



# Short-term decreased physical activity with increased sedentary behaviour causes metabolic derangements and altered body composition: effects in individuals with and without a first-degree relative with type 2 diabetes

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## Abstract

**Aims/hypothesis** Low physical activity levels and sedentary behaviour are associated with obesity, insulin resistance and type 2 diabetes. We investigated the effects of a short-term reduction in physical activity with increased sedentary behaviour on metabolic profiles and body composition, comparing the effects in individuals with first-degree relatives with type 2 diabetes (FDR+ve) vs those without (FDR–ve).

**Methods** Forty-five habitually active participants (16 FDR+ve [10 female, 6 male] and 29 FDR–ve [18 female, 11 male]; age 36 ± 14 years) were assessed at baseline, after 14 days of step reduction and 14 days after resuming normal activity. We determined physical activity (using a SenseWear armband), cardiorespiratory fitness ( $\dot{V}O_{2peak}$ ), body composition (dual-energy x-ray absorptiometry/magnetic resonance spectroscopy) and multi-organ insulin sensitivity (OGTT) at each time point. Statistical analysis was performed using a two-factor between-groups ANCOVA, with data presented as mean ± SD or (95% CI).

**Results** There were no significant between-group differences in physical activity either at baseline or following step reduction. During the step-reduction phase, average daily step count decreased by 10,285 steps (95% CI 9389, 11,182;  $p < 0.001$ ), a reduction of 81 ± 8%, increasing sedentary time by 223 min/day (151, 295;  $p < 0.001$ ). Pooling data from both groups, following step reduction there was a significant decrease in whole-body insulin sensitivity (Matsuda index) ( $p < 0.001$ ), muscle insulin sensitivity index ( $p < 0.001$ ), cardiorespiratory fitness ( $p = 0.002$ ) and lower limb lean mass ( $p = 0.004$ ). Further, there was a significant increase in total body fat ( $p < 0.001$ ), liver fat ( $p = 0.001$ ) and LDL-cholesterol ( $p = 0.013$ ), with a borderline significant increase in NEFA AUC during the OGTT ( $p = 0.050$ ). Four significant between-group differences were apparent: following step reduction, FDR+ve participants accumulated 1.5% more android fat (0.4, 2.6;  $p = 0.008$ ) and increased triacylglycerol by 0.3 mmol/l (0.1, 0.6;  $p = 0.044$ ). After resuming normal activity, FDR+ve participants engaged in lower amounts of vigorous activity ( $p = 0.006$ ) and had lower muscle insulin sensitivity ( $p = 0.023$ ). All other changes were reversed with no significant between-group differences.

**Conclusions/interpretation** A short-term reduction in physical activity with increased sedentary behaviour leads to a reversible reduction in multi-organ insulin sensitivity and cardiorespiratory fitness, with concomitant increases in central and liver fat and dyslipidaemia. The effects are broadly similar in FDR+ve and FDR–ve individuals. Public health recommendations promoting physical activity should incorporate advice to avoid periods of sedentary behaviour.

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## Research in context

### What is already known about this subject?

- Low physical activity levels and sedentary behaviour are major risk factors for obesity, the metabolic syndrome and type 2 diabetes
- First-degree relatives of individuals with type 2 diabetes have a threefold higher risk of developing type 2 diabetes compared with those with no family history

### What is the key question?

- How does a short-term decrease in physical activity affect metabolic health/body composition and are the associated changes more pronounced in individuals with a first-degree relative with type 2 diabetes vs individuals without?

### What are the new findings?

- A reduction in physical activity with increased sedentary behaviour and decreased cardiorespiratory fitness leads to unfavourable changes in body composition with loss of lean mass and accretion of body fat (particularly abdominal and liver fat). Metabolic decompensation was evident, with development of whole-body insulin resistance
- Overall, there was no convincing evidence that the genetic background of type 2 diabetes influences the magnitude of the metabolic derangements or the change in body composition

### How might this impact on clinical practice in the foreseeable future?

- These findings emphasise the need for healthcare professionals to educate individuals about the harmful effects of sedentary behaviour/low activity levels and furthermore highlight the importance of a habitually active lifestyle while demonstrating how even subtle changes in physical activity can positively or negatively influence health

**Keywords** Body composition · Cardiorespiratory fitness · Glucose tolerance · Insulin resistance · Liver fat · Physical activity · Sedentary behaviour · Skeletal muscle · Type 2 diabetes

### Abbreviations

<sup>1</sup> H-MRS	Proton magnetic resonance spectroscopy
Adipo-IR	Adipose tissue insulin resistance
DBP	Diastolic BP
DXA	Dual-energy x-ray absorptiometry
FDR+ve	Individuals with a first-degree relative with type 2 diabetes
FDR−ve	Individuals with no first-degree relatives with type 2 diabetes
IHCL	Intrahepatocellular lipid
IMCL	Intramyocellular lipid
METS	Metabolic equivalents
SBP	Systolic BP
TEE	Total energy expenditure
VIF	Variance inflation factor

## Introduction

There is an abundance of epidemiological evidence which indicates that physical inactivity (defined as an insufficient physical activity level to meet physical activity guidelines) and sedentary behaviour (defined as any waking behaviour characterised by an energy expenditure <1.5 metabolic

equivalents (METS), while in a sitting, reclining or lying posture) are major causal factors in the development of obesity, insulin resistance and type 2 diabetes [1–4]. However, such data provide no mechanistic insight into these pathophysiological changes. One plausible paradigm suggests that a transition to physical inactivity and/or increased sedentary time causes a reduction in skeletal muscle insulin sensitivity, contributing to a repartitioning of energy substrates into storage, increasing central fat accumulation and ectopic storage within the liver and other organs, causing further insulin resistance [5–9]. As peripheral insulin resistance progresses, continued ectopic fat accumulation within the liver and pancreas precipitates development of the metabolic syndrome, a progressive decline in beta cell function and, ultimately, type 2 diabetes [10].

To date, mechanistic studies in humans pertaining to reduced physical activity levels have used extreme experimental models including prolonged bed rest [11], limb immobilisation [12] and cessation of exercise in exercise-trained volunteers [13]. These models are not physiologically representative of habitual activity levels in free-living individuals, and thus should be interpreted cautiously. More recently, an alternative experimental model has been developed in which active (~10,000 steps/day) healthy volunteers, who do not participate in regular exercise, transition to an inactive lifestyle

(reducing to ~1500 steps/day) for brief periods (~14 days). This approach reflects societal changes in physical activity levels (i.e. reduced physical activity and more sedentary time) [14, 15]. Step-reduction models have demonstrated that reduced physical activity results in detrimental physiological changes, including reduced cardiorespiratory fitness, accumulation of central fat, loss of skeletal muscle mass with associated anabolic resistance, and reductions in peripheral insulin sensitivity [16–20]. These changes are reversible with resumption of normal activity in young [18], but not older adults [20]. Importantly, such research provides a mechanistic basis for the consequences of increased sedentary behaviour and aids our understanding of the development of metabolic disease.

First-degree relatives of individuals with type 2 diabetes (FDR+ve) have a threefold increased risk of developing the disease compared with those without a family history (FDR–ve) [21]; this risk can further be increased with low physical activity levels [22]. No previous studies have employed a step-reduction protocol to examine whether individuals genetically predisposed to type 2 diabetes are more susceptible to the adverse metabolic consequences of an inactive lifestyle compared with those who are not. Given that group-specific physical activity guidelines have been proposed for other high-risk groups (e.g. South Asians) and that the exercise dose–response curve clearly differs between populations [23], this is a very pertinent research question. Furthermore, only two studies have investigated whether the detrimental effects of step reduction are reversed when habitual physical activity is resumed: one induced overfeeding during step reduction [18] and the other was conducted in older adults [20].

The primary aim of this study was to investigate the metabolic consequences of short-term decreased physical activity with increased sedentary behaviour in free-living individuals and determine whether more metabolic decompensation is observed in FDR+ve individuals.

## Methods

### Participants

Habitually active participants with no history/current engagement in regular structured exercise (>2 h/week) or highly physical employment (determined by questionnaire) were recruited from local advertisements across hospital departments and university campuses. Exclusions included: cardiovascular, respiratory, kidney, liver and/or endocrine complications, smokers and those consuming >14 units/week of alcohol consumption. A first-degree relative was classed as a parent, sibling or child, and participants were divided into two groups: the FDR+ve group included participants with a first-degree relative diagnosed with type 2 diabetes according to medical

records; the FDR–ve group included those with no first-degree relative diagnosed with type 2 diabetes.

The study conformed to the Declaration of Helsinki and was approved by the local research ethics committee (14/NW/1147 and 14/NW/1145). All participants were informed of the methods verbally and in writing before providing written informed consent prior to any assessments being performed. Prior to each visit, participants were required to fast overnight for >8 h, abstain from alcohol and caffeine for 24 h and from exercise for 48 h.

### Study design

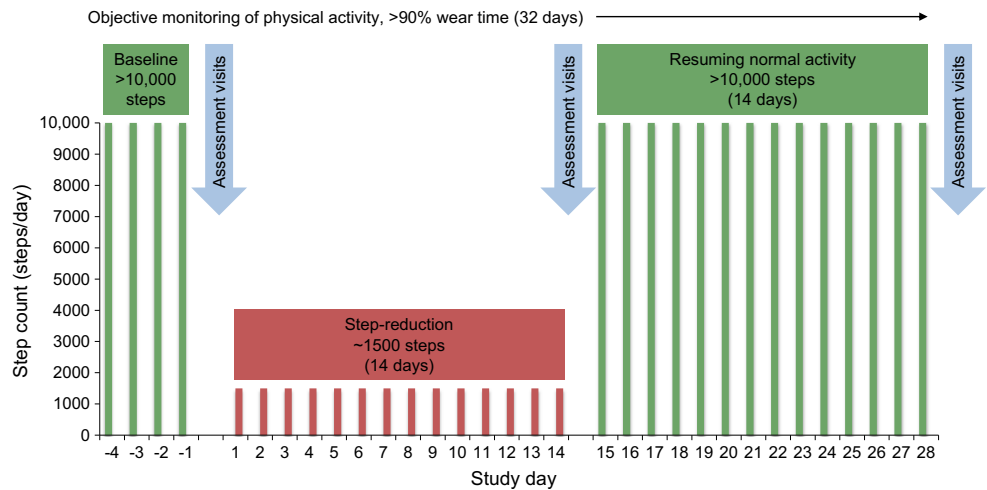
Physical activity was assessed in participants' free-living environment. A mean daily step count >10,000 was required for participants to be eligible. Screening was blinded and consisted of monitoring from midnight to midnight on 4 consecutive days, including 1 weekend day. If eligible, participants underwent their initial assessment visits before being instructed to reduce their activity to ~1500 steps for 14 days, after which the assessments were repeated. Habitual activity was then resumed for a further 14 days before participants underwent their final assessment visits (Fig. 1). There were two assessment visits at each time point: (1) at University Hospital Aintree for anthropometry, fasting bloods, OGTT and  $\dot{V}O_{2peak}$ ; and (2) at University of Liverpool for dual-energy x-ray absorptiometry (DXA) and proton magnetic resonance spectroscopy ( $^1H$ -MRS). The study took place between December 2014 and August 2017, and 16 FDR+ve and 29 FDR–ve participants successfully completed the intervention with all measures available (Fig. 2). Dietary records were taken in the 4 days preceding an assessment visit; participants were instructed to maintain their usual dietary habits throughout the study.

### Experimental measures

**Anthropometric measurements** Weight, height and waist and hip circumferences were measured. Participants then rested for 5 min before BP was determined from an average of three measures.

**Biochemical measurements** Blood samples were collected and analysed using the Olympus AU2700 analyser (Beckman Coulter, High Wycombe, UK) with standard proprietary reagents as follows: glucose with hexokinase, total cholesterol and HDL-cholesterol with cholesterol esterase/oxidase and triacylglycerol with glycerol kinase. LDL-cholesterol was calculated according to the Friedewald formula. Insulin was measured using radioimmunoassay (Invitrogen, Paisley, UK). HOMA-IR was calculated using fasting glucose and insulin concentrations [24]. NEFA was measured using a NEFA assay kit (Randox Daytona, County

**Fig. 1** Study design for step-reduction intervention. Complete objective assessment of physical activity throughout screening (baseline), step reduction and resumption of normal activity; x-axis represents study day and y-axis daily average steps



Antrim, UK). Fasting NEFA and insulin concentrations were used to estimate adipose tissue insulin resistance (adipo-IR) [25].

**OGTT** After fasting blood samples were collected, a 75 g glucose solution was consumed within 5 min and post-ingestion blood samples were drawn at 30, 60, 90 and 120 min. Glucose, insulin and NEFA responses were calculated as AUC. Matsuda index was calculated to estimate whole-body insulin sensitivity; indices of hepatic insulin resistance and skeletal muscle insulin sensitivity were determined as previously described [26, 27].

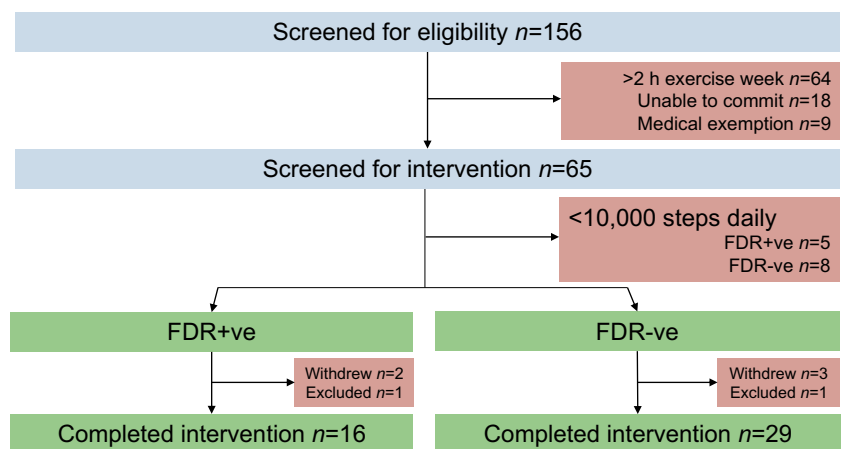
**Cardiorespiratory fitness** A  $\dot{V}O_{2peak}$  cardiopulmonary exercise test (CPET) was performed on a treadmill (Model 770CE, RAM Medisoft Group, Manchester, UK) in a temperature-controlled room. The CPET provided breath-by-breath monitoring and analysis of expiratory gases and ventilation as well as continuous electrocardiographic monitoring (Love Medical Cardiopulmonary Diagnostics, Manchester, UK). The modified Bruce protocol was employed: after an initial 2 min warm up at 2.2 km/h on a flat gradient, stepwise increments in speed and gradient were employed each min.  $\dot{V}O_{2peak}$  was determined by any of: respiratory exchange ratio > 1.15; heart rate > 90%

predicted maximum; plateau in  $\dot{V}O_2$ ; or exhaustion.  $\dot{V}O_{2peak}$  data acquired includes: absolute (l/min), relative to total body mass ( $ml\ min^{-1}\ kg^{-1}$ ), and relative to lean body mass ( $ml\ min^{-1}\ kg^{-1}$ ; termed ' $\dot{V}O_{2peak}\ lean$ ').

**Physical activity monitoring** Physical activity was tracked throughout using a SenseWear mini armband (BodyMedia, Pittsburgh, PA, USA). Instructions were that the armband was to be worn at all possible times, and the inclusion criterion was >90% wear time, which was monitored using SenseWear Professional software (version 8.0). Data collected from the armband included: daily average step count; total energy expenditure; active energy expenditure; and time spent in domains of physical activity, including sleep, lying, sedentary (<1.5 METS), light (1.5–3 METS), moderate (3–6 METS), vigorous (6–9 METS) and very vigorous (>9 METS).

**Dietary analysis** Total energy consumption, carbohydrate, protein and fat content were determined from dietary records by a registered nutritionist (KLM) using Nutritics (Nutrition Analysis Software for Professionals; <https://www.nutritics.com/p/home>; accessed 17 July 2017).

**Fig. 2** Screening, recruitment, retention and completion numbers of FDR+ve and FDR–ve individuals in the study



**DXA** Whole-body scans were performed in line with manufacturer's guidelines using Lunar iDXA (GE Healthcare, Amersham, UK) and were analysed using the instrument's software (version 13.60.033) to determine body fat, lean body mass and bone mineral density. Each scan session was preceded by a calibration routine, using multiple quality-control phantoms that simulate soft tissue and bone.

**<sup>1</sup>H-MRS** Liver and skeletal muscle fat were determined using a 1.5 T Siemens Symphony MRI scanner (Siemens Medical Solutions, Erlangen, Germany) as previously described [28–30]. In brief, intrahepatocellular lipid (IHCL; liver fat) is expressed as the percentage of CH<sub>2</sub> lipid signal amplitude relative to water signal amplitude and intramyocellular lipid (IMCL; skeletal muscle fat) is expressed as CH<sub>2</sub> lipid amplitude relative to total creatine amplitude.

### Sample size calculation

The primary outcome variable for this study was the difference in insulin sensitivity change between the two groups. Based on previous data [18], using Minitab 16 (<https://www.minitab.com/en-us/products/minitab/>), a sample size of 50 was calculated to have ≥80% power to detect a standardised mean difference of 0.89 for insulin sensitivity using Matsuda index with 5% significance level, assuming a 20% drop-out rate (20 participants per group required).

### Statistical analysis

All data were explored for normality using visual inspection of frequency distribution, and logarithmically transformed where appropriate. Independent *t* tests were used to determine baseline differences between FDR+ve and FDR−ve individuals. BMI, waist circumference, hip circumference, fasting insulin, HOMA-IR and adipo-IR were significantly different; coefficient of determination ( $R^2$ ) was calculated from Pearson correlation coefficients to evaluate collinearity between these variables using the variance inflation factor (VIF). BMI had evidence of collinearity with body mass ( $R^2 = 0.645$ ; VIF = 2.8), waist circumference ( $R^2 = 0.703$ ; VIF = 3.4) and hip circumference ( $R^2 = 0.719$ ; VIF = 3.6); BMI was selected as a representative covariate. HOMA-IR had evidence of collinearity with fasting insulin ( $R^2 = 0.943$ ; VIF = 17.5) and adipo-IR ( $R^2 = 0.639$ ; VIF = 2.8); HOMA-IR was selected as a representative covariate. The groups' responses to the intervention were compared by calculating  $\Delta$  values and analysed using a two-factor between-groups (group  $\times$  time) ANCOVA with respective baseline data, BMI and HOMA-IR values entered as covariates. The term intervention refers to step reduction and resumption of normal activity. Statistically significant interactions were assessed using the least significant difference (LSD) approach to multiple pairwise comparisons, and

interactions were used to explore within-group change over time and between-group differences at the specific time points (i.e. step reduction and resumption of normal activity).

We performed sensitivity analysis on significant outcomes using the Bonferroni procedure and found limited variation in variables being adjusted from statistically significant to non-significant.

Paired sample *t* tests were used to assess differences between baseline and resumption of activity because baseline data was entered as a covariate in the ANCOVA models. The  $\alpha$  level of statistical significance was set at  $p < 0.05$ . Data are presented as mean  $\pm$  SD or (95% CI). Data are presented as outcomes for the entire study group (pooled FDR+ve and FDR−ve); differences between and within groups and changes over time are explicitly noted. Where required, data were logarithmically transformed using  $\log_{10}$ . Data were back transformed to original units for reporting. Statistical analysis was performed using SPSS for Windows (Version 24.0, SPSS, Chicago, IL, USA).

## Results

### Baseline characteristics

A total of 45 healthy participants (28 female, 17 male) were included: 16 FDR+ve (10 female, 6 male) and 29 FDR−ve (18 female, 11 male) (Table 1). The mean age of all the participants was  $36 \pm 14$  years, with no statistical difference between the two groups ( $p = 0.166$ ). FDR+ve participants had significantly higher overall body mass, BMI, waist circumference and hip circumference ( $p < 0.05$ ). Fasting insulin, HOMA-IR and adipo-IR were significantly higher in FDR−ve participants. No other variables were statistically different.

### Changes in pattern of physical activity

Mean daily step count was similar between the two groups at baseline: FDR+ve,  $12524 \pm 2137$  vs FDR−ve,  $13036 \pm 2481$  steps ( $p = 0.391$ ). The intervention induced significant changes in all activity measures (Fig. 3). During the step-reduction period, average daily step count decreased by 10285 steps (95% CI 9389, 11182;  $p < 0.001$ ), a reduction of  $81 \pm 8\%$ . In parallel, daily sedentary time increased by an average of 223 min (151, 295;  $p < 0.001$ ) and daily total energy expenditure (TEE) decreased by an average of 2697 kJ (3008, 2385;  $p < 0.001$ ). All activity  $>1.5$  METS (i.e. light, moderate and vigorous) decreased during the step-reduction period (all  $p < 0.001$ ). There were no statistical differences between the groups in average daily step count, TEE or sedentary, light or moderate activity at any of the time points ( $p > 0.05$ ). Time spent sleeping was not significantly different between groups and did not change throughout ( $p > 0.05$ ). Vigorous activity

**Table 1** FDR+ve and FDR–ve participant characteristics at baseline

Characteristic	FDR+ve (n = 16)	FDR–ve (n = 29)	p value
<b>Clinical</b>			
Age (years)	40 ± 14	33 ± 13	0.166
Body mass (kg)	79.3 ± 13.4	68.9 ± 9.8	0.011*
BMI (kg/m <sup>2</sup> )	27 ± 5	24 ± 3	0.018*
Waist circumference (cm) <sup>a</sup>	93 ± 13	84 ± 10	0.029*
Hip circumference (cm) <sup>a</sup>	103 ± 11	95 ± 8	0.015*
Waist:hip ratio	0.91 ± 0.09	0.89 ± 0.07	0.052
SBP (mmHg)	122 ± 14	121 ± 14	0.932
DBP (mmHg)	74 ± 8	76 ± 10	0.438
<b>Glucose regulation</b>			
Whole-body IS <sup>b</sup>	4.1 ± 1.9	3.2 ± 1.9	0.155
Muscle IS index	0.07 ± 0.06	0.06 ± 0.04	0.749
Hepatic IR index	33.4 ± 9.7	40.5 ± 9.4	0.058
NEFA AUC (mmol/l × min)	27 ± 12	24 ± 11	0.422
Fasting glucose (mmol/l)	5.0 ± 0.6	5.0 ± 0.5	0.994
Fasting insulin (pmol/l) <sup>a</sup>	83 ± 35	139 ± 63	0.002*
HOMA-IR <sup>a</sup>	2.8 ± 1.1	4.3 ± 2.1	0.003*
Adipo-IR <sup>a</sup>	41.6 ± 26.4	88.2 ± 55.6	0.004*
Glucose AUC (mmol/l × min)	780 ± 235	799 ± 177	0.928
Insulin AUC (pmol/l × min)	57368 ± 27486	63833 ± 20069	0.468
<b>Lipid profile</b>			
Cholesterol (mmol/l)	5.1 ± 1.1	4.9 ± 0.8	0.681
Triacylglycerol (mmol/l)	1.0 ± 0.5	1.0 ± 0.6	0.524
HDL-cholesterol (mmol/l)	1.8 ± 0.6	1.8 ± 0.5	0.907
LDL-cholesterol (mmol/l)	2.8 ± 1.1	2.6 ± 0.8	0.475
Cholesterol:HDL-cholesterol ratio	3 ± 1	3 ± 1	0.400
<b>Body composition</b>			
Total body fat (%)	33 ± 10	30 ± 8	0.279
Android fat (%)	36 ± 12	30 ± 11	0.134
Gynoid fat (%)	35 ± 11	34 ± 9	0.581
Total lean mass (kg)	50.5 ± 9.1	46.1 ± 8.9	0.150
Leg lean mass (kg)	17.5 ± 3.3	16.0 ± 3.3	0.164
Arm lean mass (kg)	5.8 ± 1.8	5.5 ± 1.6	0.352
IHCL (%) <sup>a</sup>	3.0 ± 3.6	0.7 ± 1.0	0.064
IMCL (%)	9.1 ± 5.0	6.4 ± 2.6	0.109
<b>Cardiorespiratory fitness and physical activity</b>			
$\dot{V}O_2$ (l/min)	2.5 ± 0.4	2.4 ± 0.6	0.676
$\dot{V}O_{2peak}$ (ml min <sup>-1</sup> kg <sup>-1</sup> )	32.1 ± 6.6	35.3 ± 6.7	0.136
$\dot{V}O_{2peak}$ lean (ml min <sup>-1</sup> kg <sup>-1</sup> )	50.1 ± 5.3	52.6 ± 6.8	0.189
Energy expenditure (kJ/day)	12251 ± 2101	11383 ± 2913	0.408
Daily steps (steps)	12524 ± 2137	13036 ± 2481	0.391
Daily sedentary time (min)	606 ± 93	586 ± 116	0.528
Daily light activity (min)	231 ± 78	240 ± 76	0.650
Daily moderate activity (min)	130 ± 41	139 ± 61	0.306
Daily vigorous activity (min)	21 ± 27	24 ± 31	0.550

Data shown are mean ± SD and p values between groups

<sup>a</sup> Variables analysed following logarithmic transformation

<sup>b</sup> Matsuda index

\*  $p < 0.05$

IR, insulin resistance; IS, insulin sensitivity

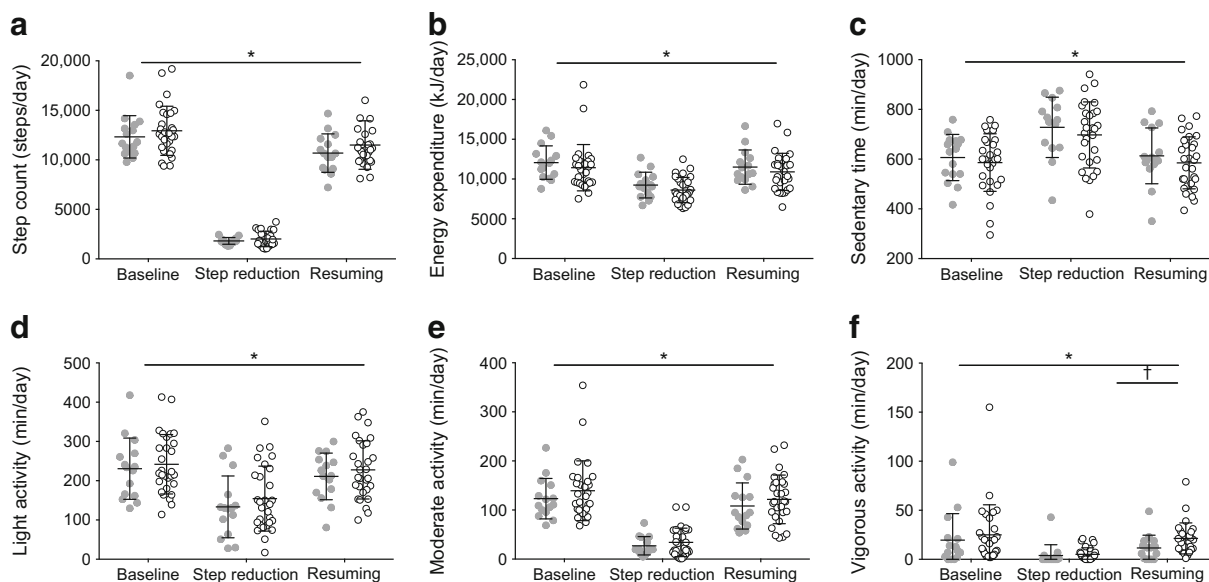
was significantly higher in FDR–ve participants ( $p = 0.001$ ); when explored, the between-group difference was not present at step reduction ( $p = 0.988$ ) but rather at resumption of activity ( $p = 0.006$ ), with FDR–ve participants increasing their vigorous activity more than FDR+ve participants at this stage (Fig. 3f).

There were no significant differences in any of the physical activity measures between baseline and following resumption

of activity, suggesting habitual physical activity levels were successfully restored ( $p > 0.05$ ).

### Dietary analysis

Total energy consumption did not change throughout ( $p = 0.330$ ) and there was no difference between groups ( $p = 0.372$ ). Mean ± SD macronutrient percentages were 56 ±



**Fig. 3** Physical activity data for FDR+ve and FDR–ve individuals at baseline, following step reduction and resuming activity, including daily average step count (a), TEE (b), daily sedentary time (<1.5 METS) (c), daily light activity (1.5–3 METS) (d), daily moderate activity (3–6

METS) (e) and daily vigorous activity (>6 METS) (f). Data are presented as mean  $\pm$  SD. Grey circles, FDR+ve; white circles, FDR–ve. \* $p < 0.05$  main effect of time; † $p < 0.05$  between groups

15% carbohydrate,  $24 \pm 10\%$  protein, and  $20 \pm 9\%$  fat, and these did not change throughout ( $p = 0.235$ ,  $p = 0.268$ ,  $p = 0.924$ , respectively) and there was no difference between groups ( $p = 0.660$ ,  $p = 0.179$ ,  $p = 0.177$ , respectively).

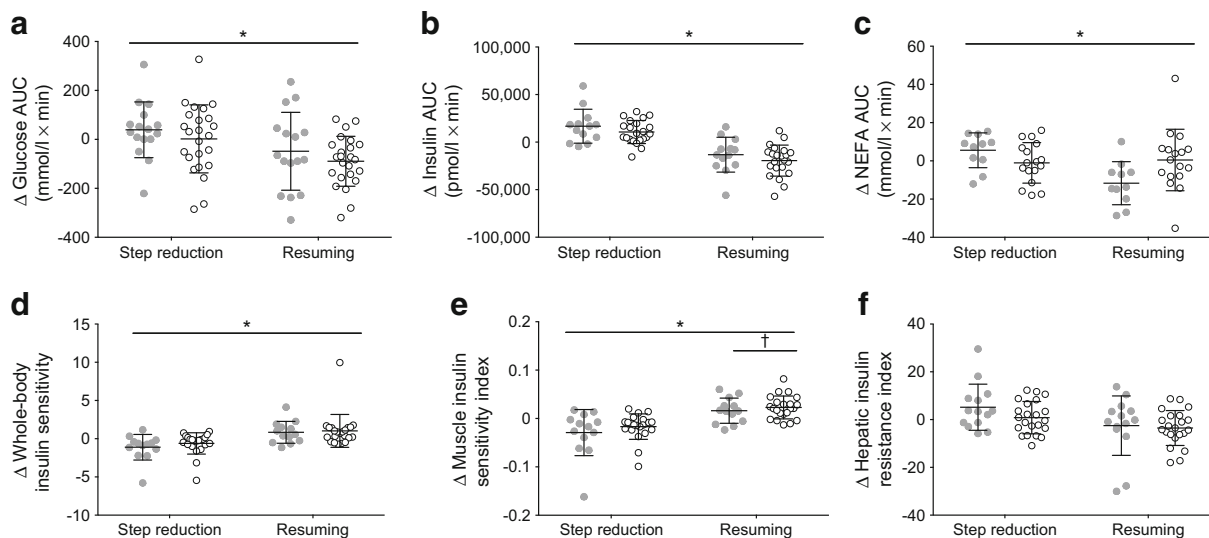
following step reduction ( $p < 0.001$ ), and this was accompanied by a significant increase in glucose AUC ( $p = 0.025$ ) and insulin AUC ( $p < 0.001$ ). However, no between-group differences in these measures were observed (Fig. 4).

### Whole-body insulin sensitivity

In both groups, the primary outcome measure of whole-body insulin sensitivity (Matsuda index) significantly declined

### Skeletal muscle insulin sensitivity

Muscle insulin sensitivity was significantly reduced following step reduction ( $p < 0.001$ ). Overall, there was a significant



**Fig. 4** Metabolic responses of FDR+ve and FDR–ve individuals at baseline, following step reduction and resuming activity. Derived from 2 h OGTT: glucose AUC (a), insulin AUC (b), NEFA AUC (c), whole-body insulin sensitivity (Matsuda index) (d), muscle insulin sensitivity index

(e) and hepatic insulin resistance index (f). Data are presented as mean  $\pm$  SD. Grey circles, FDR+ve; white circles, FDR–ve. \* $p < 0.05$  main effect of time; † $p < 0.05$  between groups

between-group difference of 0.015 (95% CI 0.006, 0.023;  $p = 0.001$ ), with FDR+ve participants displaying lower levels of muscle insulin sensitivity. Following resumption of activity, there was a significant difference of 0.023 (0.003, 0.042;  $p = 0.023$ ) between the two groups, with FDR+ve individuals displaying lower muscle insulin sensitivity (Fig. 4e).

### Adipose tissue insulin resistance

The intervention had a borderline significant effect on NEFA AUC ( $p = 0.050$ ; Fig. 4c).

### Hepatic insulin resistance

The intervention had no significant effect on hepatic insulin resistance in either group ( $p = 0.060$ ), but hepatic insulin resistance index was, on average, 3.8 greater in FDR+ve individuals across the intervention (95% CI 1.7, 6.4;  $p = 0.007$ ) (Fig. 4f).

### Clinical characteristics

Body mass, BMI and waist to hip ratio did not change in either group ( $p = 0.611$ ,  $p = 0.553$  and  $p = 0.385$ , respectively). Pooled waist and hip circumference measures increased by 0.7 cm (95% CI 0.4, 1.0) and 0.4 cm (0.1, 0.7), respectively ( $p < 0.001$ ) after step reduction. Systolic BP (SBP) increased by 4 mmHg (0, 8;  $p = 0.037$ ) following step reduction, while diastolic BP (DBP) remained unchanged ( $p = 0.982$ ). There were no between-group differences in any of the changes in clinical characteristic.

### Fasting glucose metabolism

Fasting glucose ( $p = 0.294$ ) and adipo-IR ( $p = 0.067$ ) were not significantly altered by the intervention. Fasting insulin ( $p = 0.016$ ) and HOMA-IR ( $p = 0.027$ ) significantly changed over time.

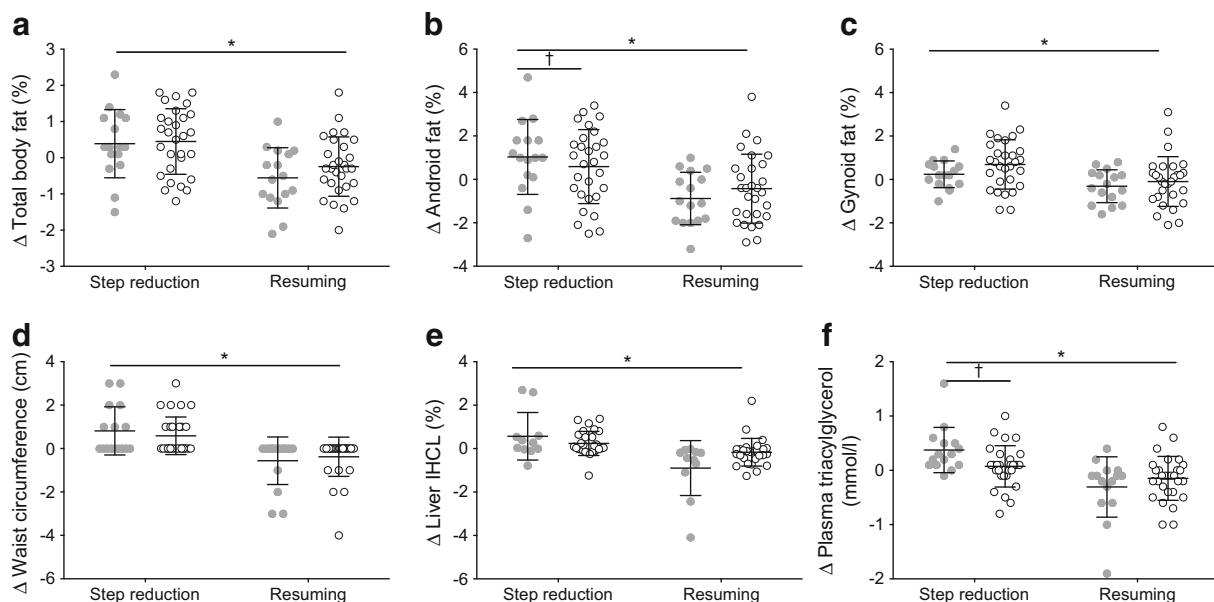
### Lipid profile

Total cholesterol significantly increased by 0.5 mmol/l (95% CI 0.1, 0.9;  $p = 0.041$ ) following step reduction: HDL-cholesterol remained unchanged ( $p = 0.641$ ) while LDL-cholesterol significantly increased by 0.3 mmol/l (0.1, 0.6;  $p = 0.013$ ). Triacylglycerol pooled outcomes also increased following step reduction by 0.5 mmol/l (0.2, 0.7;  $p = 0.002$ ) but a between-group difference was present, with FDR+ve participants having a 0.3 mmol/l greater change (0.1, 0.6;  $p = 0.044$ ) (Fig. 4).

### Body composition

**Lean body mass** Following step reduction, total lean mass decreased by 0.3 kg (95% CI 0.1, 0.6;  $p = 0.005$ ), and lower limb lean mass by 0.2 kg (0.1, 0.3;  $p = 0.004$ ), but there was no significant change in arm lean mass ( $p = 0.502$ ).

**Regional fat mass** Step reduction induced similar changes in both groups: total body fat increased by 0.9% (95% CI 0.6, 1.3;  $p < 0.001$ ) (Fig. 5a), android fat increased by 1.7% (1.1, 2.3;  $p < 0.001$ ) (Fig. 5b), and gynoid fat increased by 0.6% (0.3, 0.8;  $p = 0.001$ ) (Fig. 5c). A between-group difference was



**Fig. 5** Responses of FDR+ve and FDR-ve individuals following step reduction and resumption of activity. Change in: (a) total body fat; (b) android (waist) fat; (c) gynoid (hip) fat; (d) waist circumference; (e)

IHCL; and (f) plasma triacylglycerol. Data are presented as mean  $\pm$  SD. Grey circles, FDR+ve; white circles, FDR-ve. \* $p < 0.05$  main effect of time; † $p < 0.05$  between groups



found, with FDR+ve individuals accumulating 1.5% more android fat (0.4, 2.6;  $p = 0.008$ ) following step reduction.

**Liver and skeletal muscle fat** Following the period of reduced activity, IHCL (liver fat) increased significantly by 0.7% (95% CI 0.2, 1.2;  $p = 0.001$ ). Changes in IMCL (skeletal muscle fat) did not reach statistical significance ( $p = 0.094$ ).

### Cardiorespiratory fitness

The period of reduced physical activity significantly lowered cardiorespiratory fitness across the study population. This was the case whichever way fitness was expressed (absolute, relative to body weight or lean body mass):  $\dot{V}O_2$  by 0.3 l/min (95% CI 0.5, 1.0;  $p = 0.002$ ),  $\dot{V}O_{2peak}$  per kg body mass by

2.2 ml min<sup>-1</sup> kg<sup>-1</sup> (0.9, 3.6;  $p = 0.002$ ) and  $\dot{V}O_{2peak}$  per kg lean body mass by 2.9 ml min<sup>-1</sup> kg<sup>-1</sup> (0.9, 5.0;  $p = 0.006$ ), with no between-group differences for any of these measures.

### Return to normal activity

All clinical, biochemical, body composition and cardiorespiratory fitness measures returned to baseline on resumption of habitual physical activity (Table 2).

### Within-group comparisons

Several within-group responses were different in terms of reaching statistical significance. All measures of cardiorespiratory fitness (absolute, relative to body weight and relative to

**Table 2** Change in variables for FDR+ve and FDR–ve individuals on step reduction and resumption of activity

Variable	FDR+ve		FDR–ve		Between-group difference		<i>p</i> value Group [Time]
	Δ Step reduction	Δ Resuming activity	Δ Step reduction	Δ Resuming activity	Step reduction	Resuming	
<b>Clinical characteristics</b>							
Body mass (kg)	−0.1 ± 1.0	−0.1 ± 1.2	0.1 ± 1.0	0.3 ± 1.2	−0.2 (−0.8, 0.5)	−0.4 (−1.2, 0.4)	0.241 [0.611]
BMI (kg/m <sup>2</sup> )	0 ± 0.3	0 ± 0.4	0 ± 0.3	0 ± 0.4	0 (−0.3, 0.2)	0 (−0.4, 0.2)	0.369 [0.553]
Waist circumference (cm) <sup>a</sup>	0.6 ± 1.0	−0.3 ± 0.9	0.7 ± 1.0	−0.4 ± 0.8	−0.1 (−0.8, 0.6)	0.1 (−0.5, 0.7)	0.934 [ $<0.005$ ] <sup>*</sup>
Hip circumference (cm) <sup>a</sup>	0.3 ± 0.5	−0.3 ± 0.6	0.1 ± 0.5	−0.1 ± 0.6	0.2 (−0.2, 0.5)	−0.2 (−0.7, 0.2)	0.670 [ $<0.005$ ] <sup>*</sup>
SBP (mmHg)	−1 ± 9	−3 ± 8	2 ± 8	−4 ± 8	−2 (−8, 4)	1 (−4, 7)	0.654 [0.037] <sup>*</sup>
DBP (mmHg)	−1 ± 8	−2 ± 9	−1 ± 8	−1 ± 8	1 (−5, 6)	−1 (−7, 5)	0.846 [0.982]
<b>Glucose metabolism</b>							
Fasting glucose (mmol/l)	−0.1 ± 0.5	0.1 ± 0.4	−0.1 ± 0.5	−0.1 ± 0.4	0.1 (−0.3, 0.3)	0.2 (−0.1, 0.5)	0.171 [0.294]
Fasting insulin (pmol/l) <sup>a</sup>	34 ± 9	−35 ± 11	17 ± 8	−11 ± 11	18 (−24, 59)	−25 (−79, 30)	0.669 [0.016] <sup>*</sup>
HOMA-IR <sup>a</sup>	1.1 ± 2.1	−1.1 ± 2.6	0.4 ± 2.1	−0.4 ± 2.6	0.7 (−0.7, 2.1)	−0.7 (−2.5, 1.0)	0.911 [0.027] <sup>*</sup>
Whole-body IS <sup>b</sup>	−0.7 ± 1.2	0.3 ± 2.3	−0.9 ± 1.2	1.3 ± 2.2	0.1 (−0.7, 1.0)	−1.1 (−2.7, 0.5)	0.254 [ $<0.005$ ] <sup>*</sup>
<b>Lipid profile</b>							
Cholesterol (mmol/l)	0.2 ± 0.8	−0.2 ± 0.9	0.2 ± 0.8	−0.3 ± 0.8	−0.1 (−0.6, 0.4)	0.9 (−0.5, 0.7)	0.962 [0.041] <sup>*</sup>
HDL-cholesterol (mmol/l)	−0.1 ± 0.3	0 ± 0.4	0 ± 0.3	0 ± 0.4	0 (−0.2, 0.2)	0 (−0.3, 0.2)	0.495 [0.641]
LDL-cholesterol (mmol/l)	0.8 ± 0.5	−0.1 ± 0.6	1.2 ± 0.5	−0.2 ± 0.5	−0.2 (−0.5, 0.2)	0.2 (−0.2, 0.5)	0.930 [0.013] <sup>*</sup>
<b>Body composition</b>							
Total lean mass (kg)	−0.6 ± 0.9	0.4 ± 1.2	−0.1 ± 0.9	0.4 ± 1.1	−0.4 (−1.0, 0.2)	0.2 (−0.6, 1.0)	0.598 [0.005] <sup>*</sup>
Leg lean mass (kg)	−0.1 ± 0.4	0.2 ± 0.5	−0.2 ± 0.4	0.2 ± 0.5	0.2 (−0.1, 0.4)	−0.1 (−0.4, 0.3)	0.589 [0.004] <sup>*</sup>
Arm lean mass (kg)	0.1 ± 0.2	0.1 ± 0.2	−0.1 ± 0.2	0.1 ± 0.2	0.1 (−0.1, 0.2)	0.1 (−0.1, 0.2)	0.607 [0.502]
<b>Cardiorespiratory fitness</b>							
$\dot{V}O_2$ (l/min)	−0.2 ± 0.3	0.3 ± 0.4	−0.1 ± 0.3	0.1 ± 0.4	−0.1 (−0.3, 0.1)	0.2 (−0.1, 0.5)	0.376 [0.002] <sup>*</sup>
$\dot{V}O_{2peak}$ (ml min <sup>-1</sup> kg <sup>-1</sup> )	−3.0 ± 4.8	3.6 ± 5.6	−1.4 ± 4.6	0.9 ± 5.5	−1.6 (−4.8, 1.6)	2.7 (−1.1, 6.7)	0.370 [0.002] <sup>*</sup>
$\dot{V}O_{2peak}$ lean (ml min <sup>-1</sup> kg <sup>-1</sup> )	−4.0 ± 7.3	4.9 ± 8.5	−1.9 ± 6.9	1.1 ± 8.1	−2.2 (−7.1, 2.7)	3.9 (−1.8, 9.6)	0.394 [0.006] <sup>*</sup>

Data shown are: mean change ± SD; between-group difference (95% CI); and *p* values for main group difference [pooled effect of time]. Values are presented to the nearest accuracy of measurement; where values do not demonstrate a change, data are presented with one decimal place; ‘0’ is not absolute 0 but values 0 ± 0.05

<sup>a</sup> Variables analysed following logarithmic transformation

<sup>b</sup> Matsuda index

IS, insulin sensitivity

lean body) were significant in FDR+ve but not FDR–ve participants. For fasting insulin: FDR+ve  $70 \pm 130$  pmol/l ( $p = 0.046$ ) vs FDR–ve  $27 \pm 127$  pmol/l ( $p = 0.278$ ). For NEFA AUC: FDR+ve  $17 \pm 29$  mmol/l  $\times$  min ( $p = 0.017$ ) vs FDR–ve  $2 \pm 30$  mmol/l  $\times$  min ( $p = 0.715$ ).

## Discussion

The results of this study demonstrate that a short-term reduction in physical activity with increased sedentary behaviour, resulting in a decline in cardiorespiratory fitness, leads to unfavourable changes in body composition with reduced lower limb lean body mass, accretion of body fat, particularly centrally, and of liver fat. These changes are small, but nevertheless large enough to be clinically significant and thus to pose a serious health threat over a longer term [31]. The metabolic sequelae of these changes include whole-body insulin resistance and dyslipidaemia (increased triacylglycerol and LDL-cholesterol). Importantly, participants in the FDR+ve group demonstrated similar decompensation in whole-body insulin sensitivity when compared with those in the FDR–ve group. However, some differences were noted: greater rise in plasma triacylglycerol, more android fat deposition and reduced skeletal muscle insulin sensitivity. Although the changes observed in this short period were reversed on resumption of normal physical activity, the pattern of the changes provides mechanistic insight into how chronically increased sedentary behaviour contributes to development of the metabolic syndrome, type 2 diabetes, non-alcoholic fatty liver disease and, potentially, cardiovascular disease [32, 33].

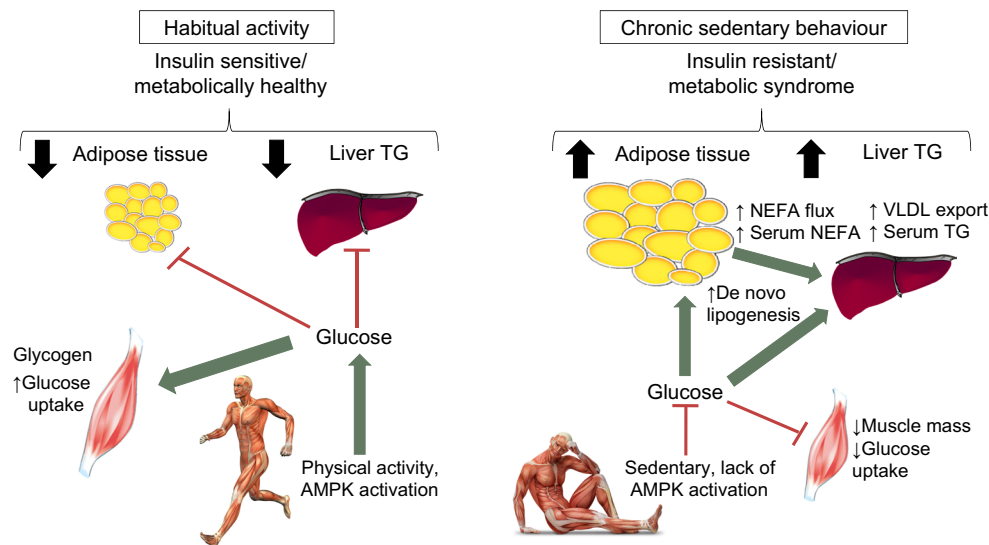
Previous research has focused on increasing habitual physical activity or structured exercise, yet little is known about the consequences of decreasing habitual physical activity. We used validated measures of tissue-specific insulin sensitivity to demonstrate that inactivity resulted in decreased skeletal muscle insulin sensitivity. Skeletal muscle insulin sensitivity was significantly altered in both groups; notably, in FDR+ve participants, muscle insulin sensitivity did not return to baseline levels on resumption of habitual activity. While this may be an intrinsic defect, the FDR+ve individuals also had lower levels of vigorous activity on resumption of activity. As vigorous activity induces greater uptake of skeletal muscle glucose [34], this could have influenced this between-group difference.

We also observed increased adipose tissue insulin resistance with increased sedentary time, with lesser suppression of lipolysis and greater release of NEFA during the OGTT. We observed expansion of the adipose tissue mass and small but important increases in liver fat. This peripheral insulin resistance and accumulation of liver fat was accompanied by a rise in plasma triacylglycerol, LDL-cholesterol and an increase in BP, all components of the metabolic syndrome (although no

individuals developed it). These data are consistent with the hypothesis that increased sedentary time results in a reduced metabolic demand of skeletal muscle, leading to lower glucose uptake (by the development of peripheral insulin resistance) and repartitioning of energy substrates into storage in adipose tissue and in the liver by ‘overspill’, so that energy will be available when activity resumes [7]. This paradigm is supported by measurements of postprandial muscle and liver glycogen/lipid ( $^{13}\text{C}/^1\text{H}$ -MRS) and de novo lipogenesis (deuterated water,  $^2\text{H}_2\text{O}$ ), which show that skeletal muscle insulin resistance promotes hepatic steatosis and hyperlipidaemia [5, 8]. Moreover, this process can be reversed with exercise [6]. We propose that increased sedentary time leads to central and hepatic fat accumulation, development of insulin resistance and emergence of components of the metabolic syndrome, including dyslipidaemia, by a similar mechanism (Fig. 6).

This step-reduction study somewhat corroborates previous reports regarding the effects on cardiorespiratory fitness and body composition [16, 17]. The effects of step reduction are amplified when combined with overfeeding [18], leading to undesirable metabolic changes, including impaired insulin sensitivity independent of changes in body composition. In our study, the detrimental changes were recovered on resumption of habitual activity. However, with overfeeding, body composition did not return to normal [18] and in older adults, glycaemic control and myofibrillar protein synthesis remained disrupted [20]. We believe our findings represent a significant advance for several reasons. Previous step-reduction studies have been limited in sample size, the largest comparable studies being in ten young men [16, 17]; the present study is more representative of the general population, involving 45 individuals, both male and female, with a mean age of 36 years. We acknowledge their relatively high levels of habitual activity, despite being formally untrained. Complete objective monitoring of physical activity throughout ensured that the baseline criteria of  $>10,000$  steps/day was met and likewise  $\sim 1500$  steps/day ( $>80\%$  step reduction) during inactivity; habitual activity was monitored during the final 14 days, with no instruction or guidance; further, we characterised the time participants spent in domains of activity. Moreover, the participants were comprehensively phenotyped, including all measures of the metabolic syndrome, region-specific distribution and quantification of lean muscle and adipose tissue by DXA, and liver fat was quantified by  $^1\text{H}$ -MRS, a highly sensitive non-invasive method [35].

The study is also the first to examine whether FDR+ve people are more susceptible to the detrimental effects of reduced physical activity than FDR–ve individuals. The findings do not suggest that FDR+ve individuals show greater adverse changes with decreased physical activity compared with FDR–ve individuals when we consider the effects on the primary outcome, whole-body insulin sensitivity. However, there were differences in a subset of measures, with



**Fig. 6** Mechanisms of habitual activity vs chronic sedentary behaviour. A consequence of sedentary behaviour is lack of AMP-activated protein kinase (AMPK) activation and glucose uptake from skeletal muscle; this triggers insulin resistance and provides a substrate for de novo lipogenesis in adipose tissue and liver. Consequently, there is expansion of adipose tissue mass, an increase in NEFA flux and serum NEFA, intra-hepatic

lipid accumulation and increased lipid exports as VLDL triacylglycerol particles and serum triacylglycerol with systemic insulin resistance. On the contrary, being habitually active promotes AMPK activation and uptake of glucose in skeletal muscle; insulin sensitivity is therefore preserved and less glucose is diverted to metabolically unfavourable depots. TG, triacylglycerol

greater increases in triacylglycerol and android fat following step reduction and a more marked change in cardiorespiratory fitness. These differences may be important, not only associated with an increased risk for future development of insulin resistance and type 2 diabetes, but also long term for cardiovascular disease and overall mortality [36]. The presence of good metabolic health in these relatively active individuals at baseline, the decline with increased sedentary time and restoration after resumption of habitual physical activity all, to some extent, validate current physical activity recommendations. The findings reinforce the importance of meeting the physical activity recommendations for all participants.

We acknowledge limitations to the study and its design. The target recruitment for FDR+ve participants was 20. The difficulty recruiting for this study was noteworthy. Physically active people were often reluctant to induce physiological harm, even for a brief period; subsequently, between-group comparisons may have been underpowered. We have presented evidence of within-group variation to highlight where degree of response is varied between the two groups, but recognise that this does not constitute a between-group difference. Furthermore, the practical intrusion of ‘real life’ (i.e. occupation, caring commitments) during the inactivity phase also explains why not all individuals could precisely reduce to 1500 steps/day.

We note the lack of a control group: an additional group, comprising participants who continue their habitual physical activity and having the same serial measurements, would have provided useful insight. Additionally, the gold standard measure of insulin sensitivity is the hyperinsulinaemic–

euglycaemic clamp. Owing to the participant burden of conducting serial clamps, we opted to assess whole-body insulin sensitivity from an OGTT using the Matsuda index, which has been shown to correlate with hyperinsulinaemic–euglycaemic clamp [26]. Calculations of hepatic insulin resistance and skeletal muscle insulin sensitivity have also been validated [27]. The indices derived from OGTT are valid, repeatable and easily assessable measures of insulin sensitivity, which is advantageous for direct comparison with follow-up studies. Future studies may wish to incorporate low- and high-dose hyperinsulinaemic–euglycaemic clamps to measure precisely hepatic and skeletal muscle insulin sensitivity and consider dynamic measurements of de novo lipogenesis and of VLDL triacylglycerol kinetics to determine effects on liver fat and lipoprotein metabolism. These data provide a good rationale to determine the molecular and tissue-specific adaptations in skeletal muscle and subcutaneous adipose tissue in response to short-term physical inactivity.

In summary, by taking untrained individuals with a habitually active lifestyle (>10,000 steps/day) and drastically reducing their step count, this study provides direct evidence of a number of unfavourable adaptations to body composition and cardiometabolic risks with increased sedentary behaviour. These changes are reversed with resumption of habitual activity. The data suggest a mechanistic framework for understanding the deleterious cardiometabolic adaptations that occur with chronic physical inactivity and sedentary behaviour. Furthermore, both FDR+ve and FDR–ve individuals are susceptible to the risks of increased sedentary behaviour. The study findings reinforce the health benefits of meeting

physical activity recommendations and emphasise the importance of reducing daily sedentary time.

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**Contribution statement** DJC, GJK and JPHW conceived the study or parts of the study. KABD, VSS, JAN and DJC generated the data. GJK analysed the MRS data. KLM analysed the nutritional data. JCGH and JAH acquired DXA data. KABD and AT statistically analysed and interpreted the data. All authors participated in preparation of the manuscript and approved the final version for publication. KBD is the guarantor of this work.

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