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Title: Influence of traumatic lower-limb amputation on physical activity, body composition and cardiometabolic risks: A descriptive preliminary study

Short Running Title: Outcomes following traumatic amputation

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Influence of traumatic lower-limb amputation on physical activity, body composition and cardiometabolic risks: A descriptive preliminary study

ABSTRACT

Introduction: Following traumatic lower-limb amputation (LLA), humans are predisposed to numerous unfavourable changes in health, including the development of secondary chronic health conditions, including metabolic disorders and cardiovascular disease.

Objective: To determine within and between group differences in cardiometabolic component risks, body composition and physical activity (PA) in individuals with traumatic unilateral and bilateral LLA, compared to non-injured controls.

Design: Prospective observational cohort study.

Setting: A military complex trauma rehabilitation centre.

Participants: Sixteen males with traumatic LLA (8 unilateral, mean age 30±5yrs and 8 bilateral, mean age 29±3yrs). Thirteen active age-matched males with no LLA (28±5yrs) acted as controls and performed habitual activities of daily living.

Intervention: Participants with LLA attended two 4-week periods of in-patient rehabilitation, separated by two 6-week periods of home-based recovery.

Main Outcome Measures: Venous blood samples were taken prior to and following a 75g oral glucose load, for determination of biomarkers, including insulin and glucose, at baseline and 20-weeks. Body composition (dual X-ray absorptiometry) was measured at baseline, 10 and 20-weeks. Daily PA was recorded using a triaxial accelerometer for 7-days during in-patient rehabilitation and whilst at home. Energy expenditure was estimated using population-specific equations.

Results: Bilateral-LLA demonstrated more unfavourable mean body composition values, lower PA and increased cardiometabolic health risk compared to control. Cardiometabolic syndrome was identified in 63% of bilateral-LLA. No statistically significant differences in cardiometabolic component risk factors, body composition and estimated daily PA were reported between unilateral-LLA and control ($p>0.05$). Whilst at home, mean PA counts.day^{-1} reduced by 17% ($p=0.018$) and 42% ($p=0.001$) in the unilateral and bilateral-LLA groups, respectively.

Conclusions: Despite extensive in-patient rehabilitation, cardiometabolic component risks are elevated in bilateral-LLA, but are comparable between unilateral-LLA and active non-injured controls. Innovative strategies that improve/support the long-term PA and cardiometabolic health of severely injured bilateral-LLA are warranted.

Keywords: Amputation, Trauma Rehabilitation, Function, Physical Activity, Cardiometabolic Health

INTRODUCTION

Severe traumatic injuries, such as lower-limb amputation (LLA), can result in extensive long-term healthcare and rehabilitation needs. The restoration of physical function and maintenance of physical activity (PA) is considered a vital therapeutic component in the short-term rehabilitation and long-term recovery of individuals following LLA.[1] Current evidence suggests an increased prevalence of physical inactivity and reduced functional status in individuals with LLA.[2] Despite advanced prosthetic technology and rehabilitative care, current efforts have been unsuccessful in mitigating the effects of severe traumatic injury.

Following traumatic LLA, humans are predisposed to numerous unfavourable changes in health and well-being.[3] These include changes in body composition, characterised by lower limb skeletal muscle atrophy and the development of central and peripheral adiposity.[4] Such adaptations are commonly associated with, and thought to lead to, the development of secondary chronic health conditions, including cardiovascular disease and type 2 diabetes mellitus[5] Changes in clinically relevant systemic biomarkers of these conditions, including changes in blood lipid profiles, inflammatory cytokines, insulin and glucose concentrations/sensitivity, might provide insight into the early development and ensuing risk of chronic disease in this population.[5-7]

Unfortunately the majority of studies investigating cardiometabolic component risks following traumatic LLA are now over 20-years old,[8-11] which pre-dates many recent developments in prosthetic design/technology and multidisciplinary rehabilitation care. It therefore remains unclear if previously physically active and healthy individuals with LLA have compromised cardiometabolic health following intensive exercise-based rehabilitation. The aim of this longitudinal cohort study was therefore to determine whether: i) cardiometabolic component risks are elevated among the more severely injured bilateral LLA, compared to unilateral LLA and healthy age-matched non-injured controls; ii) body fat, particularly visceral adipose tissue (VAT), is greater among bilateral-LLA compared to unilateral-LLA and control and; iii) estimates of physical activity energy expenditure would be lower in LLA compared to control and; iv) physical activity energy expenditure is further compromised when individuals with LLA are at home compared to in-patient rehabilitation.

METHODS

Study Design

A descriptive 20-week longitudinal observational cohort study design was used to compare the effect of two 4-week in-patient admissions interspersed by two 6-week blocks of active recovery at home. Physical activity behaviour, body composition and cardiometabolic component risks in individuals with traumatic unilateral-LLA and bilateral-LLA, compared to an active control group without LLA were assessed. The trial protocol was approved by the UK Ministry of Defence Research Ethics Committee (Reference number: 512/MODREC/14) and conforms to Helsinki Declaration. All participants provided written informed consent. Figure 1 provides a schematic representation of the study design detailing the measurements taken over the 20-week observation period. The complex trauma rehabilitation care pathway at the UK Defence Medical Rehabilitation Centre (DMRC) has been described previously.[12] Whilst at home, patients were provided with an individualised exercise programme and encouraged to participate in regular activities of daily living (ADL), whilst wearing their prosthetic limb(s).[12]

[INSERT FIGURE 1 ABOUT HERE]

Study Participants

Participant characteristics are provided in Table 1. Participants who met the following eligibility criteria were included in the study; male, aged 18 to 50 years, with traumatic unilateral or bilateral-LLA, known to require a further three in-patient rehabilitation admissions at DMRC (enabling the monitoring of the final 20 weeks of the rehabilitation care pathway). Exclusion criteria included: known medical discharge/last admission date that did not allow for three in-patient admissions to DMRC, planned surgery during the data collection study period (potential risk of systemic inflammatory response and interrupted

progression of physical rehabilitation), severe traumatic brain injury, insufficient wound healing around the residuum (screened by physician) or unable to ambulate using a prosthesis (screened by physiotherapist). Ninety-seven percent of UK military service personnel admitted to DMRC with traumatic LLA are men.[12] At the time of recruitment, no women were attending the DMRC complex trauma rehabilitation care pathway with a LLA who had multiple in-patients admissions remaining. Retrospectively, no females were admitted to DMRC who met the eligibility criteria during the data collection period.

[INSERT TABLE 1 ABOUT HERE]

A convenient sample of age-matched, non-injured active males, employed within physically active roles in the UK Ministry of Defence (i.e. physiotherapists and exercise rehabilitation instructors), who engaged in aerobic or resistance-based training at least three times per week, were recruited to act as normative controls. Being employed in physically active roles, the control group are likely to closer resemble the PA status demonstrated pre-injury in both LLA groups (front line infantry roles), allowing us to consider the impact of traumatic limb loss in a previously physical active population. Due to their physically demanding job roles and regular engagement in structured physical exercise, the control group are not representative of the wider general population.

Outcomes Measures

Biomarkers of cardiometabolic component risk

Blood Sampling and Oral Glucose Tolerance Test

Given that skeletal muscle is an important glucose disposal site during postprandial conditions,[5] it is currently unclear what impact the effects of muscle atrophy and/or diminished lower-limb muscle strength following LLA may have on metabolic health. Blood sampling occurred on two separate occasions during the study; within 3-days of commencing in-patient rehabilitation (baseline admission) and at the 20-weeks follow-up (Figure 1). All participants reported to the laboratory in the morning following an overnight fast (≥10 hours). A cannula was inserted into the antecubital vein and a 25ml blood sample drawn. Within 5-minutes of the baseline blood sample being drawn, participants consumed 140g of a carbohydrate supplement equivalent to 75g of glucose. Blood samples were then obtained every 15 minutes for the first hour and every 30 minutes during the second hour. Following centrifugation of whole blood, plasma and serum samples were subsequently dispensed into 0.5ml aliquots using a pipette and immediately stored in a freezer at -80ºC.

Blood Analyses

All blood analyses were performed in duplicate using a batch analysis. Concentrations of total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, triacylglycerol, non-esterified fatty acids (NEFA), plasma glucose and C-reactive protein (CRP), were conducted on a Daytona analyser, according to manufacturer instructions, using commercially available assays. Enzyme-linked immunosorbent assays (ELISA) were used to measure serum insulin concentrations. Absorption was determined using a microplate reader at the wavelengths specified by the manufacturer.

Derived Indices

In order to simplify data analyses and facilitate the interpretation of a complex data,[13,14] serial measurements of glucose and insulin responses following oral glucose tolerance test, were

converted into simple summary statistics,[15] such as incremental area under the curve (iAUC)[16] and insulin sensitivity index (ISIMatsuda).[17] The Homeostasis Model Assessment (HOMA) calculator, incorporating the updated HOMA-2 model,[18] was also used to derive fasting estimates of pancreatic β-cell function, insulin resistance and sensitivity, both at rest and post exercise. Metabolic syndrome will be determined using established cut-point criteria from the International Diabetes Foundation.[19] These criteria (for men) include; increased 'abdominal' obesity (waist circumference ≥94 cm), hypertriglyceridemia (≥1.7 mmol/L), reduced HDL cholesterol (<1.03 mmol/L), hyperglycaemia (fasted plasma glucose ≥5.6 mmol/L), hypertension (blood pressure ≥130/85 mm Hg).

Body Composition

Body composition was determined using a dual-energy X-ray absorptiometry (DXA) and administered at 3 time points; baseline, 10-weeks and 20-weeks. DXA scans were performed within three days of arriving at DMRC for each respective in-patient admission and time matched for controls. Scans were analysed for total mass, fat mass, lean mass, percentage body fat, android/gynoid fat percentage and VAT area following the guidelines described in the user manual. The android region represents the proportion of fat around the abdomen, whilst the gynoid region represents the *gluteofemoral fat depot*. Android and gynoid fat distribution is commonly estimated via the anthropometric assessment of waist-to-hip ratio. Body height was measured and recorded to the nearest centimetre using a stadiometer. For individuals with bilateral-LLA, pre-injury body height was used. Waist and hip circumference were taken at baseline only. Waist measurements were taken at the midway point between the lowest rib and the top of the iliac crest, with hip circumference taken as the widest part of the buttocks. The mean of three measurements were used.

Estimating Daily Ambulatory Physical Activity Parameters

Participants wore an Actigraph GT3X+ triaxial accelerometer on the hip of their shortest residual limb (right hip for controls) using an elasticated belt, which has previously been validated as an accurate method for estimating ambulatory physical activity energy expenditure with population specific LLA equations.[20] Individuals with LLA wore the device for 7 continuous days during in-patient rehabilitation at DMRC and 7 continuous days during active recovery at home. Control participants wore their device for 7 continuous days during a normal week of employment. All participants were told to remove the monitor before participating in any water-based activity (i.e., hydrotherapy, showering) and during sleeping hours. After 7 days the participants returned the device and the data were downloaded onto the ActiLife version 6 software, for subsequent analyses. Wear time validation analysis, an integral part of the ActiLife software, was performed using the Troiano equation prior to converting to an excel file for analysis.[21] The ActiLife software defined non-wear time by an interval of at least 60 consecutive minutes whereby vector magnitude values remained constantly at zero. Based on a typical 8-hours sleep pattern and making allowances for water-based activities, >14 hours (87.5% of potentially available data) from a 16-hour waking day was considered an appropriate cut-point for a valid day. Vector magnitude data from the Actigraph was plotted against the corresponding 24-hr timestamp and visually inspected. Non-wear time vector magnitude data were excluded from the daily averages. An arbitrary unit called a 'physical activity count' (PAC), calculated through summing the change in raw acceleration values measured during a specific interval of time or 'epoch' will be used as a surrogate marker of PA levels per day.

Functional Outcomes

In order to provide an insight into the physical functional status of the LLA participants, a variety of functional outcomes were captured at baseline. These variables were: 6-minute

walk distance,[22] amputee mobility predictor (AMP)[23] and a DMRC specific question relating to mobility.[24]

Statistical Analysis

Mixed model analysis of variance (ANOVA) tests with Bonferroni *post-hoc* analysis were performed to assess differences between the 3 groups (unilateral-LLA, bilateral-LLA, control) at baseline and over time for cardiometabolic component risk biomarkers and body composition outcomes. In order to simplify data analysis and facilitate the interpretation of complex data set, serial measurements of glucose and insulin responses to the oral glucose tolerance test at baseline and follow-up were converted into simple summary statistics [i.e., fasting and peak concentrations, time to peak, iAUC and estimates of insulin resistance and sensitivity [15]]. ANOVA were performed irrespective of any minor deviations from a normal distribution, but with Greenhouse-Geisser corrections applied to intra-individual contrasts where $\varepsilon < 0.75$ and the Huynh-Feldt corrections applied for less severe asphericity.[25] Where significant interactions were observed, one-way ANOVAs (for 3 group or time-point comparisons) with Bonferroni *post-hoc* analysis or multiple t-tests were applied to determine the location of variance both between time points within each group relative to baseline.

Physical activity counts and physical activity energy expenditure responses within and between amputation groups were determined by a two-way (group [unilateral, bilateral] x environment [Rehabilitation, Home]) mixed-model ANOVA. Where significant interactions were observed between the two amputation groups, multiple t-tests were applied to determine differences between groups and/or environments. As the control group wore the device in one environment only (during employment) a one-way ANOVA was used to determine the differences in PA between the three groups (i.e., both amputation groups during a week of

rehabilitation vs. control participants at work; and both amputation groups during a week at home versus controls at work). All data are presented in text and tables as mean ± SD, whereas error bars on figures represent standard error of the mean (SEM). Mean and the lower and upper 95% CI of the change (Δ) were calculated for all biomarkers of cardiometabolic health and PA measures between environments. Statistical significance was set at *a priori* of $\alpha \leq 0.05$. All analyses were performed using IBM® SPSS® Statistics 22 for Windows. Standardised effect sizes (Cohens *d*) were also calculated to facilitate the interpretation of the substantive significance within groups, with thresholds of >0.2 (small), >0.5 (moderate) and >0.8 (large) used[26].

RESULTS

Participant Characteristics

Table 1 describes the injury characteristics of the LLA groups, highlighting differences in injury severity. All participants with a through and/or above knee LLA, in both groups, wore a Genium prosthetic device. At baseline, individuals with bilateral-LLA had received a significantly greater total length of rehabilitation (39±15 versus 14±8 months, d=2.08, $p<0.05$) and number of admissions (15±15 versus 6±3, d=0.83, $p<0.001$) compared to unilateral-LLA, respectively.

Cardiometabolic component risk biomarkers

Lipid Profile, Inflammation and Blood Pressure

Individuals with bilateral-LLA demonstrated significantly greater TC:HDL ratio (d=1.88, $p<0.001$), triacylglycerol (d=1.49, p=0.001), CRP (d=1.33, p=0.002), systolic blood pressure (BP) ($p<0.001$) and diastolic BP (d=3.26, p=0.002) compared to control (Table 2). Bilateral-

LLA also demonstrated significant greater TC:HDL ratio (d=1.96, p=0.002), triacylglycerol (d=1.49, p=0.003), NEFA (d=1.18, p=0.030) and CRP (d=1.35, p=0.004), systolic BP (d=1.70, p=0.001) compared to unilateral-LLA (Table 2). No statistically significant differences in lipid profile or inflammation were reported between unilateral-LLA and active normative control (p>0.05). While systolic BP (d=1.57, p=0.020) was significantly greater in the unilateral-LLA group, values were within the normal range. There were no significant interaction effects or main effects of time for any lipid profile or inflammatory biomarker (p>0.05).

[INSERT TABLE 2 ABOUT HERE]

Fasted blood Insulin and Glucose levels and Oral Glucose Tolerance Test

No significant main effect of group was demonstrated for fasted serum insulin concentrations at baseline (p>0.05). A significantly greater insulinemic response following a 75g glucose load was demonstrated in bilateral-LLA compared to controls at 45 minutes (d=1.06, p=0.024), 60 minutes (d=1.37, p=0.017), 90 minutes (d=1.98, p<0.001) 120 minutes (d=1.50, p=0.001), with greater peak insulin values (d=1.13, p=0.015), and iAUC (d=1.61, p=0.002) (Figure 2). Bilateral-LLA demonstrated significantly greater iAUC (d=1.43, p=0.017) compared to individuals with unilateral-LLA. No statistically significant differences in insulinemic response to the oral glucose tolerance test were demonstrated between unilateral-LLA and control (d<0.05 and p>0.05). There were no interaction effect on serum insulin responses during the oral glucose tolerance test (p>0.05).

No significant main effect of group was demonstrated at baseline for fasting concentrations of plasma glucose (p>0.05) (Table 2). Following a 75g oral glucose tolerance test challenge,

significantly greater plasma glucose concentrations were demonstrated in bilateral-LLA at 60-minutes post-glucose ingestion (d=1.74, p=0.010) and greater mean time to peak glucose (d=1.56, p=0.016) when compared to controls (Figure 2). No statistically significant differences in glycaemic response to the oral glucose tolerance test were observed between unilateral-LLA and control (d<0.05 and p>0.05). No interaction effects were apparent for plasma glucose response to the orl glucose tolerance test after 20 weeks (p>0.05).

Cardiometabolic Syndrome

Cardiometabolic syndrome was identified in 63% of bilateral-LLA. Cardiometabolic syndrome was not demonstrated in any individuals with unilateral-LLA or the normative control group.

Indices of Insulin Sensitivity/Resistance

Bilateral-LLA demonstrated significantly greater fasted estimates of pancreatic β-cell function (HOMA2-β, d=1.29, p=0.030), and significantly less insulin sensitivity (ISI-Matsuda, d=1.68, p=0.005) compared to control (Figure 2). No significant differences were observed between unilateral-LLA and control (p>0.05). No significant interaction effects were demonstrated for any indices of insulin sensitivity or resistance (p>0.05) (Figure 2).

[INSERT FIGURE 2 ABOUT HERE]

Body Composition

Waist circumference and waist to hip ratio values for each group were as follows; unilateral-LLA, 84±6 cm and 0.87±0.04; bilateral-LLA, 101±22cm and 0.93±0.10; control, 83±5 cm and 0.83±0.03, respectively. At baseline, bilateral-LLA demonstrated significant greater fat

mass (d=1.27, p=0.005), percent body fat (d=1.94, p<0.001), android fat percent (d=1.82, p<0.001), gynoid fat percent (d=1.92, p<0.001), VAT area (2.11, p<0.001), waist circumference (d=1.13, p=0.010) and waist:hip ratio (d=1.35, p=0.001) and lower lean mass (d=1.18, p=0.026) compared to control. Bilateral-LLA also demonstrated significantly larger waist circumference (d=1.05, p=0.014) and VAT area (d=1.67, p=0.046) compared to unilateral-LLA. There were no statistically significant differences reported between unilateral-LLA and control on any measure of body composition at baseline (P>0.05). Despite varying levels (transtibial and transfemoral) and numbers of amputation(s) there were no significant differences in total body mass between groups (Figure 3A). However, Figure 3B and 3C clearly demonstrate a different regional distribution of tissues (fat and muscle) between the three groups. There was a significant interaction effect (F=2.949, p=0.029) for android:gynoid ratio. This was caused by a significant difference in android fat percentage change over time between unilateral-LLA and control (p=0.015), where android fat percentage reduced over time in the unilateral-LLA group.

[INSERT FIGURE 3 ABOUT HERE]

Physical Activity

There were no differences in the number of valid days (>14hours) recorded or mean wear time between groups or environments (i.e. rehabilitation versus home) (p>0.05). Unilaterl-LLA recorded 5±1 valid days in each environment with mean daily wear times of 918±41 minutes during rehabilitation and 916±55 minutes whilst at home. The bilateral-LLA recorded 6±1 valid days in each environment with mean daily wear times of 918±45 minutes during rehabilitation and 904±42 minutes whilst at home. The control group recorded 5±1 valid days with mean daily wear times of 934±40 minutes during work.

During in-patient rehabilitation, *Post-hoc* analyses revealed significantly lower PAC·day^{-1} (d=1.44, p=0.009) and estimated daily physical activity energy expenditure (d=4.50, p<0.001) in bilateral-LLA when compared to control (Figure 4). Bilateral-LLA also demonstrated significantly lower estimated daily physical activity energy expenditure compared to unilateral-LLA (d=5.4, p<0.001), but no significant difference in mean PAC.day^{-1} (p=0.142). No statistically significant differences in mean PAC.day^{-1} and estimated daily physical activity energy expenditure were demonstrated between unilateral-LLA and control (p>0.05).

[INSERT FIGURE 4 ABOUT HERE]

During time spent at home, *Post-hoc* analyses revealed significantly reduced PAC.day^{-1} (d=2.75, p<0.001) and estimated daily physical activity energy expenditure (d=5.85, p<0.001) in bilateral-LLA compared to control (at work) (Figure 4). Unilateral-LLA also demonstrated significantly reduced PAC.day^{-1} (d=1.13, p=0.049) and estimated daily physical activity energy expenditure (d=1.71, p=0.002) compared to control. Bilateral-LLA recorded significantly reduced PAC.day^{-1} (d=2.76, p=0.008) and estimated daily physical activity energy expenditure (d=6.00, p<0.001) compared to unilateral-LLA.

There was a reduction in PAC.day^{-1} and mean daily physical activity energy expenditure (kcal.d^{-1}) during habitual free-living at home in both LLA groups. However, the difference demonstrated between these two environments was greatest in bilateral-LLA. Unilateral-LLA reduced their daily PAC whilst at home by 17% (*d*=1.26, p=0.018) and their physical activity energy expenditure by 13% (*d*=1.21, p=0.019). Bilateral-LLA reduced their mean PAC.day^{-1}

by 42% (*d*=2.53, p=0.001) and their daily physical activity energy expenditure by 47% (*d*=2.51, p=0.001). There were no significant interaction effects demonstrated between any PA measurements.

DISCUSSION

This study investigated the influence of LLA severity (unilateral versus bilateral-LLA) on cardiometabolic component risk, PA and body composition in UK military personnel at the end of their complex trauma rehabilitation care pathway. Individuals with bilateral-LLA demonstrated lower ambulatory PA and elevated cardiometabolic component risks compared to control, including higher waist circumference, VAT area, percentage body fat, TC:HDL cholesterol, triacylglycerol concentrations, systemic inflammation (CRP), systolic BP, insulin iAUC, fasting estimates of β-cell function; and reduced lean muscle mass and insulin sensitivity. The bilateral-LLA group also had lower ambulatory PA and demonstrated elevated cardiometabolic component risks when compared to unilateral-LLA, including greater waist circumference, VAT, TC:HDL ratio, triacylglycerol concentration, NEFA, CRP and insulin iAUC. These are particularly compelling findings considering the relatively short duration (39±15 months) since their injuries occurred. Individuals with unilateral-LLA demonstrated low effect size ($d<0.05$) and no statistically significant differences in any cardiometabolic risk profile to healthy active age-matched control, which may reflect a positive adaptation to physical rehabilitation. To our knowledge this is the first study to demonstrate comparable health outcomes between individuals with unilateral-LLA and physically active healthy adults.

Mechanisms likely to be contributing towards the elevated cardiometabolic component risks in bilateral-LLA could be the less favourable body composition values and lower free-living

PA, particularly whilst at home. It is well documented that skeletal muscle atrophy, diminished muscle strength and increased visceral fat mass commonly occur following LLA.[4,27,28] Despite losing healthy tissue from both lower-limbs, the total body mass of bilateral-LLA recorded at baseline is comparable to the control group, however their body fat content was >2-fold greater (24 kg versus 11 kg, respectively). Of concern, the location of this higher body fat content appears to be central (waist circumference: 101±22 cm, android fat percentage: 30 ± 11%, and VAT area 117 ± 47cm^2), which is a known risk factor for future cardiovascular and metabolic related disorders[29] and prosthetic mobility.[30,31] The majority of participants with bilateral-LLA were transfemoral (above knee) amputees (Table 1), the level of amputation and length of the residual limb are significant factors in the severity of muscle atrophy,[28] but also in the loss of functional performance.[24] While it is not obvious from the results of this study how much muscle mass each participant lost following their bilateral injury and subsequent surgery, using control as a reference, we estimate that the bilateral-LLA lost a mean 9 kg (or 14%) of metabolically active tissue (66kg versus 57kg, respectively). Skeletal muscle is a critical glucose and fat disposal site during postprandial conditions.[5,32] Therefore the reduced amount of whole body lean muscle tissue evident among bilateral-LLA may have considerable implications for their short to long-term metabolic health. Significant muscle atrophy and diminished muscle strength following amputation will not only reduce resting metabolic rate, but also reduce physical function, engagement in ADL and overall physical activity energy expenditure,[33] as was demonstrated in this study.

The elevated cardiometabolic component risks evident in the bilateral-LLA could be a consequence of their increased injury severity (see list of secondary injuries in Table 1). Stewart *et al.* (2015)[34] predicted each 5-point increment in injury severity score was

associated with a 6%, 13% and 13% increase in incidence rates of hypertension, diabetes mellitus and coronary artery disease, respectively. The estimated incidence rates of hypertension, diabetes mellitus and coronary artery diseases for the most severely injured patients (injury severity score [ISS] > 25) were 2.5 to 4-fold higher than published rates for the overall US military population, respectively.[35,36] Similar to our own study, these projected outcomes were observed in as little as 1 to 3 years following injury.

We found a reduction in PAC.day^{-1} of 42% (d=2.53) and physical activity energy expenditure by 47% (d=2.51) when bilateral-LLA were at home compared to in-patient rehabilitation. This discrepancy was less pronounced in unilateral-LLA with PAC.day^{-1} and physical activity energy expenditure reductions of 17% (d = 1.26) and 13% (d = 1.21), respectively. Therefore, confirming recovery environment (i.e. rehabilitation versus home) and injury severity (number of amputations) both affect daily PAC and physical activity energy expenditure in individuals with LLA. Another recent study in UK military LLA supports these observations, with mean daily step count significantly reduced (39%) from 2258±192 steps.d^{-1} during in-patient rehabilitation to 1387±363 steps.d^{-}1 while at home.[37] One reason the authors provided for this discrepancy between environments is there may be more occasions at home when it's considered more appropriate for an individual with a LLA to use a wheelchair to mobilise as oppose to ambulating in their prosthesis.[37]

Access to advanced prosthetic technology in combination with prolonged interdisciplinary rehabilitation is likely to have supported the positive outcomes demonstrated by unilateral-LLA. By maintaining higher levels of PA and associated energy expenditure, may have helped to elicit their favourable body composition values. As cardiometabolic health is, in part, regulated by lean muscle and adipose tissue, and these body composition values

remained unchanged in all three groups throughout the 20 weeks observation, it is not surprising that no significant time related changes in components of cardiometabolic risk occurred during this study.

Limitations

An unavoidable limitation of the study design was the inability to standardise the length of time since injury, the amount of rehabilitation exposure prior to participating in the trial and the homogeneity within the LLA groups (i.e,, below versus above knee amputation). This was due to the availability of eligible participants at the time of recruitment. However, by recruiting participants with 3 admissions remaining prior to their discharge from in-patient care, we were able to comment on the health and well-being of severely injured military personnel with LLA at the end of their rehabilitation care pathway. In the context of measuring the impact traumatic LLA on objective markers of cardiometabolic risk, the current study is one of the largest, although the sample size of individuals with LLA remains relatively small (n=16). Despite the small sample size and the large variance in lower-limb injury severity within groups, significant differences were still demonstrated between groups. This heterogeneity of injury may be considered beneficial as the range of functional abilities improves the external validity of the findings, making them more relevant to the wider LLA population. Due to the multiple outcome measures and analysis performed, there is an elevated risk of type 1 error. However, this was an exploratory/descriptive study that aimed to facilitate better understanding of health determinants of LLA at the end of the complex trauma care pathway and is balanced by the comprehensive assessment of cardiometabolic component risk completed with every participant. Positioning the accelerometer at the hip captured changes in ambulatory-based PA (a primary goal of rehabilitation). However, physical activity energy expenditure values may have been underestimated using this

anatomical location in the bilateral-LLA group as the device would be unable to accurately capture physical activity energy expenditure during time spent performing wheelchair propulsion or upper-limb resistance exercise. Military personnel are predominantly male, aged 20 to 40 years old and have undergone extensive physical training in the course of their career. As a higher functioning LLA group, with access to advance rehabilitation and prosthetic provision, these finding may not be immediately applicable to the wider LLA population.

Clinical Implications

These findings highlight the importance of long-term monitoring of the most severely injured military personnel, especially bilateral-LLA. Despite access to advance prosthetic provision and prolonged rehabilitative care (15±15 in-patient admissions over 39±15 months), bilateral-LLA demonstrated unfavourable body composition and elevated biomarkers of cardiometabolic risk compared to unilateral-LLA and control. Large differences occurred in PA levels between in-patient rehabilitation and home. These data emphasise the importance of maintaining an active lifestyle in the home environment. To facilitate this, a home-based exercise/nutritional intervention is perhaps the most likely approach to ensure a longer term maintenance or improvement in cardiometabolic component risks, body composition and physical function. The results presented in this study reflect the health and well-being status of UK military personnel at the end of the complex trauma rehabilitation pathway. The longer term impacts of transitioning away from a structured defence rehabilitation care pathway to independent care remain unknown and warrant further investigation.[38]

CONCLUSION

Despite extensive in-patient rehabilitation, cardiometabolic component risk biomarkers are elevated in traumatic bilateral-LLA, but considered 'healthy/normal' for unilateral-LLA and active healthy control. This increased risk among bilateral-LLA was characterised by higher total body fat content, visceral and gynoid fat tissue, impaired systemic lipid profile, systemic inflammation and impaired post-load insulin sensitivity. Interestingly, this unfavourable cardiometabolic risk profile was accompanied with lower PA and associated energy expenditure, which was even further reduced when recovering at home compared to the in-patient rehabilitation environment. Strategies that improve/support the long-term health and well-being of severely injured bilateral-LLA are necessary, with a particular focus on long-term healthy and active aging.

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Table 1: Baseline participant characteristics: injury and functional status

	Unilateral LLA	Bilateral LLA	Normative Controls
Number	8	8	13
Age (years)	30 ± 5	29 ± 3	28 ± 5
Injury Characteristics			
Time Since Amputation			
<1 year	3 (37.5)	-	-
1 to 2 years	4 (50)	2 (25)	-
2 to 3 years	1 (12.5)	2 (25)	-
3 to 4 years	-	2 (25)	-
>4 years	-	2 (25)	-
Level of Amputation			
Below Knee	6 (75)	-	-
Through Knee	1 (12.5)	-	-
Above Knee	1(12.5)	-	-
Bilateral - Below knee	-	1 (12.5)	-
Bilateral - Below/Above Knee	-	1 (12.5)	-
Bilateral - Through Knee	-	2 (25)	-
Bilateral - Above Knee	-	4 (50)	-
Secondary Injuries			
Fractures	3 (37.5)	8 (100)	-
Nerve damage	1 (12.5)	2 (25)	-
Soft tissue or vascular trauma	6 (75)	8 (100)	-
Location of Secondary Injuries			
Head/neck/face	1 (12.5)	5 (62.5)	-
Chest / upper back	1 (12.5)	5 (62.5)	-

Upper Limbs	1 (12.5)	8 (100)	-
Spine	-	3 (37.5)	-
Abdomen	1 (12.5)	4 (50)	-
Pelvis	2 (25)	6 (75)	-
Lower-Limbs	7 (87.5)	8 (100)	-
Functional Outcomes			
Physical Function			
6 MWD (metres) *	574 ± 66	337 ± 85	705 ± 32
AMP †	46 ± 1	40 ± 4	-
DMRC - Mobility			
Able to run independently	1 (12.5%)	0 (0%)	13 (100%)
Able to walk independently	8 (100%)	5 (62.5%)	13 (100%)
Requires a walking aid / adaptation	0 (0%)	3 (37.5%)	0 (0%)

Data are presented as mean ± SD for continuous variables or as number of participants (%) for categorical variables.

Abbreviations: 6-MWD = six-minute walk distance, AMP = amputee mobility predictor, DMRC = Defence Medical Rehabilitation Centre.

* Significant differences between individuals with unilateral and bilateral amputation, unilateral amputation and normative controls, and bilateral amputation and normative controls ($p < 0.001$).

† Significant difference between individuals with unilateral and bilateral amputation ($p < 0.05$).

Table 2: Cardiometabolic component risk markers for unilateral and bilateral lower-limb amputees (LLA) and normative controls at baseline and change score after 20 weeks.

Marker	Unilateral-LLA (n = 8)		Bilateral-LLA (n = 8)		Control (n = 13)	
	Baseline	**Δ (95% CI)**	**Baseline**	**Δ (95% CI)**	**Baseline**	**Δ (95% CI)**
Total Cholesterol, mmol·L^{-1}	3.1 ± 1.1	-0.1 (-0.8 to 0.6)	3.6 ± 0.4	**-0.7 (-1.3 to -0.1) ***	3.2 ± 0.9	-0.2 (-0.5 to 0.2)
HDL Cholesterol, mmol·L^{-1}	0.9 ± 0.3	-0.0 (-0.3 to 0.2)	0.8 ± 0.1	-0.1 (-0.3 to 0.0)	1.1 ± 0.4	-0.1 (-0.2 to 0.1)
LDL Cholesterol, mmol·L^{-1} [a]	1.9 ± 1.0	-0.1 (-0.6 to 0.4)	2.6 ± 0.4	**-0.6 (-1.0 to -0.2) ***	2.0 ± 0.6	-0.2 (0.4 to 0.1)
TC:HDL ratio	**3.3 ± 0.6** §	0.1 (-0.1 to 0.4)	**4.8 ± 0.9** ‡§	-0.1 (-0.4 to 0.2)	**3.2 ± 0.8** ‡	0.0 (-0.3 to 0.3)
Triacylglycerol, mmol·L^{-1}	**0.7 ± 0.3** §	0.2 (0.0 to 0.3)	**1.8 ± 1.0** ‡§	-0.1 (-0.8 to 0.5)	**0.7 ± 0.3** ‡	0.2 (0.0 to 0.4)
NEFA, mmol·L^{-1}	**0.3 ± 0.2** §	0.1 (-0.1 to 0.3)	**0.6 ± 0.3** §	-0.1 (-0.4 to 0.1)	0.4 ± 0.1	0.1 (-0.2 to 0.1)
C-Reactive Protein, mg.L^{-1}	**0.40 ± 0.28** §	0.3 (-0.2 to 0.7)	**2.85 ± 2.56** ‡§	-0.4 (-2.8 to 2.1)	**0.40 ± 0.48** ‡	0.2 (-0.4 to 0.8)
Systolic BP (mm Hg)	**125 ± 6** §	**-7 (-11 to -3)** †	**137 ± 8** §	-4 (-8 to 0)	118 ± 2	-1 (-2 to 0)
Diastolic BP (mm Hg)	69 ± 10	-3 (-11 to 6)	78 ± 9	-2 (-8 to 5)	63 ± 7	1 (-2 to -2)
Fasted Glucose, mmol·L^{-1}	5.7 ± 0.4	-0.1 (-0.4 to 0.2)	5.5 ± 0.6	0.1 (-0.2 to 0.5)	5.5 ± 0.4	0.0 (-0.2 to 0.3)
Peak Glucose, mmol·L^{-1}	9.9 ± 1.5	-0.6 (-1.8 to 0.6)	10.1 ± 1.5	-0.1 (-2 to 1.9)	9.4 ± 1.4	0.1 (-1.0 to 1.2)
Time to peak Glucose (mins)	35.6 ± 11.2	1.9 (-12.2 to 16.0)	**45.0 ± 8.1** ‡	-9.4 (-22.7 to 4.0)	**33.5 ± 6.6** ‡	0.0 (-7.4 to 7.4)
Fasted Insulin, pmol·L^{-1}	30.5 ± 25.4	2.1 (-4.4 to 8.7)	49.2 ± 36.3	-7.5 (-30.9 to 15.9)	22.4 ± 9.4	0.7 (-6.1 to 7.5)
Peak Insulin, pmol·L^{-1}	282.2 ± 99.3	-27.7 (-84.3 to 29.0)	**482.5 ± 254.2** ‡	-38.3 (-133.6 to 56.9)	**258.7 ± 116.1**‡	3.6 (-49.4 to 56.5)
Time to Peak Insulin (minutes)	43.1 ± 12.5	0.0 (-11.6 to 11.6)	48.8 ±13.3	-7.5 (-25.2 to 10.2)	39.2 ± 9.8	-2.3 (-8.6 to 3.9)

Data presented as mean ± SD and mean change Δ (95% lower and upper CI).

Abbreviations: HDL = high-density lipoprotein, LDL = low density lipoprotein, TC:HDL total cholesterol : high-density lipoprotein, NEFA = nonesterified fatty acid, BP = blood pressure, AUC = area under the curve, HOMA2-IR = homeostasis model assessment for insulin resistance, HOMA2-β homeostasis model assessment for β-cell function, ISI = Insulin Sensitivity Index.

* Significant difference between baseline and follow-up in individuals with bilateral LLA ($p < 0.05$)

† Significant difference between baseline and follow-up in individuals with unilateral LLA ($p < 0.05$)

‡ Significant difference between baseline bilateral LLA and normative controls ($p < 0.05$)

§ Significant difference between baseline bilateral LLA and unilateral LLA ($p < 0.05$)

[a] Calculated with the use of the Friedwald equation [LDL cholesterol = total cholesterol - HDL cholesterol - (triacylglycerol/2.2)].

Figure Legends

Figure 1: Schematic description of the longitudinal observational cohort study design

Figure 2: Summary of indices of metabolic health derived using plasma glucose and serum insulin during an oral glucose tolerance test. Mean glucose area under curve (AUC) values **(A)**, mean insulin AUC values **(B)**, mean values of insulin resistance **(C)**, mean values indicating pancreatic β-cell function **(D)**, mean insulin sensitivity **(E)**, mean Matsuda insulin sensitivity Index **(F)**. Circles represent values recorded at baseline. Squares represent values recorded at 20 week follow-up. White shapes represent unilateral amputees, black shapes represent bilateral amputees and grey shapes represent normative controls.

* Significant difference between unilateral amputation and bilateral amputation ($p<0.05$).

† Significant difference between bilateral amputation and control ($p<0.05$)

Figure 3: Body composition at baseline, 10 weeks and 20 weeks follow-up in unilateral (n = 8) and bilateral (n = 8) lower-limb amputees and normative controls (n = 13). Body mass (kg) (**A**), lean muscle mass (kg) (**B**), fat mass (kg) (**C**), percentage body fat (**D**), percentage android fat (**E**), percentage gynoid fat (**F**), android: gynoid ratio (**G**), visceral adipose tissue (VAT) area (cm^2) (**H**). Black dots represent bilateral amputees, white dots represent unilateral amputees, grey dots represent normative controls. Data are presented as mean and standard error.

* Significant difference between individuals with bilateral amputation and controls ($p<0.05$).

†Significant difference between individuals with bilateral amputation and unilateral amputation ($p<0.05$).

Figure 4: Estimated daily physical activity (PA) levels of individuals with unilateral and bilateral lower-limb amputation (LLA) during in-patient rehabilitation and whilst at home and uninjured normative controls during work. Daily Physical Activity Counts (PAC) (**A**); estimated physical activity energy expenditure (PAEE) (**B**). Circles represent values during rehabilitation. Squares represent values at home. White circles/squares represent values of PA within unilateral LLA. Black circles/squares represent values of PA within bilateral LLA. Grey triangles represent values of PA for normative controls during a working week. Individual data points reflect mean scores for each participant. Mean ± SD data for daily PAC are as follows; unilateral LLA 645084 ± 86078 during rehabilitation and 534248 ± 90125 while at home; bilateral LLA, 492569 ± 72750 during rehabilitation and 283357 ± 91406 while at home; control, 707632 ± 197909 during work. Mean ± SD data for daily physical activity energy expenditure are as follows; unilateral LLA, 839 ± 88 kcal during rehabilitation and 733 ± 87 kcal while at home; bilateral LLA, 410 ± 68 kcal during rehabilitation and 217 ± 85 kcal while at home; control, 948 ± 155 during a working week. The cross-validated population specific prediction models developed for estimating physical activity energy expenditure (22) in Figure 4B are:

[a] Unilateral LLA physical activity energy expenditure = $(0.000979 \times PAC \cdot min^{-1}) + 0.225548$

[b] Bilateral LLA physical activity energy expenditure = $(0.000929 \times PAC \cdot min^{-1}) - 0.051541$

[c] Normative control physical activity energy expenditure = $(0.000776 \times PAC \cdot min^{-1}) + 0.427097$

* Significant difference between individuals with unilateral amputation and bilateral amputation during rehabilitation ($p < 0.05$)

† Significant difference between individuals with unilateral amputation and bilateral amputation whilst at home ($p < 0.05$)

‡ Significant difference between individuals with bilateral amputation during rehabilitation and normative controls ($p < 0.05$)

§ Significant difference between individuals with bilateral amputation at home and normative controls ($p < 0.05$)

¶ Significant difference between individuals with unilateral amputation at home and normative controls ($p < 0.05$)