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Immobilisation induces sizeable and sustained reductions in forearm glucose uptake in just 24 h but does not change lipid uptake in healthy men

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Key points

- The trajectory, magnitude and localisation of metabolic perturbations caused by immobilisation (IMM) are unresolved.
- Forearm glucose uptake (FGU) in response to glucose feeding was determined in healthy men before and during 72 h of forearm IMM, and the same measurements were made in the non-IMM contralateral limb at baseline and 72 h. In a similar study design, FGU and forearm lipid uptake were determined after a high fat mixed-meal (HFMM) in IMM and non-IMM limbs.
- FGU was reduced by 38%, 57% and 46% following 24, 48 and 72 h IMM, respectively, but was unchanged in the non-IMM limb. A similar FGU response to IMM was observed after a HFMM, and forearm lipid uptake was unchanged.
- A sizeable reduction in FGU occurs in just 24 h of IMM, which is sustained thereafter and specific to the IMM limb, making unloading *per se* the likely rapid driver of dysregulation.

Abstract The trajectory and magnitude of metabolic perturbations caused by muscle disuse are unknown yet central to understanding the mechanistic basis of immobilisation-associated metabolic dysregulation. To address this gap, forearm glucose uptake (FGU) was determined in 10 healthy men (age 24.9 ± 0.6 years, weight 71.9 ± 2.6 kg, BMI 22.6 ± 0.6 kg/m^2) during a 180 min oral glucose challenge before (0) and after 24, 48 and 72 h of arm immobilisation, and before and after 72 h in the contralateral non-immobilised arm (Study A). FGU was decreased from baseline at 24 h (38%, $P = 0.04$), 48 h (57%, $P = 0.01$) and 72 h (46%, $P = 0.06$) of immobilisation, and was also 63% less than the non-immobilised limb at 72 h ($P = 0.002$). In a second study, FGU and forearm lipid uptake were determined in nine healthy men (age 22.4 ± 1.3 years, weight 71.4 ± 2.8 kg, BMI 22.6 ± 0.8 kg/m^2) during a 420 min mixed-meal challenge before (0) and after 24 and 48 h of arm immobilisation and before and after 72 h in the contralateral non-immobilised arm (Study B). FGU responses were similar to Study A, and forearm lipid uptake was unchanged from pre-immobilisation in both arms over the study. A sizeable decrement in FGU in response to glucose feeding occurred within 24 h of immobilisation that was sustained and specific to the immobilised limb. Increasing lipid availability had no additional impact on the rate or magnitude of these responses or on lipid

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uptake. These findings highlight a lack of muscle contraction *per se* as a fast-acting physiological insult to FGU.

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Introduction

The general public is largely unaware of the insidious health risks associated with not moving (WHO, 2009). Epidemiological evidence reveals a number of factors associated with increased risk of poor metabolic health and functional decline, and physical inactivity appears to be the dominant risk factor (Matthews *et al.* 2012; de Rezende *et al.* 2014), being linked to increased risk of all-cause mortality (Katzmarzyk *et al.* 2009), cause-specific mortality (Katzmarzyk *et al.* 2009; Wilmot *et al.* 2012), cardiovascular disease (Stamatakis *et al.* 2011) and poor metabolic health (Hu *et al.* 2003; Ford *et al.* 2010). A large scale (3720 men and 1412 women) 16-year follow-up study, in which a total of 450 deaths were recorded, pointed to physical inactivity *per se* as the central driver of mortality risk (Pulsford *et al.* 2015). It is of genuine concern therefore that physical inactivity and sedentary behaviours are commonplace world-wide, which will have become exacerbated during the current coronavirus (SARS-CoV-2) pandemic. That being said, the mechanistic understanding of the metabolic and physiological impacts of inactivity is incomplete, not least insight from human volunteer research involving longitudinal study designs and active/inactive limb level measurements.

From a mechanistic standpoint physical inactivity has been reported to reduce insulin-stimulated whole-body glucose disposal, which is most dominant at a peripheral muscle level (Mikines *et al.* 1991), and to promote impairments in lipid trafficking and increase intramuscular lipid content (Dolkas & Greenleaf, 1977; Stuart *et al.* 1988; Smorawinski *et al.* 2000; Bergouignan *et al.* 2006, 2011; Cree *et al.* 2010). However, little is known about the temporal relationship or mechanisms linking physical inactivity with these metabolic perturbations, with the vast majority of research focusing on two pre- to post-inactivity time points. For example, Biensø *et al.* (2012) reported lower skeletal muscle GLUT 4 and hexokinase II protein content, in addition to reduced ability of insulin to activate glycogen synthase and phosphorylate Akt under euglycaemic insulin clamp conditions after 7 days of bed rest, pointing to immobilisation induced reductions in leg glucose uptake arising because of lower content/activity of key proteins regulating glucose transport, phosphorylation and storage. Nevertheless, instead of being causative in the reduction in limb glucose disposal observed during immobilisation, these muscle level changes could at least to some extent reflect an adaptive response to reduced muscle glucose uptake, utilisation and storage *per se* during immobilisation, which is unknown. This latter response would require immobilisation-induced deficits in limb blood glucose uptake to be rapid and precede muscle level adaptation. However, detailed information on the time frame and magnitude of change in limb glucose uptake during immobilisation, particularly in the context of a non-immobilised control limb, is missing. What is known is that substantial reductions in peripheral glucose disposal are evident after 2 days of forearm immobilisation (Dirks *et al.* 2019) and 3 days of bed rest (Lipman *et al.* 1970) in young, healthy volunteers, which is not evident at a whole-body level after 1 day of bed rest (Dirks *et al.* 2018).

Inactivity is also reported to negatively impact on whole-body lipid uptake. Kim *et al.* (2016) demonstrated the incremental area under the curve for serum triglycerides after a high dietary fat tolerance test was 26% greater following 2 days of prolonged sitting (14.3 h/day) compared to interrupted sitting or active walking in healthy, lean subjects. These findings suggest that periods of reduced physical activity quickly lead to changes in post-prandial lipid uptake that could have negative health consequences linked to endothelial dysfunction and the establishment of an atherogenic environment (Zilversmit, 1995; Lopez-Miranda *et al.* 2006). However, the precise site of these inactivity-induced effects is uncertain given lipid disposal involves the liver, muscle and adipose tissue. At present the strict time frame and magnitude of impact of limb immobilisation on limb lipid uptake is unclear. However, it is known that resistance to insulin-mediated whole-body glucose disposal positively associates with the magnitude of postprandial lipaemia (Jeppesen *et al.* 1995).

Here we addressed gaps in the literature by leveraging the findings from two studies aimed at investigating temporal changes in post-prandial limb glucose and lipid uptake over the course of several days of forearm immobilisation, and using a study design incorporating a non-immobilised contralateral limb exposed to the same feeding-induced changes. We hypothesised that short-term forearm immobilisation would inhibit insulin-stimulated limb glucose uptake and this would be specific to the immobilised limb. However, we could

not predict the trajectory and magnitude of any effect. We also hypothesised any immobilisation-induced temporal decline in forearm glucose uptake would positively associate with a concurrent reduction in forearm lipid uptake.

Methods

Ethical approval

This project was approved by the University of Nottingham Medical School Ethics Committee (reference number: J14112016) and was conducted in accordance with the standards set by the latest version of the *Declaration of Helsinki*, except for registration in a database. Informed, written consent was obtained from all volunteers before participation and all understood they were free to leave the study at any point.

Participants

Following routine medical screening (height, body mass, blood pressure, 12-lead electrocardiogram (ECG), blood testing, including full blood cell count, urea and electrolytes, hepatic, renal and clotting function), 10 healthy men (age 24.9 ± 0.6 years, weight 71.9 ± 2.6 kg, body mass index (BMI) 23.1 ± 0.6 kg/m^2) completed Study A, while nine healthy men (age 22.4 ± 1.3 years, weight 71.4 ± 2.8 kg, BMI 22.6 ± 0.8 kg/m^2) completed Study B. Exclusion criteria for participation in both studies included any metabolic or endocrine abnormalities, history of varicose veins or deep vein thrombosis, regular medications, clinically significant abnormalities detected by ECG or blood tests, BMI below 18.5 and above 25.0 kg/m^2, and ages below 18 or above 35 years. All participants were instructed to avoid any strenuous exercise during the study.

Experimental protocols

Study A (Fig. 1). Following an overnight fast and having abstained from alcohol, caffeine and strenuous exercise for the previous 24 h, participants attended a thermoneutral laboratory (room temperature 25°C, 50% humidity) on four separate occasions (pre-immobilisation and at 24, 48 and 72 h of immobilisation) to determine forearm glucose uptake. Following local anaesthesia (1% lidocaine hydrochloride, B. Braun, Melsungen, Germany), a 20-gauge cannula (BD Venflon, Becton Dickinson Infusion Therapy, Helsingborg, Sweden) was inserted in a retrograde direction in a dorsal foot vein, which was placed in a hot-air warming unit (55°C; University of Nottingham Medical Engineering Unit, Queens Medical Centre, Nottingham) to allow sampling of arterialised-venous blood. Arterialised-venous cannulation provides a safer alternative to arterial

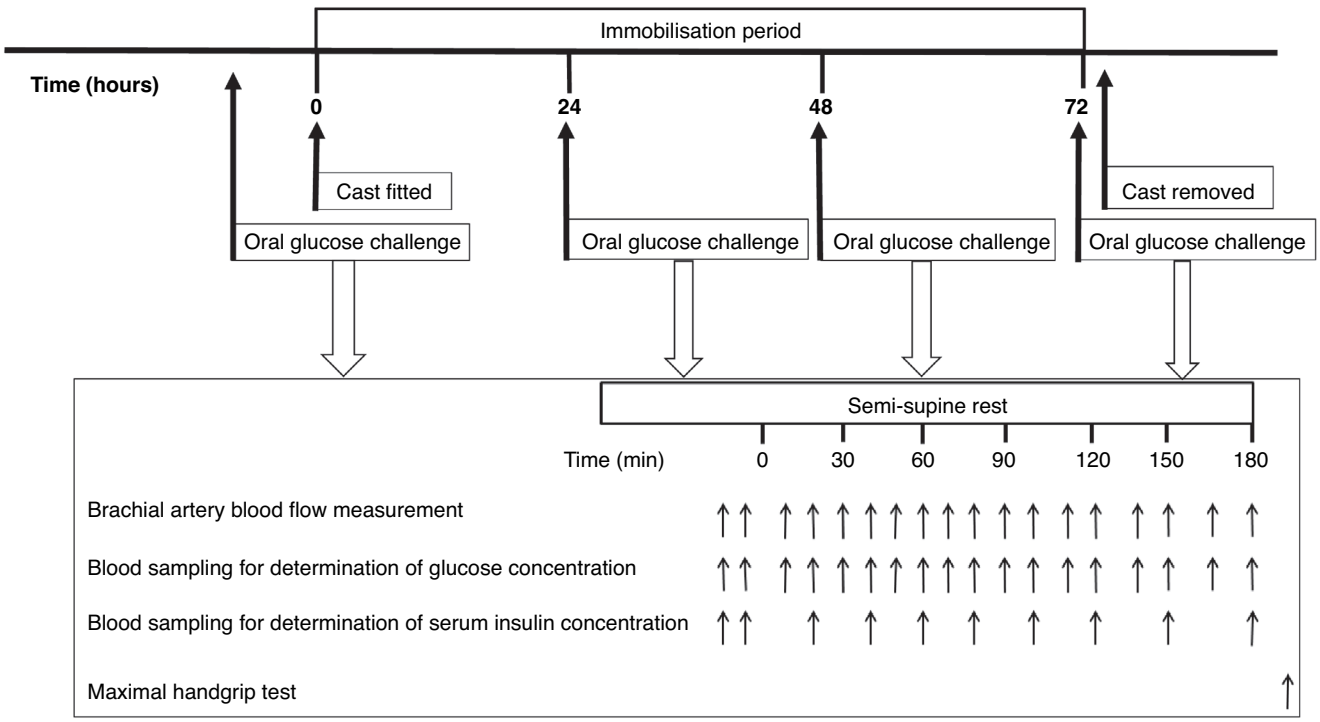

Figure 1. Overview of the experimental protocol for Study A

cannulation, particularly when repeated cannulation is required, and the difference in whole blood acid–base status and glucose concentration between an arterialised-venous sample and an arterial sample is negligible (Forster *et al.* 1972; Liu *et al.* 1992). Additionally, use of a warm-air box is known to have minimal effect on whole body temperature and skin and forearm blood flow in the contralateral heated limb (Gallen & Macdonald, 1990).

A 20-gauge retrograde cannula was also advanced, using guided ultrasound (Aplio SSA-770A; Toshiba Medical Systems, Crawley, UK), so that the tip lay within the perforating branch of the median cubital vein of each arm to allow deep venous sampling from the forearm muscle bed (Betry *et al.* 2020). Positioning of the cannula was confirmed by ultrasound B-mode imaging before starting the experimental protocol, and at the end of each study visit. If necessary, Doppler mode ultrasonography in conjunction with a 0.9% saline solution flush was used as a second confirmation of the cannula position; if the cannula was within the perforating vein, turbulent blood flow velocity was detected in the perforating vein (Betry *et al.* 2020).

All cannulae had three-way taps attached to allow blood sampling and were kept patent with a slow 0.9% saline infusion (Baxter Healthcare Ltd, Newbury, UK). Previous investigation in our laboratory demonstrated glucose uptake in the non-immobilised (NIM) arm was unlikely to change over time, and therefore to minimise cannulation failure associated with repeated deep-venous cannulation only the foot and immobilised (IMM) arm were cannulated at 24 and 48 h time points.

Before and at 24, 48 and 72 h of unilateral arm immobilisation (see section 'Forearm immobilisation' below) subjects underwent a 3 h oral glucose challenge (75 g dextrose in 250 ml water). Measurements of brachial artery blood flow were made simultaneously with blood sample collection before and throughout the oral glucose challenge. Blood samples were drawn concurrently from all three cannulae every 10 min for the initial 2 h and every 15 min for the final hour of the oral glucose challenge. An additional 1 ml of arterialised-venous blood was sampled before and every 20 min for the first 2 h and every 30 min for the final hour of the oral glucose challenge to determine serum insulin concentration. Grip strength was measured at the end of the oral glucose challenge prior to and after 72 h of immobilisation, using a hand grip dynamometer (University of Nottingham Medical Engineering Unit). At the end of the final laboratory visit, participants' forearm cast was removed and the volunteers were fed.

Study B (Fig. 2). Participants reported to a thermoneutral laboratory, having fasted overnight and abstained from alcohol, caffeine and strenuous exercise for the previous 24 h on three separate occasions (pre-immobilisation and at 24 and 48 h of immobilisation) to determine forearm

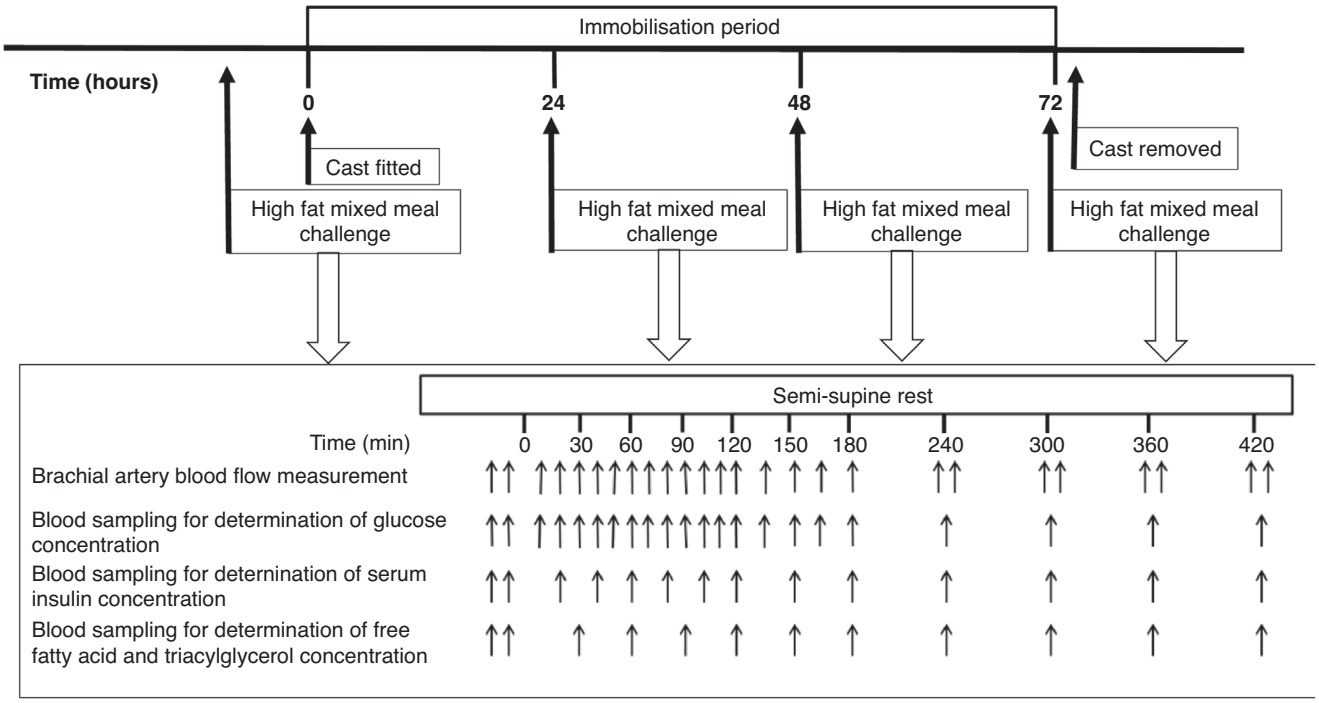

Figure 2. Overview of the experimental protocol for Study B

glucose uptake and lipid uptake. Cannulation of a dorsal foot vein and the perforating branch of the median cubital vein in each arm was performed following the same procedures described for Study A. Due to complications with repeated retrograde cannulation of the perforating branch of the median cubital vein in the IMM arm on consecutive days, only six volunteers completed a fourth experimental visit at 72 h after cast fitting to allow assessment of forearm glucose uptake and lipid uptake in the NIM arm at this specific time point.

After baseline blood samples were obtained, subjects consumed a high fat mixed-meal (Ensure Shake, Abbott Nutrition, Maidenhead, UK, along with beans on toast; 16.7 kcal kg^{-1} body mass, comprising 1.21 g (47% energy) fat, 1.11 g (43% energy) carbohydrate and 0.27 g (10% energy) protein per kg body mass; Kim *et al.* 2016) within 15 min to begin the 7 h mixed-meal challenge. Participants were also provided with water (2 ml kg^{-1} body weight) to rinse their mouth following the test meal. Measurements of brachial artery blood flow were made simultaneously with blood sample collection before and throughout the high fat mixed-meal challenge. Blood samples for determination of whole blood glucose concentration were drawn concurrently from all three cannulae before, every 10 min for the initial 2 h, every 15 min for the third hour and every 60 min for the final 4 h of the mixed-meal challenge. An additional 2 ml of blood was drawn before, every 30 min for the initial 3 h and every 60 min for the final 4 h of the mixed-meal challenge for determination of plasma free fatty acid, serum triglyceride and glycerol concentration. Arterialised-venous blood for serum insulin determination was drawn before and every 20 min for the initial 2 h, every 30 min for the third hour and every 60 min for the final 4 h of the mixed-meal challenge. At the end of the final laboratory visit, participants' forearm cast was removed and the volunteers were fed.

Forearm immobilisation. Within 7 days of the pre-immobilisation visit in both studies, participants had a fibre-glass arm cast fitted. This cast extended from the second phalanges of the fingers (also encasing the thumb) to below the elbow of the non-dominant arm to prohibit any gripping action. The IMM arm was also supported by a broad-arm sling, which volunteers were repeatedly instructed to wear at all times, except when bathing and sleeping. The dominant arm served as the control NIM limb.

Brachial artery blood flow. Brachial artery blood flow was measured concurrently with blood sampling by Doppler ultrasound imaging (Aplio SSA-770A Diagnostic ultrasound system, Toshiba Medical Systems). Luminal diameter measurements were made using online video callipers at a perpendicular angle to the axis of the vessel. Blood velocity was measured using the pulsed-wave Doppler signal at an insonation angle of 60°.

Blood analyses

Blood glucose. Directly after blood collection, 0.5 ml from each sample was added to a microtube (Sarstedt, Nümbrecht, Germany), containing heparin and fluoride to inhibit glycolysis, and placed on a roller (Stuart Roller Mixer SRT6, Bibby Scientific, Stone, UK) for 3 min, before being analysed for whole-blood glucose concentration using an automated glucose analyser (YSI 2300 STATplus, Yellow Springs Instruments, Yellow Springs, OH, USA).

Serum insulin. Blood from each sample was added to serum separating tubes (BD Vacutainer SST II) and allowed to clot over 30 min at room temperature before being centrifuged at 3000 g for 10 min. Serum was then stored at −80°C and, at a later date, serum insulin concentration was determined using a competitive radioactive immunoassay (Millipore, St. Louis, USA).

Plasma free fatty acid concentration. One millilitre from each blood sample was added to lithium–heparin coated anticoagulant BD vacutainers pre-filled with 7.5 μl of ethylene glycol tetraacetic acid-gluthathione before centrifugation for 10 min at 3000 g. Plasma was then pipetted into microtubes (Sarstedt) containing 5 μl tetrahydrolipstatin to prevent *in vitro* lipolysis (Krebs *et al.* 2000), before being stored at −80°C until subsequent free fatty acid analysis using a colorimetric assay (Wako Chemicals GmbH, Neuss, Germany).

Serum triglyceride and glycerol. One millilitre from each blood sample was added to serum separating tubes and allowed to clot for 30 min before being centrifuged for 10 min at 3000 g. Serum was then aliquoted into screw-top mictotubes (Sarstedt) before being stored at −80°C and analysed for triglyceride (reagents Horiba Medical, Montpellier, France) and glycerol (reagents Sigma-Aldrich, St Louis, MO, USA) concentrations at a later date. Both analyses were spectrophotometric based assays run on a Pentra 400 analyser (Horiba Medical).

Calculations and statistical analyses

The arterialised venous–venous substrate difference was calculated as the difference between the arterialised-venous sample (from the heated foot) and the deep venous brachial sample from each arm. Using mean blood velocity and arterial diameter, brachial artery blood flow was calculated in ml min^{-1} as brachial artery blood flow = MBV × πr^2, where MBV is mean blood velocity and r is brachial artery radius. Using Fick's principle (Zierler, 1961) forearm glucose (Study A and

B), glycerol (Study B) and free fatty acid (Study B) uptake and triglyceride disposal (Study B) were calculated for each sample as: brachial artery blood flow × ([AV] – [V]), where [AV] is the arterialised-venous and [V] is the deep-venous metabolite concentration.

Area under analyte × time curve analysis was calculated for forearm glucose (Study A and B), glycerol (Study B), and free fatty acid (Study B) uptake, triglyceride disposal (Study B), brachial artery blood flow (Study A and B), arterialised venous–venous substrate difference (Study A and B) and serum insulin concentration (Study A and B) and employed curve integration using the trapezium rule.

All statistics and graphs were analysed using GraphPad Prism (version 6.05; GraphPad Software Inc., La Jolla, CA, USA). A two-way analysis of variance (ANOVA) was used to compare all time-dependent variables to detect main effects of immobilisation on blood glucose, serum triglyceride, glycerol, plasma free fatty acid and brachial artery blood flow. Significant main effects were further investigated using a Sidak's *post hoc* test. One-way repeated measures ANOVA with Dunnett's *post hoc* analysis was performed to assess a single variable over time and Student's *t*-test was used to compare two time points. Statistical significance was set at $P < 0.05$, and data are presented as means ± standard deviation (SD).

Results

Study A

Brachial artery blood flow. Figure 3 shows brachial artery blood flow over the course of 180 min following the ingestion of 75 g of glucose ($t = 0$ min) in the NIM arm (Fig. 3*A*) and the contralateral IMM arm (Fig. 3*B*) before (0 h) and after 24, 48 and 72 h. Although blood flow tended to increase over time on each visit following glucose ingestion, there was no difference in blood flow at any specific time point when comparing visits within each limb. Considering the overall response on each visit (AUC in (ml/min)·180 min, Table 1), the brachial artery blood flow response was no different between arms prior to immobilisation ($P = 0.86$) and was unchanged after 72 h in the NIM arm ($P = 0.59$). However, the brachial artery blood flow response in the IMM limb at 24 h was greater than before immobilisation ($P = 0.04$), but was not different at 48 or 72 h ($P = 0.70$ and $P = 0.97$, respectively).

Forearm glucose uptake. Figure 4 illustrates the area under the curve for forearm glucose uptake at baseline (0) and after 24, 48 and 72 h of immobilisation and at baseline and 72 h in the contralateral non-immobilised limb. The data are shown as means ± SD and individual values for each volunteer. Forearm glucose uptake was no different between arms prior to immobilisation ($P = 0.58$), and did not change following 72 h in the NIM limb ($P = 0.16$). However, in the IMM limb forearm glucose uptake declined from baseline at 24 h (38%, $P = 0.04$), 48 h (57%, $P = 0.01$) and 72 h (46%, $P = 0.06$) of immobilisation. Forearm glucose uptake was also less in the IMM arm compared to the NIM arm after 72 h of immobilisation ($P = 0.002$).

Serum insulin. The area under the curve for arterialised-venous serum insulin concentration during each oral glucose challenge over the course of Study A

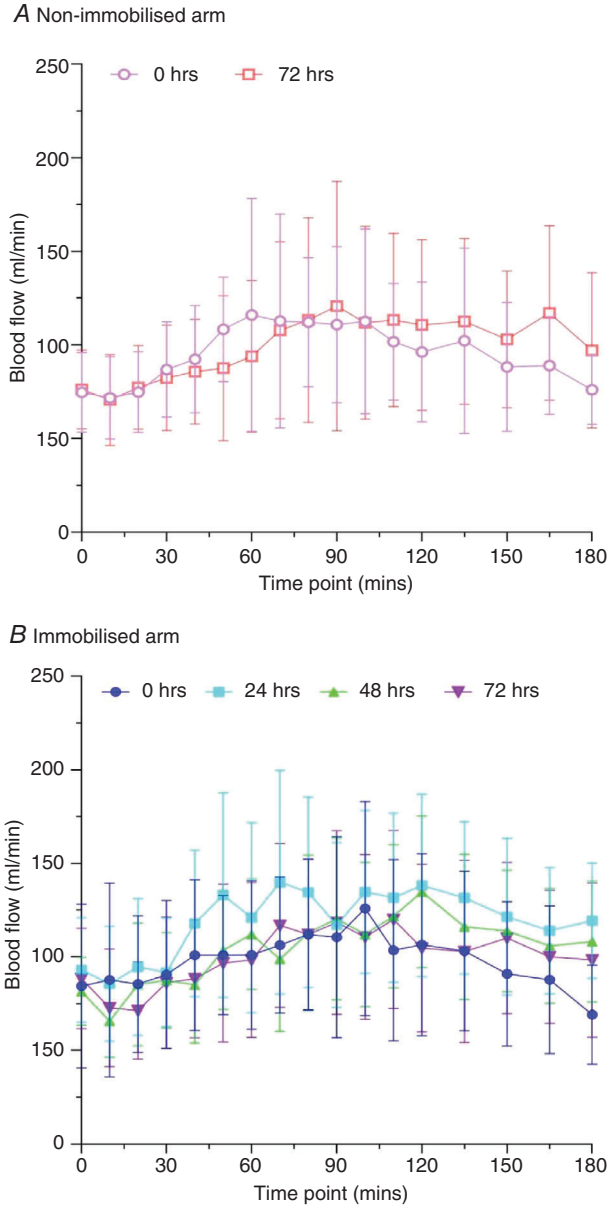

Figure 3. Brachial artery blood flow over the course of 180 min following the ingestion of 75 g of glucose ($t = 0$ min) in a non-immobilised forearm (*A*) and the contralateral immobilised forearm (*B*) before (0 h) and after 24, 48 and 72 h
Values are means ± SD ($n = 10$). Data for each individual volunteer has been made available as supporting information.

Table 1. Overall brachial artery blood flow response (AUC in (ml/min)·180 min) following an oral glucose challenge measured at baseline (0) and 72 h in a non-immobilised limb and at baseline (0) and after 24, 48 and 72 h in the contralateral immobilised limb

	Time (h)			
	0	24	48	72
Non-immobilised limb	17.3 ± 5.3			18.1 ± 6.2
Immobilised limb	17.8 ± 6.8	21.6 ± 6.3*	19.0 ± 5.0	18.1 ± 6.2

Values are means ± SD ($n = 10$). *$P = 0.04$, significantly different from corresponding baseline value.

did not change from the pre-immobilisation state (6733 ± 2724 (mIU/l)·180 min) after 24 h (8326 ± 3622 (mIU/l)·180 min, $P = 0.18$), 48 h (8963 ± 4164 (mIU/l)·180 min, $P = 0.14$) and 72 h (88 241 ± 3491 (mIU/l)·180 min, $P = 0.16$) of arm immobilisation.

Grip strength. Grip strength was no different between arms prior to immobilisation (39.34 ± 3.79 and 39.48 ± 3.86 kg in the NIM and IMM arms respectively, $P = 0.99$). Grip strength was unchanged from baseline after 72 h in the NIM limb (41.09 ± 4.34 kg, $P = 0.38$) and IMM limb (37.28 ± 3.43 kg, $P = 0.16$), but there was a difference between limbs at this time point ($P = 0.02$).

Study B

Brachial artery blood flow. Figure 5 shows brachial artery blood flow over the course of 420 min following the

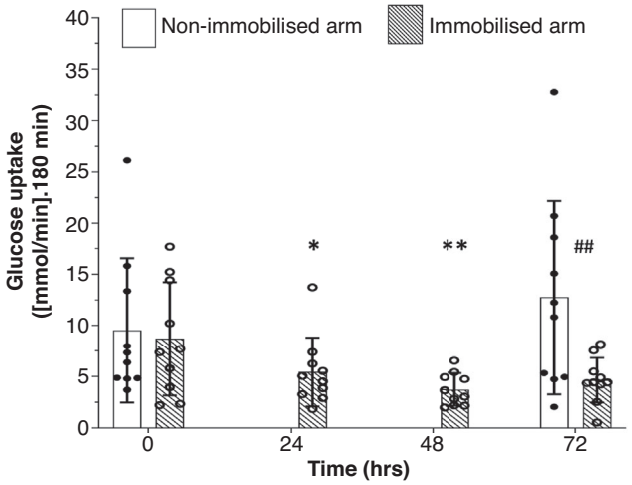

Figure 4. Area under the curve for forearm glucose uptake over the course of 180 min following the ingestion of 75 g of glucose at baseline (0) and after 24, 48 and 72 h of immobilisation and at baseline and 72 h in the contralateral non-immobilised limb
Values are means ± SD and individual values ($n = 10$ volunteers). *$P < 0.05$, **$P = 0.01$, significantly different from corresponding baseline value in the immobilised arm. ##$P < 0.01$, significantly different between arms at 72 h.

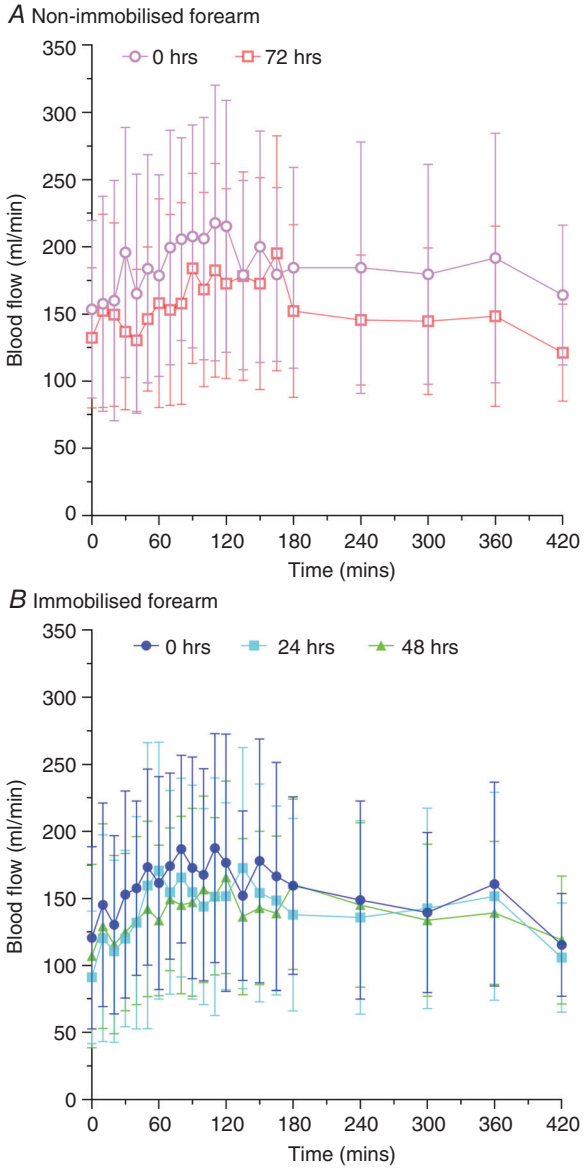

Figure 5. Brachial artery blood flow over the course of 420 min following the ingestion of a mixed meal ($t = 0$ min) in a non-immobilised forearm (*A*) and the contralateral immobilised forearm (*B*) before (0 h) and after 24, 48 and 72 h
Values are mean ± SD ($n = 9$ at 0, 24, 48 h in both arms and $n = 6$ at 72 h in the non-immobilised arm). Data for each individual volunteer have been made available as supporting information.

Table 2. Overall brachial artery blood flow response (AUC in (ml/min)·420 min) following an oral mixed-meal challenge at baseline (0) and 72 h in the non-immobilised limb and at baseline (0) and after 24 and 48 h of immobilisation

	Time (h)			
	0	24	48	72
Non-immobilised limb	74.5 ± 31.6			63.8 ± 23.2
Immobilised limb	64.7 ± 26.4	59.5 ± 27.8	58.9 ± 22.4	

Values are means ± SD ($n = 9$, apart from $n = 6$ in the non-immobilised limb at 72 h).

ingestion of a mixed meal ($t = 0$ min) in the NIM arm (Fig. 5*A*) and the contralateral IMM arm (Fig. 5*B*) before (0 h) and after 24, 48 and 72 h. Blood flow tended to increase over the initial 180 min on each visit following mixed meal ingestion, but there was no difference in blood flow at any specific time point when comparing visits within each limb. Studying the overall response on each visit (AUC in (ml/min)·420 min, Table 2), the brachial artery blood flow response to the high fat mixed-meal challenge was no different between arms prior to immobilisation ($P = 0.95$) and showed a tendency to be lower after 72 h in the NIM arm compared to baseline ($P = 0.06$). The overall brachial artery blood flow response in the IMM arm tended to be lower than baseline after 24 and 48 h immobilisation, but was not significantly different from baseline ($P = 0.065$ and $P = 0.07$, respectively).

Forearm glucose uptake. Figure 6 illustrates area under the curve of forearm glucose uptake over the course of a 420 min high fat mixed-meal challenge at baseline (0) and after 72 h in the non-immobilised limb, and at baseline (0) and after 24 and 48 h of immobilisation in the contralateral limb. The data are shown as means ± SD and individual values for each volunteer. There was no difference in forearm glucose uptake from baseline in the non-immobilised arm after 72 h ($P = 0.65$). However, in the contralateral immobilised limb, forearm glucose uptake was less than pre-immobilisation after 24 h (46%, $P = 0.01$) and 48 h (51%, $P = 0.03$) of immobilisation.

Serum insulin. The area under the curve for arterialised-venous serum insulin concentration during each mixed-meal challenge over the course of Study B was no different from the pre-immobilisation state (10,295 ± 2858 (mIU/l)·420 min) following 24 h (10,595 ± 2852 (mIU/l)·420 min, $P = 0.94$) and 48 h (10,653 ± 2391 (mIU/l)·420 min, $P = 0.91$) of arm immobilisation.

Fasting plasma free fatty acid, serum triglyceride and free glycerol concentration. Table 3 displays arterialised-venous plasma free fatty acid, serum triglyceride and free glycerol concentrations, along with deep-venous free fatty acid, triglyceride and free glycerol concentrations from the non-immobilised and immobilised arms following an overnight fast. Immobilisation resulted in a decline in fasting state arterialised-venous and deep-venous free fatty acid concentrations from baseline on all laboratory visits, although the magnitude of decline was quantitatively small (Table 3). Fasting state arterialised-venous serum triglyceride concentration was also less than baseline after 48 h immobilisation, but again the magnitude of decline was quantitatively small (Table 3).

Forearm free fatty acid uptake and free glycerol uptake and triglyceride disposal. Forearm free fatty acid and free glycerol uptake, and triglyceride disposal over the course

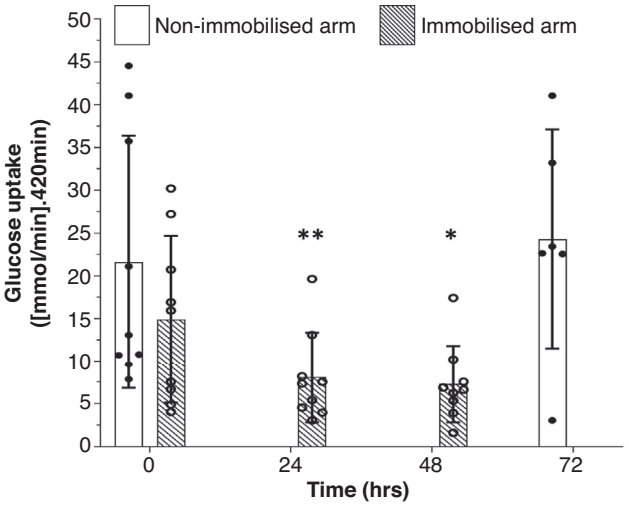

Figure 6. Area under the curve for forearm glucose uptake over the course of 420 min following the ingestion of a mixed meal at baseline (0) and after 24 and 48 h of immobilisation and at baseline and 72 h in the contralateral non-immobilised limb
Values are means ± SD and individual values ($n = 9$ at 0, 24, 48 h in both arms and $n = 6$ at 72 h in the non-immobilised arm). There were no differences between time points when comparing the non-immobilised arm. $*P < 0.05$, $**P = 0.01$, significantly different from corresponding baseline value in the immobilised arm.

Table 3. Free fatty acid (FFA; mmol/l), triglyceride (TAG; mmol/l) and free glycerol (GLY; μmol/l) concentrations in arterialised-venous blood samples from a heated foot and deep-venous blood samples from a non-immobilised (NIM) and immobilised (IMM) arm following an overnight fast, before (0), and at 24, 48 and 72 h of forearm immobilisation

	Arterialised-venous				Venous NIM		Venous IMM		
	0	24	48	72	0	72	0	24	48
FFA	0.47 ± 0.14	0.32 ± 0.17 *P = 0.017	0.22 ± 0.13 *P = 0.006	0.22 ± 0.06 *P = 0.008	0.40 ± 0.10	0.20 ± 0.10 *P = 0.009	0.40 ± 0.17	0.25 ± 0.11 *P = 0.017	0.18 ± 0.08 *P = 0.006
TAG	0.72 ± 0.21	0.70 ± 0.19 P = 0.965	0.58 ± 0.11 *P = 0.040	0.58 ± 0.11 P = 0.089	0.68 ± 0.18	0.58 ± 0.13; P = 0.068	0.68 ± 0.21	0.67 ± 0.17; P = 0.930	0.58 ± 0.11; P = 0.143
GLY	10 ± 5	8 ± 8 P = 0.762	7 ± 5 P = 0.236	5 ± 5 P = 0.021	8 ± 6	4 ± 4 P = 0.152	8 ± 8	5 ± 4 P = 0.149	5 ± 4 P = 0.482

Values represent means ± SD (n = 9, apart from n = 6 in the arterialised-venous and non-immobilised limb at 72 h). ∗Significantly different from corresponding baseline value (P-value presented in table).

Table 4. Free fatty acid uptake ((mmol/l)·420 min) over a 420 min mixed-meal challenge at baseline (0) and 72 h in the non-immobilised limb and at baseline (0) and after 24 and 48 h in the immobilised limb

	Time (h)			
	0	24	48	72
Non-immobilised limb	3.25 ± 2.13			2.34 ± 1.54
Immobilised limb	2.04 ± 1.36	2.04 ± 1.16	2.14 ± 0.99	

Values represent means ± SD (n = 9, apart from n = 6 in the non-immobilised limb at 72 h).

Table 5. Triglyceride disposal ((mmol/l)·420 min) over a 420 min mixed-meal challenge at baseline (0) and 72 h in the non-immobilised limb and at baseline (0) and after 24 and 48 h in the immobilised limb

	Time (h)			
	0	24	48	72
Non-immobilised limb	3.86 ± 1.61			3.62 ± 1.85
Immobilised limb	2.82 ± 1.34	3.14 ± 2.72	4.71 ± 3.76	

Values represent means ± SD (n = 9, apart from n = 6 in the non-immobilised limb at 72 h).

Table 6. Free glycerol uptake ((μmol/l)·420 min) over a 420 min mixed-meal challenge at baseline (0) and 72 h in the non-immobilised limb and at baseline (0) and after 24 and 48 h in the immobilised limb

	Time (h)			
	0	24	48	72
Non-immobilised limb	105 ± 84			272 ± 324
Immobilised limb	120 ± 70	111 ± 82	215 ± 137*	

Values represent means ± SD (n = 9, apart from n = 6 in the non-immobilised limb at 72 h). *P = 0.03, significantly different from corresponding baseline value.

of a 420 min high fat mixed-meal challenge is shown in Tables 4, 5 and 6, respectively. The forearm free fatty acid uptake response was similar between arms prior to immobilisation (P = 0.26) and was unchanged in the NIM arm after 72 h (P = 0.23). Similarly, the forearm free fatty acid uptake response in the IMM limb was no different from before immobilisation after 24 and 48 h immobilisation (P = 0.99 and P = 0.95, respectively). The triglyceride disposal response was similar between arms prior to immobilisation (P = 0.33), and was unchanged

in the NIM arm after 72 h ($P = 0.75$). Triglyceride disposal in the IMM limb was no different from before immobilisation after 24 and 48 h immobilisation ($P = 0.65$ and $P = 0.19$, respectively). The free glycerol uptake response was similar between arms prior to immobilisation ($P = 0.80$) and was unchanged after 72 h in the NIM arm ($P = 0.33$). The free glycerol uptake response in the IMM arm was similar to baseline following 24 h ($P = 0.92$) of immobilisation, but free glycerol uptake increased from baseline at 48 h of immobilisation ($P = 0.03$).

Discussion

Decrements in whole-body glucose uptake during physical inactivity are well documented, but investigations into regional and temporal responses are sparse. The collective novel findings of the present studies are that a 40–45% blunting of feeding-induced forearm glucose uptake occurs in as little as 24 h of immobilisation, and in the absence of a concurrent decline in brachial artery blood flow or a change in arterialised-venous serum insulin response. Furthermore, this magnitude of immobilisation-induced deficit in forearm glucose uptake was sustained thereafter and localised to the immobilised limb, demonstrating the lack of contraction *per se* as being the primary driver of this rapid dysregulation. A further novel finding was that forearm lipid uptake following a mixed-meal challenge was unaffected by immobilisation.

Numerous studies have reported reductions in whole-body glucose uptake in response to an oral glucose challenge or an hyperinsulinaemic euglycaemic clamp within 3–7 days of bed rest in healthy volunteers (Stuart *et al.* 1988; Mikines *et al.* 1991; Yanagibori *et al.* 1994; Smorawinski *et al.* 2000; Hamburg *et al.* 2007), which is evident regionally in the leg (Mikines *et al.* 1991) and forearm (Lipman *et al.* 1970) in healthy volunteers, and also in the leg of patients with cervical spinal cord lesions (Dela *et al.* 2000). In contrast however, Dirks *et al.* (2018) reported a single day of bed rest in healthy volunteers had no measurable impact on insulin sensitivity and whole-body substrate oxidation during an oral glucose challenge, or 24 h glycaemic control or muscle mRNA expression of genes involved in insulin signalling and lipid storage/oxidation. This suggests more than 1 day is required for whole-body adaptation to bed rest to occur and/or that whole-body responses do not accurately reflect limb level adaptations to bed rest at this time point. In support of this latter line of reasoning, the study of Mikines *et al.* (1991), in which whole-body and leg glucose uptake were determined concurrently under insulin clamp conditions following 7 days of bed rest and over a range of insulin concentrations, revealed leg glucose uptake to be the most negatively affected by inactivity.

The temporal impact of immobilisation on limb glucose uptake has not been systematically investigated to date, particularly under conditions in which a non-immobilised 'control' limb has been included in the experimental design. Consequently, as far as we are aware, this is the first study to document a reduction in limb glucose uptake as a result of immobilisation in as short a time as 24 h, and furthermore this suppression was of a magnitude that reflects unambiguous physiological adaptation to immobilisation. Indeed, the extent of decline in forearm glucose uptake in both studies at 24 h was similar to the deficit sustained at 48 of immobilisation (Fig. 7), and thereafter in Study A (Fig. 4). Additionally, there was no reduction in glucose uptake in the non-immobilised limb (Figs 4 and 6). This latter observation also highlights the localised nature of immobilisation and supports the proposition that inactivity-induced decrements in glucose uptake primarily reflects reductions in muscle glucose uptake in response to reduced contraction. Indeed, acute exercise during (Lipman *et al.* 1972) and after (Biensø *et al.* 2012) bed rest has been shown to improve the blunting of glucose disposal in humans. Of note, the investigation of the arm, rather than the leg, intuitively seems to have been more appropriate because the contralateral non-immobilised arm was unlikely to have been

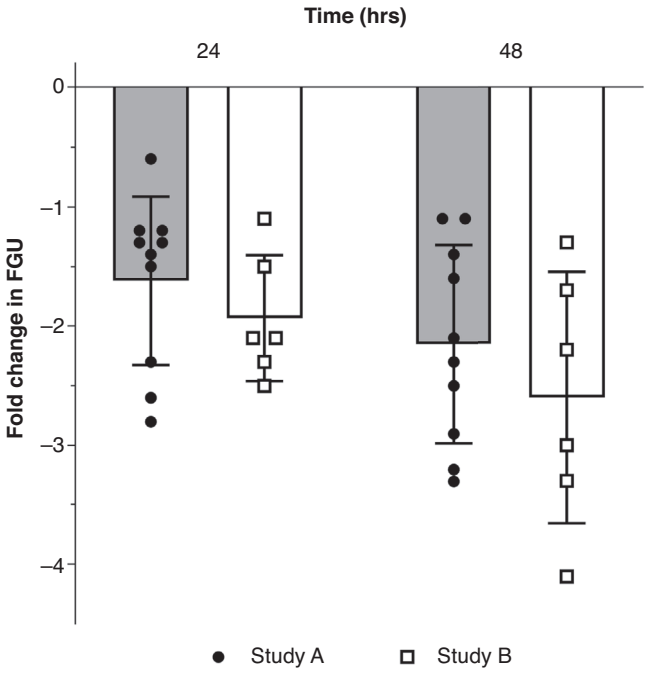

Figure 7. Fold changes in forearm glucose uptake (FGU) from baseline at 24 and 48 h of forearm immobilisation during a 180 min oral glucose challenge (Study A, $n = 10$) and 420 min high fat mixed-meal challenge (Study B, $n = 9$)
Values are means ± SD and individual values.

negatively influenced by immobilisation of the contralateral limb to the same extent as a leg might have been. On the other hand, the leg has a much greater muscle mass and therefore the impact of immobilisation on glucose homeostasis would presumably be easier to detect in the leg.

The mechanism(s) behind immobilisation-induced reductions in whole-body/limb glucose uptake is currently unclear. As the majority of glucose uptake occurs in skeletal muscle (DeFronzo & Tripathy, 2009), it can be assumed that this blunting effect reflects muscle specific inhibition of glucose uptake. As outlined earlier, Biensø *et al*. (2012) demonstrated the decline in leg glucose uptake following 7 days of bed rest was associated with reductions in the content/activity of key proteins regulating glucose transport, phosphorylation and storage. However, the rapid rate and magnitude of decline in glucose uptake during immobilisation in the present study strongly suggests that at least some of the protein-level responses reported by Biensø *et al*. are likely to have occurred secondarily to the reduction in limb glucose uptake, rather than being responsible for it. Thus, whilst the precise mechanism(s) are not yet apparent, it is clear from this study that inactivity is a physiological insult, and its effects on glucose uptake manifest very quickly. This rapid, negative effect of inactivity on glucose uptake occurs in the absence of a concurrent decline in brachial artery blood flow or a change in arterialised-venous serum insulin response suggesting it is of intra-muscular origin.

Increased intramyocellular lipid content has also been implicated in the inhibition of skeletal muscle glucose uptake associated with immobilisation of 10–28 days, with the accumulation of lipid intermediates suggested to interfere with insulin signalling (Cree *et al*. 2010; Sonne *et al*. 2010). However, it seems unlikely that intramyocellular lipid accumulation could be responsible for the impairment of limb glucose uptake reported during short-term immobilisation, particularly within 24 h, given 7 days of bed rest resulted in whole-body insulin resistance in the absence of a measurable change in intramyocellular lipid content (Dirks *et al*. 2016).

As a slight aside, it has been demonstrated that whole-body and muscle insulin sensitivity is blunted when lipid availability is acutely increased (Schrezenmeir *et al*. 1993), most likely as a result of free fatty acid-mediated inhibition of glucose transport caused by decreased insulin receptor substrate 1 and phosphatidylinositol 3-kinase activity (Dresner *et al*. 1999) and inhibition of downstream insulin signalling (Griffin *et al*. 1999) in humans. However, few studies have addressed the impact of increased lipid supply on insulin-stimulated glucose uptake during immobilisation, which Study B was able to address. Boden *et al*. (1991) reported an acute 55% decrease in rates of glucose disposal 2–4 h after intravenous infusion of lipid and heparin during euglycaemic hyperinsulinaemia in healthy men, demonstrating acutely increasing intravascular lipid availability diminished whole-body glucose disposal. Conversely, the present findings demonstrate the diminution of the forearm glucose uptake in response to feeding over the course of 48 h immobilisation was unaffected by acutely increasing dietary lipid intake (Fig. 7). This observation is in line with the suggestion that elevated plasma free fatty acids must be first reincorporated into lipid stores within the muscle fibre before muscle resistance to insulin action develops (Boden *et al*. 2001). Irrespective of the mechanism, recent evidence showing increasing dietary fat intake for 7 days concurrent with forearm immobilisation had no further impact on forearm glucose balance after 2 and 7 days of immobilisation, when compared to an isocaloric control diet, demonstrates a sustained increase in lipid supply has no additive impact on insulin-stimulated glucose uptake during immobilisation (Dirks *et al*. 2019). Overall, it would appear that a lack of muscle contraction *per se* is the primary driver of impaired forearm glucose uptake during arm immobilisation.

High-fat dietary intervention strategies have been commonly used to modulate post-prandial lipid metabolism (Lopez-Miranda *et al*. 2006). Furthermore, Kim *et al*. (2016) demonstrated that 2–4 days of prolonged sitting (>14 h/day) amplified the post-prandial plasma lipaemic response to a high fat test meal compared to active volunteers, which occurred independent of energy balance. The authors also demonstrated that prolonged sitting attenuated the ability of acute whole-body exercise (treadmill for 1 h ∼67% maximal oxygen uptake) to blunt post-prandial lipaemia or increase relative fat oxidation, regardless of energy balance. We therefore hypothesised that forearm immobilisation would attenuate forearm lipid uptake from baseline after 24 and 48 h immobilisation, and also when compared to the contralateral non-immobilised limb. However, as illustrated in Tables 4 and 5, neither forearm free fatty acid uptake nor triglyceride disposal was affected by immobilisation or appeared to be different between limbs. This observation was surprising considering organ-specific fatty acid accumulation over the 6 h following acute administration of labelled dietary fatty acids has been shown to occur in liver, heart, skeletal muscle, and subcutaneous and abdominal adipose tissues using positron emission tomography coupled to computed tomography (Labbé *et al*. 2011). The lack of effect of immobilisation on free-fatty acid uptake and triglyceride disposal in the present study is presumably explained by the relatively low blood lipid uptake kinetics compared to glucose, coupled with the volume of muscle immobilised being relatively small compared to the whole-body sink available for lipid disposal. In support of this suggestion, and in keeping with the present findings, Dirks *et al*. (2019), using the immobilised forearm model, reported no impact of 7 days

of high-fat dietary intake on forearm fatty acid balance in response to an acute mixed macronutrient drink when compared to fatty acid balance across an immobilised forearm during 7 days of controlled dietary intake.

In conclusion, a major decrement in forearm glucose uptake in response to oral glucose feeding occurred within 24 h of immobilisation that was sustained and specific to the immobilised limb, and was not explained by deficits in brachial artery blood flow or the arterialised-venous serum insulin response. Furthermore, the rate of onset and magnitude of this immobilisation-induced reduction in limb glucose uptake was not influenced by acutely increasing dietary lipid availability. Collectively, these findings strongly point to the lack of contraction *per se* as being the primary driver of a rapid and localised reduction in limb glucose disposal during immobilisation, and future studies should focus on the intracellular events regulating this physiological process.

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Additional information

Data availability statement

The data that support the findings of this study can be made available on a collaborative basis upon reasonable request. The data that support the findings depicted in Figs 3 and 5 of this study are available in the Supporting information of this article.

Competing interests

The authors have no conflicts of interest.

Author contributions

A.B., F.B.S. and P.L.G. designed Study A and A.B. and P.L.G. designed Study B. A.B. undertook volunteer recruitment and organised all screening and study visits, which were conducted by A.B., A.N. and J.M. A.B. and S.C. performed the wet laboratory analyses and A.B. performed data and statistical analyses. A.B., F.B.S. and P.L.G. interpreted the data for Study A and A.B. and P.L.G. for Study B. A.B. and P.L.G. wrote the manuscript and all authors revised subsequent versions. All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or

integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Keywords

fuel metabolism, immobilisation, metabolic dysfunction, physical inactivity

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Statistical Summary Document
Supplementary blood flow data Study A
Supplementary blood flow data Study B