effects of time (0 weeks and 10 weeks) and group

(control and leucine) on the dependent variables (muscle strength, muscle volume, C-reactive protein, fat and carbohydrate oxidation, resting energy expenditure, body fat percentage, sum of skinfolds, and perceptions of wellbeing). In case of a significant interaction, post hoc tests were performed between groups (independent t-tests) and between time points (paired t-tests). A bonferroni correction was performed to adjust for multiple comparisons. Data were presented as mean ± SD, mean difference with 95% confidence intervals and Cohen's D effect size.

Results

Initial recruitment to the study began in August 2018 and post-testing was completed in October 2018. Of the initial 24 participants recruited, one participant withdrew outlining personal reasons, one withdrew due to inability to take the supplement and one was not included in the final analysis because of non-compliance with the protocol (leucine: n = 10; control: n = 11) (Supplementary Figure 1). This resulted in 88% compliance to the study. Final group characteristics are presented in **Table 1**.

Dietary analysis

Nineteen participants (n = 8 leucine; n = 11 control) completed three days of food diaries within the first two weeks of the study (n = 2 diaries were incomplete). Independent samples t-tests revealed there were no differences in the mean daily total energy intake (kcal, MJ) and macronutrient contributions (g, % of total energy intake) of participants' typical diets between groups (**Table 2**).

Muscle strength, volume and CRP

One participant was not included in the analysis for muscle strength and muscle volume as they were unable to perform the isometric strength test and clear images were not obtained for

muscle volume. Blood samples for CRP analysis were not taken from two participants in the leucine group and three participants in the control group due to non-compliance. Independent t-tests revealed there were no baseline differences between groups for muscle strength (p = 0.084), muscle volume (p = 0.452) or CRP (p = 0.594). Results of the ANOVA demonstrated significant interaction effects for muscle strength (p = 0.019) muscle volume (p < 0.001) and CRP (p = 0.045). Post hoc tests demonstrated that after 10 weeks of leucine supplementation, muscle strength, muscle volume and CRP were significantly higher in the leucine group (p < 0.001) compared to the control group (p > 0.05) (**Table 3**).

Substrate oxidation, resting energy expenditure and body composition

The results of independent t-tests revealed no baseline differences in fat oxidation (p = 0.506), carbohydrate oxidation (p = 0.095), resting energy expenditure (p = 0.319), body fat (p = 0.958) or sum of skinfolds (p = 0.098). Results of the ANOVA's revealed no changes between groups or over time for fat oxidation (p = 0.662) carbohydrate oxidation (p = 0.307) or resting energy expenditure (p = 0.218) (respiratory exchange ratio: Pre = 0.88 ± 0.07; Post = 0.89 ± 0.07). Skinfold measures were not possible on one participant in the leucine group and two in the control group. For all other participants, there were no changes in body fat (p = 0.451) or the sum of skinfolds (p = 0.174) between groups after 10 weeks of leucine supplementation (**Table 3**).

Wellbeing

Independent t-tests revealed no baseline differences between groups for wellbeing variables (p > 0.05). The results of the ANOVA demonstrated significant interaction effects for muscle soreness (p = 0.010), stress levels (p = 0.011), mood (p = 0.048) and general wellbeing (p = 0.035). Post hoc tests demonstrated that after 10 weeks of leucine supplementation, muscle soreness and stress levels were significantly lower in the leucine group (p < 0.01), with no changes in the control group (p > 0.05). In addition, post hoc tests revealed that ratings of mood

and general wellbeing were significantly greater in the leucine group after 10 weeks of supplementation ($p < 0.05$), with no changes in the control group ($p > 0.05$) (**Table 3)**. There were no changes in ratings of fatigue ($p = 0.770$) or sleep quality ($p = 0.924$), between groups after 10 weeks of leucine supplementation (**Table 3**).

Discussion

This is the first study to report that 10 weeks of leucine ingestion in young adults and adolescents with moderate to severe CP significantly reduces inflammation, with concomitant improvements in muscle strength, muscle volume and perceptions of muscle soreness, stress, mood and general wellbeing.

The increases in both muscle volume and strength conferred by leucine supplementation could provide important functional changes to individuals with CP. There are few studies monitoring changes in muscle volume following dietary amino acid supplementation. One study reported increases in muscle mass following a 13-week, high-protein, leucine-enriched (6 g/day) diet among elderly sarcopenic subjects, without structured exercise interventions, lending support to the reported anabolic actions of leucine in skeletal muscle (27). To date, there has been no study to demonstrate anabolic resistance in those with CP, yet the sedentary lifestyles and prevalence of malnutrition makes this a plausible outcome (1). Physical activity is known to augment the anabolic actions of leucine-rich diets (28), thus overcoming anabolic resistive thresholds, but inducing a traditional physical activity stimulus is practically challenging among many of those with moderate to severe CP. However, the finding that strength and muscle size were increased after 10 weeks of leucine ingestion infers an anabolic effect. Not all studies have reported changes in muscle mass after leucine supplementation (29) and there is mixed evidence to support the anabolic role of leucine over total essential amino acid load

(30). However, 3 g of isolated leucine without additional amino acids, can maximally stimulate protein synthesis (30). Here, protein metabolism was not measured but we can speculate that the increase in muscle volume and strength was probably the result of leucine-mediated increases in the rates of muscle protein synthesis, and/or reductions in muscle protein breakdown.

A descriptive evaluation of individual responses to leucine supplementation in our study suggests that those who demonstrated the greatest responses had either; greater levels of gross motor function, and were more physically active (i.e. voluntary energy expenditure), or; were those with poor motor function but very high levels of spasticity (i.e. involuntary energy expenditure). However, whilst each of these energy-demanding processes is capable of augmenting anabolic singling and subsequent muscle protein synthesis in combination with the leucine supplementation (11,28,31), there is currently no valid or unified approach to monitoring daily energy expenditure and/or physical activity levels among those with severe spastic cerebral palsy during free living. For example, involuntary muscular contraction, induced by spasticity, was present in the majority of participants in this study and has been considered a source of excessive energy expenditure (28), which may augment the anabolic action of leucine, yet this cannot be objectively quantified at present. Therefore, whilst it was not possible to quantify the extent and magnitude of spasticity over a 10-week period, our results suggest the leucine response may be modulated, to some extent, by spastic episodes even in the absence of a traditional physical activity stimulus. Based on this reasoning, there is grounds for further research to develop the current understanding of energy-demanding activities (voluntary or otherwise) among those with CP and their synergistic effects with leucine supplementation for promoting muscle growth. Despite reporting changes in muscle volume of one muscle group, we did not find changes in resting metabolic rate, substrate metabolism or changes to the amount of fat mass and fat free mass. It is possible that the body

composition equations utilised were not sufficiently accurate in the current group, leading to erroneous values and a failure to detect changes in body composition. More work is necessary to confirm these findings, as well as determine the direct effects of leucine supplementation on muscle protein synthesis in CP groups.

We are the first to provide evidence of the potential systemic anti-inflammatory role of leucine supplementation among those with CP, highlighted by a significant reduction in CRP concentration across the 10-week period in the leucine group. The administration of leucine-rich amino acids is known to stimulate anti-inflammatory networks (10). Chronic inflammation has been reported among those with CP (1, 7) and increases in intermuscular adipose tissue is a probable contributor, based on our body fat estimations and the reported sedentary behaviours of non-ambulant individuals. The changes in CRP coincided with a reduction in perceived muscle soreness, which can be related to reductions in systemic inflammation. Our findings are consistent with others, whereby leucine-rich protein diets have been shown to reduce CRP in elderly subjects (15), as well as recent meta-analytic findings demonstrating the accentuated anti-inflammatory effects of whey protein diets on CRP among those with chronic low-grade inflammation (32). Therefore, we provide the first evidence that leucine could have an anti-inflammatory effect on those with CP and that this appears alongside increased muscle function, muscle mass and reduced soreness.

The observed improvements in the composite wellbeing score of the leucine group were attributable to changes in muscle soreness, stress and mood across the 10-week period. The energy intake was not different between the two groups, suggesting that the addition of leucine to the diet improved wellbeing. Those with CP face daily emotional challenges and often live with a range of comorbidities (22), which can lead to higher perceived fatigue and depleted

mood (17). Therefore, there is feasible capacity to improve the general daily wellbeing of those with CP, as demonstrated herein. There are a variety of mechanisms that link symptoms of depression, including mood states and perceived stress, to dysregulated serotonin (5-HT) within the brain. BCAAs (such as leucine) provide alternative precursors of 5-HT and can offset the depletion of others (tryptophan, TRP) (18). Indeed, supplements containing TRP and other amino acids have been shown to positively affect mood and depressive symptoms (33). The mechanistic basis of this association could be explained by the reduced blood-to-brain transfer of kynurenine reported in the mouse model following leucine treatment (34) but this requires further research in humans. However, given the adherence of the participants to the dietary regime, it is possible that this was not the underlying reason, since competitive inhibition of TRP uptake at the blood brain barrier can occur (35). Whilst the changes noted in our study are unlikely to provide a permanent solution to wellbeing problems in those with CP, it appears that leucine supplementation at least transiently alleviated low mood or stressed states.

In conclusion, ten weeks of leucine ingestion (192 mg/kg, ~9 - 15 g) provided a variety of benefits to young adults and adolescents with moderate to severe CP. The changes in muscle strength and muscle volume might provide important functional changes and could be partly explained by the reduced systemic inflammation. The improved wellbeing of the leucine-fed CP group also highlights its alternative roles and capacity to improve the quality of daily living. There is some evidence that physical activity and/or repeated involuntary muscle activity may provide superior improvements in muscle strength, muscle volume and CRP after leucine supplementation in this population, but this warrants further investigation.

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Author contributions:

1. Designed research (project conception, development of overall research plan, and study oversight): **NT, MB, MW, PW.**

2. Conducted research (hands-on conduct of the experiments and data collection): **NT, MB, PW.**

3. Provided essential reagents, or provided essential materials (applies to authors who contributed by providing animals, constructs, databases, etc., necessary for the research): **MW, PW.**

4. Analyzed data or performed statistical analysis: **MW, MB, NT.**

5. Wrote paper (only authors who made a major contribution: **NT, MB, MW.**

6. Had primary responsibility for final content: **NT, MW.**

7. All authors have read and approved the final manuscript: **NT, MB, MW, PW.**

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Table 1. Participant characteristics of adults and adolescents with CP in the leucine (n = 10) or control groups (n = 11)[1]

	Leucine group	**Control group**
Age, y	18.6 ± 1.7	18.3 ± 2.8
Sex	7 male, 3 female	5 male, 6 female
Body mass, kg	58.3 ± 20.2	48.8 ± 11.9
GMFCS level		
II	2	1
III	3	0
IV	4	7
V	1	3
Tanner level		
II	0	1
III	1	7
IV	7	2
V	2	1

[1]Values are mean ± SD, GMFCS: Gross motor function classification system.

Table 2. Daily energy and macronutrient intakes of typical diet of adults and adolescents with CP in the leucine (n = 8) or control groups (n = 11)[1]

Intake	Leucine	Control	P value
Energy Intake, kcal	1523 ± 429	1881 ± 648	0.193
MJ	6.4 ± 1.8	7.9 ± 2.7	0.193
Carbohydrate, g	160 ± 46	211 ± 70	0.092
% energy	43 ± 11	45 ± 4	0.486
Protein, g	62 ± 22	74 ± 31	0.371
% energy	16 ± 4	16 ± 3	0.680
Fat, g	70 ± 31	79 ± 33	0.551
% energy	41 ± 9	37 ± 7	0.335

[1]As determined using dietary analysis software (Nutritics Ltd, Swords, Ireland) from 3-day food diary. %, percentage of total energy intake. Values are mean ± SD.

Table 3. Dependent variables before and after 10 weeks of leucine supplementation in adults and adolescents with CP randomized to a leucine group (n = 10) or control group (n = 11)

	Leucine group			Control group			
	0 weeks	**10 weeks**	**Mean difference (95% CI), Cohen's D**	**0 weeks**	**10 weeks**	**Mean difference (95% CI), Cohen's D**	**P-interaction (group × time)**
Muscle strength, N	133.2 ± 60.9	167.0 ± 48.6*†	33.8 (79.8 to -12.2), 0.60	78.0 ± 75.7	77.0 ± 53.4	1.0 (58.4 to -56.4), -0.01	0.019
Muscle volume, cm^3	162.3 ± 22.4	168.1 ± 24.2*†	5.8 (25.3 to -13.7), 0.25	152.1 ± 35.1	151.9 ± 35.8	-0.2 (29.5 to -29.9), -0.004	0.001
Plasma CRP, mg/L	4.7 ± 4.4	1.9 ± 1.9*†	-2.8 (0.03 to -5.6), -0.78	3.6 ± 3.9	3.0 ± 2.5	-0.6 (2.1 to -3.3), -0.20	0.045
Fat oxidation, KJ/min	1.3 ± 1.1	1.1 ± 0.9	-0.2 (0.6 to -1.0), -0.22	1.3 ± 0.8	1.3 ± 0.9	0.0 (0.7 to -0.7), 0.02	0.662
Carbohydrate oxidation, KJ/min	3.6 ± 1.9	3.1 ± 1.4	-0.5 (0.9 to -1.9), -0.30	2.7 ± 1.5	3.0 ± 2.1	0.3 (1.8 to -1.2), 0.16	0.307
REE, kJ/min	2.5 ± 0.9	2.3 ± 1.0	-0.2 (0.6 to -1.0), -0.28	2.1 ± 0.9	2.3 ± 1.3	0.2 (1.1 to -0.7), 0.17	0.218
Body fat, %	35.3 ± 16.4	36.6 ± 18.1	1.3 (15.7 to -13.1), 0.08	33.3 ± 9.1	33.1 ± 9.5	-0.2 (7.6 to -8.0), -0.02	0.644
Sum of skinfolds, mm	36.9 ± 24.8	40.1 ± 25.4	3.2 (24.2 to -17.8), 0.13	21.5 ± 10.1	23.9 ± 14.0	2.4 (12.6 to -7.8), 0.19	0.174
Muscle soreness	3.7 ± 0.6	4.5 ± 0.5*†	0.8 (1.3 to 0.3), -1.84	3.9 ± 0.8	3.8 ± 0.8	-0.1 (0.6 to -0.8), -0.46	0.010
Stress levels	3.7 ± 1.0	4.6 ± 0.5*†	0.9 (1.6 to 0.2), 1.01	4.0 ± 0.7	3.8 ± 0.8	-0.2 (0.4 to -0.8), 0.57	0.011
Mood	4.0 ± 0.8	4.7 ± 0.5*†	0.7 (1.3 to 0.1), 0.94	4.1 ± 0.6	4.2 ± 0.7	0.1 (0.6 to -0.4), 0.71	0.048
Fatigue	3.3 ± 0.7	4.3 ± 0.7	1.0 (1.6 to 0.4), 1.24	3.5 ± 0.7	3.3 ± 0.7	-0.2 (0.4 to -0.8), 1.22	0.190
Sleep quality	4.0 ± 0.7	4.0 ± 0.7	0.0 (0.6 to -0.6), 0.20	4.0 ± 0.8	4.0 ± 0.8	0.0 (0.7 to -0.7), 1.22	0.614
General wellbeing	18.6 ± 2.9	22.1 ± 1.6*†	3.5 (5.5 to 1.5), -1.13	19.5 ± 3.0	19.8 ± 2.7	0.3 (2.7 to -2.1), 0.11	0.035

Values are mean ± standard deviation. [1]CRP, C-reactive protein; REE, Resting Energy Expenditure.

*Different from 0 weeks to 10 weeks, $P < 0.05$; †Different from Control at that time, $P < 0.05$