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FEEDING INFLUENCES ADIPOSE TISSUE RESPONSES TO EXERCISE IN OVERWEIGHT MEN

Running Head: Dietary status affects acute adipose responses to exercise

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ABSTRACT

Feeding profoundly affects metabolic responses to exercise in various tissues but the effect of feeding status on human adipose tissue responses to exercise has never been studied. Ten healthy overweight men aged 26 ± 5 years (mean ± SD) with a waist circumference of 105 ± 10 cm walked at 60% of maximum oxygen uptake under either FASTED or FED conditions in a randomised, counterbalanced design. Feeding comprised 648 ± 115 kcal 2 h before exercise. Blood samples were collected at regular intervals to examine changes in metabolic parameters and adipokine concentrations. Adipose tissue samples were obtained at baseline and one hour post-exercise to examine changes in adipose tissue mRNA expression and secretion of selected adipokines *ex-vivo*. Adipose tissue mRNA expression of PDK4, ATGL, HSL, FAT/CD36, GLUT4 and IRS2 in response to exercise were lower in FED compared to FASTED conditions (all $p \leq 0.05$). Post-exercise adipose IRS2 protein was affected by feeding ($p \leq 0.05$), but Akt2, AMPK, IRS1, GLUT4, PDK4 and HSL protein levels were not different. Feeding status did not impact serum and *ex-vivo* adipose secretion of IL-6, leptin or adiponectin in response to exercise. This is the first study to show that feeding prior to acute exercise affects post-exercise adipose tissue gene expression and we propose that feeding is likely to blunt long-term adipose tissue adaptation to regular exercise.

Keywords: Exercise, postprandial, adipose tissue, feeding

INTRODUCTION

It has become clear in the last 10 years or so that adipose tissue plays an active role in many physiological processes and pathological states (74, 78) and dysfunction within this tissue is characterised by tissue-specific insulin resistance, local inflammation, fibrosis, and the abnormal secretion of adipokines (36). Adipose tissue secretes dozens of mediators including the archetypal adipokines, adiponectin and leptin (16, 38, 46). Adiponectin is exclusively derived from adipose tissue and circulates in high concentrations (10−20 $mg \cdot L^{-1}$). In other cases, the quantitative amounts secreted by adipose can be substantial, for example, subcutaneous adipose contributes up to a third of circulating interleukin-6 (IL-6) (45).

Feeding has a pronounced effect on the whole-body metabolic responses to exercise and reduces the contribution of fat towards metabolism (3, 23, 77). In addition, feeding influences the skeletal muscle responses to various forms of exercise (9, 10, 13). For example, pyruvate dehydrogenase kinase isozyme 4 (PDK4) is significantly up-regulated in muscle with exercise in fasted but not fed conditions (10). Other feeding-related changes in gene expression in muscle after exercise have been reported including altered expression of glucose transporter type 4 (GLUT4), PDK4, fatty acid translocase/CD36 (FAT/CD36), carnitine palmitoyltransferase 1 (CPT-1), uncoupling protein-3 (UCP3) and AMP-activated protein kinase (AMPK) (9). Collectively, therefore, there is strong evidence that feeding affects the responses to exercise in skeletal muscle. Adipose tissue plays a crucial role during exercise (18) and this led us to speculate that pre-exercise feeding may also affect the adipose tissue responses to acute exercise.

During moderate intensity exercise, adipose tissue provides much of the energy for working skeletal muscle through the mobilisation of stored triacylglycerol (27). Exercise also initiates

63 a number of other responses in adipose tissue such as increased blood flow and altered
64 expression of various adipokines within abdominal subcutaneous adipose tissue (66). It is
65 possible that these acute exercise-induced changes could be part of the mechanism through
66 which exercise improves health (66). However, all prior studies of adipose tissue responses to
67 exercise in humans have been conducted in the fasted state (8, 19, 25, 31, 32, 39). The effect
68 of feeding status on the response of human adipose tissue to exercise has never been studied.
69 This is despite the fact that feeding has a profound effect on adipose tissue (1, 30) and that we
70 spend the vast majority (~70%) of a 24-h period in a fed state (58). Consequently, the aim of
71 the present study was to investigate whether feeding influences the adipose tissue responses
72 to exercise.

METHODS

Ethical approval

The protocol was approved by Bristol Research Ethics Committee (REC reference number: 13/SW/0020) in accordance with the *declaration of Helsinki*. This trial is registered at ClinicalTrials.gov (ID: NCT02870075). All participants provided verbal and written informed consent before taking part.

Experimental design

Ten men aged 18 to 35 years with increased central adiposity were recruited via local advertisement. Participants attended the laboratory on three occasions for initial assessment of maximum oxygen uptake ($\dot{V}O_{2max}$) and two subsequent main trials. The trial days involved walking for 60 min at 60% $\dot{V}O_{2max}$ under either FASTED or FED conditions in a randomised, counterbalanced design separated by a 3−4 week wash-out period. This intensity and duration of exercise was selected because it relies heavily on fatty acids mobilised from adipose tissue and also because it is the type of exercise recommended in recent position stands (20, 27). Blood and adipose tissue were sampled at baseline and after exercise to examine the impact of prior feeding. There are no data regarding how different dietary status (fasted *versus* fed) affects the adipose responses to exercise. However, a previous study using a similar meal showed that feeding had an enormous effect on the use of lipid during exercise – and thus this indicates that the role of adipose tissue during exercise would be potentially very different (3, 77). Based on these results, in order to see an effect on lipid oxidation during exercise with 95% power and 5% alpha, we would require between 6−8 participants. We recruited ten men to account for greater variability in other outcome measures.

Inclusion and exclusion criteria

To be eligible to take part, participants were required to be overweight with a waist circumference of 94−128 cm (75). Participants were also required to be weight stable (63) for at least 3 months (mass stable ± 3%). Participants completed a health questionnaire to exclude any existing cardiovascular and metabolic diseases and a Physical Activity Readiness Questionnaire (PAR-Q) to make sure that participants were able to exercise safely. Individuals taking any medications known to influence lipid/carbohydrate metabolism or immune function and smokers were excluded. A summary of participants' physical characteristics is shown in Table 1.

Table 1. Participant physical characteristics (n = 10)

Characteristics	Mean ± SD
Age (years)	26 ± 5
Body mass (kg)	102.4 ± 10.6
Waist circumference (cm)	105 ± 10
Hip circumference (cm)	115 ± 6
Body mass index ($kg \cdot m^{-2}$)	30.2 ± 3.7
Fat in L1-L4 region (kg)	3.4 ± 1.5
$\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	42.4 ± 6.4
Systolic blood pressure (mmHg)	132 ± 21
Diastolic blood pressure (mmHg)	73 ± 12

Fat in L1-L4 regions was determined using DEXA as previously described (22).

115 **Pre-trial assessments**

116

117 ***$\dot{V}O_{2max}$ measurement***

118 An incremental continuous treadmill test until the point of volitional fatigue was used to
119 determine $\dot{V}O_{2max}$. For most participants, a treadmill speed of 4 $km \cdot h^{-1}$ and gradient of 8.5%
120 was appropriate. This grade was maintained and the speed was increased by 1 $km \cdot h^{-1}$ after
121 every 3 min stage. One minute expired air samples were collected into Douglas bags (Hans
122 Rudolph, MO, USA) and rate of perceived exertion (RPE) and heart rate were measured in
123 the final minute of each stage and also at the point of volitional fatigue, defined as when the
124 participant indicated that only 1 min remained until fatigue. Samples were analysed for
125 relative expired fractions of oxygen and carbon dioxide (Servomex, Crowborough, UK) and
126 the total volume within the bag was measured using a dry gas meter (Harvard Apparatus,
127 Kent, UK).

128

129 ***Physical activity assessment***

130 As part of the pre-trial assessments, participants wore a combined heart rate/accelerometer
131 monitor for one week to assess their habitual physical activity energy expenditure (Actiheart,
132 Cambridge Neurotechnology Ltd., Cambridge, UK). This was attached to the chest via 2
133 adhesive ECG pads for 24 h per day except for during showering/bathing/swimming (65).

134

135 ***Body composition analysis***

136 Body mass was measured using digital scales following an overnight fast and post-void
137 (TANITA corp., Tokyo, Japan). Waist and hip circumference was assessed according to
138 World Health Organisation guidelines (75). Body composition was determined using Dual
139 Energy X-ray Absorptiometry (DEXA; Discovery, Hologic, Bedford, UK). Abdominal

subcutaneous and visceral adipose tissue mass was estimated from a central region between L1-L4 (22).

Trial days

In the 72 h prior to each main trial, participants were asked to refrain from performing any strenuous physical activity and from consuming alcohol/caffeine for 48 h prior to the main trials. A dietary record was completed 48 h before the first main trial and participants replicated this diet prior to their second main trial.

On main trial days, participants arrived at the laboratory between 8 and 9 am following a 12 h fast. After anthropometric measurements, participants rested on a bed for 15 min, followed by four 5-min expired gas sample collections to determine resting metabolic rate (RMR) (5, 11) using substrate oxidation under resting conditions (17). During exercise alternative equations were used (28).

After RMR assessment, a cannula was inserted into an antecubital forearm vein and a baseline venous blood sample was taken and allocated into tubes with either ethylenediaminetetraacetic acid (EDTA) or serum separation beads (Sarstedt Ltd, Leicester, UK). Plasma samples were centrifuged immediately at 3,465 g at 4 °C for 10 min. Serum samples were left for 45 min to coagulate before centrifugation. Subcutaneous adipose tissue samples (~1 g) were taken under local anaesthetic (1% lidocaine) from the area around the waist approximately 5 cm lateral to the umbilicus with a 14 G needle using an aspiration technique (72) followed by adipose tissue cleaning and processing as described previously (67).

Participants then either consumed a meal (FED) or remained fasting (FASTED) and cannula-derived venous blood samples were taken every 15 min for the following 60 min. A further blood sample was collected at 120 min immediately before the walking protocol. In both the FASTED and FED treatments, participants walked on the treadmill at 60% $\dot{V}O_{2max}$ for 60 min and one minute of expired air samples, RPE and heart rate were collected at 5, 20, 40 and 60 min. After finishing exercise, another blood sample was immediately collected and participants then rested for a further 60 min. At this point, a second adipose tissue and final blood sample were taken.

Meal

The meal in the FED trial was the same as previously described in detail (7). The composition of the meal was selected to reflect typical breakfasts in the UK (7). The amount was normalised to resting energy requirements (7). Briefly, the total energy provided was 648 ± 115 kcal (carbohydrate 120.1 ± 21.3 g, fat 12.7 ± 2.3 g and protein 20.9 ± 3.7 g). The meal included white bread (Brace's thick white), cornflakes (Kellogg's cornflakes), semi-skimmed milk (Sainsbury; British semi skimmed milk), orange juice (Sainsbury; 100% pure squeezed smooth orange juice), spread (Unilever; I can't believe its not butter), jam (Sainsbury; strawberry jam) and sugar (Sainsbury; British white granulated sugar). Participants were asked to consume the meal within 15 min. In the FASTED trial, participants sat quietly for a 15-min period.

Adipose tissue gene expression and culture

After cleaning and mincing the adipose tissue biopsy sample, one portion of adipose tissue (approximately 200 mg) was immediately homogenised in 5 mL TRIzol (Invitrogen, Paisley, UK) in an RNase/DNase-free sterile tube (Invitrogen, Paisley, UK) and stored at −80 °C

190 before mRNA gene expression and protein analysis. The remaining adipose tissue was used
191 for culture and four ~100 mg portions were placed in sterile culture plates (Nunc, Roskilde,
192 Denmark) with endothelial cell basal media (ECBM) (Promocell, Germany) containing 0.1%
193 fatty acid-free bovine serum albumin 100 $U \cdot mL^{-1}$ penicillin and 0.1 $mg \cdot mL^{-1}$ streptomycin
194 (Sigma-Aldrich, Gillingham, UK). Adipose tissue was incubated with a final ratio of 100 mg
195 tissue per 1 mL ECBM media for 3 h (68) at 37 °C in a 5% CO_2 and 95 ± 5% relative
196 humidity incubator (MCO-18A1C CO_2 incubator; Sanyo, Osaka, Japan). After the 3-h
197 incubation, media was transferred to sterile tubes and stored at −80 °C. Adipokine secretion
198 from adipose explants was normalised to explant adipose mass and then L1-L4 fat mass as
199 described (67).
200
201 **Real-time PCR**
202 An RNeasy Mini Kit (Qiagen, Crawley, UK) was used to extract RNA from adipose tissue as
203 described (72). Tissue samples were quantified using a Qubit 2.0 fluorimeter (Life
204 Technologies, Paisley, UK). RNA was reversed transcribed (1 μg) to cDNA using a High
205 Capacity Reverse Transcription Kit (Applied Biosystems, Warrington, UK). Organic phenol-
206 chloroform phase from the RNA extraction was kept for further protein analysis. Real-time
207 PCR was performed using a StepOne™ (Applied Biosystems, Warrington, UK). Predesigned
208 primers and probes were obtained from Applied Biosystems for the measurement of
209 expression of interleukin 6 (IL-6) (Hs00985639_m1), adiponectin (Hs00605917_m1), leptin
210 (Hs00174877_m1), interleukin 18 (IL-18) (Hs00155517_m1), tumour necrosis factor alpha
211 (TNF-α) (Hs99999043_m1), monocyte chemoattractant protein-1 (MCP-1)
212 (Hs00234140_m1), 5' AMP-activated protein kinase (AMPK) (Hs01562315_m1 and
213 Hs00178903_m1 combined), glucose transporter type 4 (GLUT4) (Hs00168966_m1),
214 hormone-sensitive lipase (HSL) (Hs00193510_m1), insulin receptor substrate 1 (IRS1)

(Hs00178563_m1), insulin receptor substrate 2 (IRS2) (Hs00275843_s1), sterol regulatory element binding protein 1c (SREBP-1c) (Hs01088691_m1), pyruvate dehydrogenase kinase isozyme (PDK4) (Hs00176875_m1), peroxisome proliferator-activated receptor γ (PPARγ) (Hs01115513_m1), peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1α) (Hs01016719_m1), RAC-alpha serine/threonine-protein kinase (Akt1) (Hs00178289_m1), adipose triglyceride lipase (ATGL) (Hs00386101_m1), fatty acid translocase (FAT)/CD36 (Hs00169627_m1), forkhead box protein O1 (FOXO1) (Hs01054576_m1), hexokinase 2 (HK2) (Hs00606086_m1), PI3K-85α (PIK3R1) (Hs00933163_m1), carnitine palmitoyltransferase 1B (CPT1B) (Hs03046298_s1), The G0/G1 switch gene 2 (G0S2) (Hs00605971_m1), peptidylpropyl isomerase A (PPIA) was used as an endogenous control (48). The comparative threshold cycle (Ct) method was used to process data where ΔCt = Ct target gene − Ct PPIA. Ct target genes were normalised to an internal calibrator (lowest ΔCt for each target gene) and baseline. The Ct values for IL-6 (31 out of 40 samples), TNF-α (16 out of 40 samples), and IL-18 (37 out of 40 samples) were frequently over 35 and thus these results are not included.

Western blotting

The adipose tissue protein fraction was isolated from the TRIzol phenol-chloroform phase following the manufacturer's protocol (TRIzol Reagent, Life Technologies). Briefly, 1 mL of organic phase was mixed with 1.5 mL isopropanol. After mixing, the samples were incubated for 10 min at room temperature, followed by 10 min centrifugation at 12,000 g at 4 °C to pellet the protein. One millilitre of protein pellet was washed using 2 mL of 0.3 M guanidine hydrochloride in 95% ethanol for 20 min incubation followed by centrifugation at 7,500 g for 5 min at 4 °C. This process was repeated 3 times. After finishing the washing procedure, 2 mL of 100% ethanol was added to the protein pellet for a further 20 min incubation at room

240 temperature before being centrifuged. The pellet was then left to air dry for 5−10 min. Then,
241 200 μL of 1% SDS was added to resuspended the pellet. The protein content of the samples
242 was determined using a BCA protein assay kit (Thermo Scientific, Waltham, USA). Proteins
243 (25 $\mu g \cdot lane^{-1}$) were separated by SDS-PAGE and transferred using a semidry electro-transfer
244 method to a nitrocellulose membrane. Western blotting analysis was performed with the
245 following antibodies: RAC-beta serine/threonine-protein kinase (Akt2)/PKBβ (Millipore)
246 (34), AMPK (Cell Signalling Technology, USA) (72), GLUT4 (26), IRS1 (Millipore) (35),
247 IRS2 (Millipore) (47), PDK4 (ABGENT, San Diego, USA) (29), HSL (Cell Signalling
248 Technology, USA) (56) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH)
249 (Proteintech, USA) (62). The images were acquired in an EPI Chemi II darkroom (UVP) and
250 bands quantified using VisionWorks LS analysis software (UVP).
251
252 **Biochemical analysis**
253 Plasma glucose and non-esterified fatty acids (NEFA) were measured using commercially
254 available assay kits and analyser (Daytona Rx; Randox, Crumlin, UK). Serum insulin
255 (Mercodia, Uppsala, Sweden) and both serum and adipose media concentrations of IL-6,
256 leptin and adiponectin (R&D systems) were measured using Enzyme-linked immunosorbent
257 assay (ELISA).
258
259 **Statistics**
260 Descriptive data are presented as means ± standard deviation (SD). The variance bars on
261 figures are presented as means with 95% confidence intervals (CI). Time series data were
262 analysed using a two-way ANOVA (trial × time) with repeated measures irrespective of
263 minor deviations from a normality of distribution (43) using SPSS version 22 (IBM, Armonk,
264 NY, USA). Where significant interactions (trial × time) were found, post hoc paired *t-tests*

were used to determine changes over time. Analysis of gene and protein expression data were conducted using logged transformed data as previously described (72). Incremental area under curve (iAUC) was calculated for insulin, glucose and NEFA using the trapezoid method (76) and analysed using paired *t-tests*. Statistical significance was set at $p \leq 0.05$.

RESULTS

Energy expenditure and substrate oxidation during FASTED and FED trials

There were modest feeding-induced differences between trials for relative exercise intensity (% $\dot{V}O_{2max}$) and exercise energy expenditure (Table 2). Fat oxidation during exercise was reduced by ~45% in the FED trial (Table 2). Pre-fed resting metabolic rate (RMR) was not different (2,103 ± 418 *versus* 2,058 ± 365 kcal·d^{-1} in FASTED and FED trials, respectively).

Table 2. Physiological responses during 60 min exercise (n = 10).

	FASTED	FED
Treadmill speed (km·h^{-1})	5.7 ± 0.7	5.7 ± 0.7
$\dot{V}O_{2max}$ (%)	59 ± 3 *	60 ± 3
Heart Rate (beat·min^{-1})	155 ± 13	155 ± 14
RPE (6−20)	12 ± 2	12 ± 2
Energy expenditure (kcal·h^{-1})	746 ± 129 *	771 ± 135
Respiratory exchange ratio ($\dot{V}CO_2$:$\dot{V}O_2$)	0.93 ± 0.03 *	0.97 ± 0.03
Carbohydrate oxidation (g·h^{-1})	147 ± 41 *	167 ± 39
Fat oxidation (g·h^{-1})	16 ± 8 *	9 ± 6

Values are means ± SD. * denotes significantly different between FASTED *versus* FED condition ($p \leq 0.05$).

Plasma glucose, NEFA and serum insulin concentrations

Trial × Time interaction effects were found for blood glucose, insulin and NEFA between FASTED and FED trials (p = 0.026, p = 0.003 and p = 0.001, respectively). As expected, iAUCs for glucose and insulin were elevated for all parameters in the FED trial (p = 0.02 and p = 0.03, respectively) (Figure 1B and D). Peak glucose and insulin concentrations in the FED trial were reached 15 min post-meal (7.1 ± 0.6 mmol·L^{-1} and 370 ± 185 pmol·L^{-1},

Figure 1A and C, respectively). NEFA concentrations were lower at most time points in the FED trial (Figure 1E).

[INSERT FIGURE 1 ABOUT HERE]

Adipose tissue mRNA expression

There was an interaction effect for adipose tissue PDK4, ATGL, HSL, FAT/CD36, GLUT4 and IRS2 (all $p \leq 0.05$) after exercising under FASTED versus FED conditions (Figure 2). These interaction effects are explained by divergent responses between FASTED and FED trials with a larger increase in FASTED conditions when compared to either a smaller change or a modest decrease in FED conditions (Figure 2). There was a time effect for HK2, MCP-1 and PGC-1α (Figure 2). The expression of the remaining genes was not significantly different between trials or over time (Figure 2).

[INSERT FIGURE 2 ABOUT HERE]

Adipose tissue protein expression

There was an interaction effect for IRS2 protein expression between trials ($p \leq 0.05$), showing approximately a 2-fold increase in FASTED conditions, and no change in FED conditions (Figure 3A). The change in the expression of the remaining proteins was not statistically different between FASTED and FED conditions. Representative Western blots are shown in Figure 3B.

[INSERT FIGURE 3 ABOUT HERE]

311 **Serum adipokine concentrations and adipose tissue secretion *ex vivo***

312 Serum IL-6 increased (Figure 4A) and serum leptin decreased (Figure 4C) in both trials ($p <$
313 0.05). There was a modest time effect for serum adiponectin (Figure 4E), although no
314 interaction effects were identified for serum IL-6, leptin and adiponectin. Furthermore, no
315 time or time × trial interactions were identified for *ex vivo* adipose explant secretion of IL-6,
316 leptin and adiponectin in response to exercise under FASTED and FED conditions (Figure 4).
317
318 [INSERT FIGURE 4 ABOUT HERE]
319

DISCUSSION

This study presents the first evidence that feeding status alters the human adipose tissue response to acute exercise and thus feeding status has the potential to influence the long-term adaptation of adipose tissue to regular exercise.

In the present study, our feeding protocol successfully manipulated systemic concentrations of glucose and insulin. In the two hours prior to exercise, there was a 168-fold and 26-fold difference in insulin and glucose iAUC, respectively. As anticipated with this study design, prior feeding increased relative carbohydrate utilisation and decreased fat oxidation during exercise (3, 23, 77). Thus, exercise in FED and FASTED trials was performed in a very different physiological state.

At the gene expression level, we found that adipose tissue responded differently to moderate-intensity exercise under FASTED *versus* FED conditions. When compared to the changes in fasted exercise conditions, feeding led to lower changes or a decrease in PDK4, ATGL, HSL, FAT/CD36, GLUT4 and IRS2 mRNA as demonstrated by interaction effects for these outcomes. Over time, acute differences in skeletal muscle gene expression with exercise conducted in the fasted *versus* the fed state have been proposed to contribute to diverse physiological adaptations (12, 70). Our data demonstrate that feeding status also alters adipose tissue responses to an acute bout of exercise. We have previously shown that weight loss leads to a large increase in genes such as PDK4 and HSL in adipose tissue (71) whereas overfeeding leads to a profound decrease in both PDK4 and HSL in adipose tissue (72). Thus, feeding has the potential to affect the acute adipose tissue responses to exercise and, given the important role of adipose tissue in health and the nature of these changes (66, 71, 72), we

propose that feeding before exercise blunts some of the health-related changes induced by exercise training.

We found a difference in adipose IRS2 protein content between FASTED and FED exercise conditions although the other measured proteins were not affected. We should highlight that our protein measurements represent only total protein content. Repeated small changes in adipose protein synthesis are likely lead to accumulated differences and functional changes in adipose phenotype over time (6, 50). Clearly, the only way to know if the acute changes observed in the current study translate into long term differences in protein content in adipose is to examine whether chronic training conducted in the fasted *versus* postprandial state leads to divergent adaptations.

Carbohydrate and lipid metabolism in adipose tissue

A primary function of PDK4 is to regulate glucose metabolism by inhibiting pyruvate dehydrogenase complex activity. Fasting and exercise increase PDK4 mRNA expression in skeletal muscle (52, 53) and insulin suppresses PDK4 mRNA and protein content in skeletal muscle (37, 40). It is possible that insulin could be responsible for the lower adipose PDK4 mRNA response in the FED trial, although higher NEFA in the FASTED trial could also have increased PDK4 expression (2, 33). Other feeding-related studies have shown that muscle PDK4 mRNA expression remained unchanged 1−4 h after exercise in the fed state (with lower NEFA concentrations), when compared with exercise in the fasted state (9, 10). Thus, exercise in the fasted state appears to increase PDK4 expression in both muscle and adipose, whereas exercise in the fed state does not.

ATGL and HSL mobilise stored fat and release it into the circulation (79). In the current study, gene expression of ATGL, HSL and FAT/CD36 in adipose were all differentially expressed in FASTED and FED exercise conditions. These interaction effects are explained by responses in the FED trial being either lower or in the opposite direction to the FASTED trial. There was also greater fat oxidation during exercise in the FASTED trial. Other studies have also shown similar responses in fasted exercise with higher fat oxidation and increased skeletal muscle FAT/CD36 gene expression (9). Moreover, these findings are consistent with the previously observed increase in adipose HSL activity reported during cycling exercise, which is blunted with nicotinic acid ingestion (73). Thus, given the nature and direction of these changes, we propose that feeding blunts at least some of the exercise-induced stimulus on adipose tissue.

We found GLUT4 mRNA, IRS2 mRNA and IRS2 protein were also differentially expressed in adipose in FASTED and FED exercise trials. These effects were subtle but consistent, and this also seems to indicate that exercise in a fed state will not generate the same change in pathways involved in glucose metabolism and signalling within adipose tissue as fasted exercise. Previous findings in skeletal muscle have found an increase in GLUT4 mRNA in fasted but not fed conditions after exercise (9, 10).

PGC-1α mRNA is a transcriptional coactivator involved in mitochondrial biogenesis (60). Acute exercise increases PGC-1α mRNA expression in human skeletal muscle (21, 54) and in rodent white adipose tissue (64). However, the impact of feeding status and/or carbohydrate variability prior to exercise on skeletal muscle PGC-1α mRNA is controversial. Some studies indicate that PGC-1α mRNA expression is up-regulated post-exercise whether acute exercise is performed in fed or fasted conditions (10, 42) but other studies show that higher

393 carbohydrate availability prior to exercise blunts PGC1-α mRNA expression in skeletal
394 muscle both at rest and post-exercise (4, 55). In the present study, the increase in adipose
395 PGC-1α mRNA expression after exercise was unaffected by feeding status. Chronic training
396 studies indicate that adipose PGC1-α mRNA is increased in humans (59) and rodents (64, 69)
397 and this has also been reported to increase PGC1-α protein content and mitochondrial
398 biogenesis in rodents (69). Whether the present results indicate an acute exercise-induced
399 increase in mitochondrial biogenesis in human adipose tissue is plausible but unclear at the
400 present time.
401
402 ***Adipokines response to exercise and impact of feeding status***
403 Previous studies have shown that circulating adipokine concentrations are affected by acute
404 exercise (8) and energy consumption during exercise alters these systemic responses (57, 61).
405 However, evidence from studies that have manipulated feeding status *prior to* exercise is
406 scarce. Zoladz et al. (80) found no difference in circulating IL-6 and leptin after a single bout
407 of exercise, in a fed or fasted state. However, the duration of exercise lasted only 12 min and
408 this might be insufficient to examine the notion that feeding status influences circulating
409 adipokines. The exercise in the present study was 60 min, and we too found no evidence that
410 pre-exercise feeding affects circulating IL-6, leptin and adiponectin. This may be due to the
411 fact that moderate intensity exercise has only a modest effect on many of these parameters
412 and thus there is little potential for feeding to interact with exercise and exert an effect (41).
413 Serum IL-6 was increased over time in both fasted and fed trials in the present study, which
414 could be partly due to the effect of exercise (51). As we did not observe changes of adipose
415 IL-6 secretion *ex-vivo*, the increase in serum IL-6 might be caused by release from skeletal
416 muscle during exercise (49), but it is also possible that reflects local production of IL-6 due to
417 prolonged cannulation (14).

Temporal and population-specific considerations

This study is the first to examine the impact of feeding on adipose tissue responses to exercise. We recruited overweight participants and this focus is a strength given that ~62% of the UK population are overweight (24). Increased adiposity has a profound effect on adipose tissue function (e.g., a down regulation of GLUT 4 mRNA (67) and reduction in postprandial adipose tissue blood flow (44)). These could be important considerations when interpreting our findings. We should also highlight that in the current study we were limited to only two adipose tissue biopsies due to a concern over potential interference from repeated sampling (15) and so we do not have a full and complete time course. For the first study of this kind and with limited sampling opportunities, a second biopsy 60 min post-exercise was considered to balance the requirement to capture pathways that rapidly change and those that are slower to respond. Subsequent studies should consider more frequent adipose sampling and/or the inclusion of additional resting trial(s). Depending on the kinetics of each response, our sample timing framework will be appropriate for some outcomes and less appropriate for others. Furthermore, in the absence of an adipose biopsy immediately prior to exercise, it is hard to establish whether some effects in the FASTED trial are due to the modestly extended fasting period or due to exercise (or the interaction between fasting and exercise). What is very apparent, however, is that exercise in FED conditions does not lead to the same changes as exercise in FASTED conditions.

Conclusion

This study provides the first evidence that the feeding status alters the response of adipose tissue to acute exercise. Several genes involved in lipid metabolism, insulin signaling and glucose transport were differentially expressed in adipose tissue when exercise was

443 performed in a fed *versus* fasted state with either lower or opposing responses after feeding.
444 Given the nature and direction of these differences, we propose that feeding is likely to blunt
445 long-term adaptations induced within adipose tissue in response to regular exercise.
446

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DISCLOSURES

The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

Yung-Chih Chen was responsible for study design and conduct, data collection, data analysis, data interpretation, statistical analysis, and manuscript revision; Rebecca L. Travers was responsible for mRNA gene expression data collection and manuscript revision. Jean-Philippe Walhin was responsible for mRNA gene expression analysis and manuscript revision. Javier T. Gonzalez was responsible for manuscript revision. Francoise Koumanov was responsible for western blotting analysis, interpretation, and manuscript revision. James Betts was responsible for study design, and manuscript revision; Dylan Thompson was responsible for funding, study design, data interpretation, and manuscript revision.

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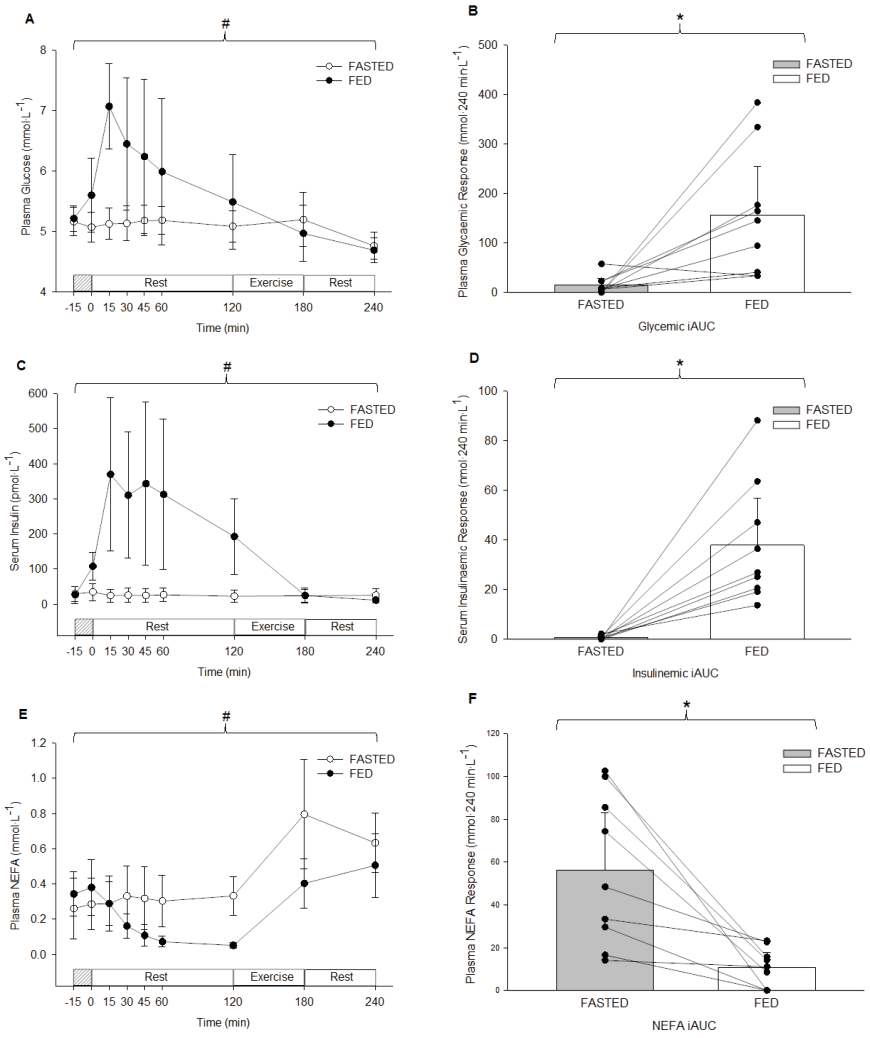
Figure legends

Figure 1. Glucose (A), insulin (C) and NEFA (E) concentrations in FASTED and FED trials and iAUC for glucose (B), insulin (D) and NEFA (F). n = 9 in both FASTED and FED trials (due to difficulty in cannulating one participant). Values are means ± 95% CI. # denotes significant interaction effect between FASTED and FED trials by two-way ANOVA with repeated measures ($p \leq 0.05$). * denotes significantly different between FASTED versus FED trials using paired *t-tests* ($p \leq 0.05$). The shaded box in (A), (C) and (E) denotes meal time.

Figure 2. Fold changes in relative gene expression in adipose tissue under FASTED and FED trials (all n = 10, except for HSL and G0S2 n = 9 owing to Ct > 35 for one participant). The dashed line indicates baseline. Data normalised to PPIA, internal calibrator and baseline. Samples that exceeded the detectable limit (Ct > 35) were excluded from the analysis. Values are means ± 95% CI. # denotes significant interaction effect between FASTED and FED trials using two-way ANOVA with repeated measures ($p \leq 0.05$). † denotes a time effect ($p \leq 0.05$). * comparison between baseline and 1 h post-exercise by paired *t-tests* ($p \leq 0.05$).

Figure 3. Fold changes in relative protein content in adipose tissue under FASTED and FED trials (all n = 8 due to lack of sufficient protein for two participants) (A). The dashed line indicates baseline. Data were normalised to GAPDH. Values are means ± 95% CI. # denotes significant interaction effect between FASTED and FED trials using two-way ANOVA with repeated measures ($p \leq 0.05$). Representative images of Western blots in adipose tissue under FASTED and FED trials (B). IRS1/2, Akt2 and AMPK represents participant number 2 and 8. GLUT4, PDK4 and HSL represents participant number 3 and 4.

Figure 4. Circulating serum IL-6 (A) leptin (C) and adiponectin (E) concentrations in FASTED and FED trials (n = 9). *Ex-vivo* adipose tissue explant protein secretion of IL-6 (B), leptin (D) and adiponectin (F) expressed relative to L1-L4 fat mass (n = 10). Values are means ± 95% CI. The shaded box in (A), (C) and (E) denotes meal time. † denotes a time effect from two-way ANOVA with repeated measures ($p \leq 0.05$).


A
#
FASTED
FED
Plasma Glucose (mmol·L-1)
Rest
Exercise
Rest
-15 0 15 30 45 60 120 180 240
Time (min)
B
*
FASTED
FED
Plasma Glycaemic Response (mmol·240 min·L-1)
FASTED
FED
Glycemic iAUC
C
#
FASTED
FED
Serum Insulin (pmol·L-1)
Rest
Exercise
Rest
-15 0 15 30 45 60 120 180 240
Time (min)
D
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FASTED
FED
Serum Insulinaemic Response (mmol·240 min·L-1)
FASTED
FED
Insulinemic iAUC
E
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FASTED
FED
Plasma NEFA (mmol·L-1)
Rest
Exercise
Rest
-15 0 15 30 45 60 120 180 240
Time (min)
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*
FASTED
FED
Plasma NEFA Response (mmol·240 min·L-1)
FASTED
FED
NEFA iAUC

Figure 1

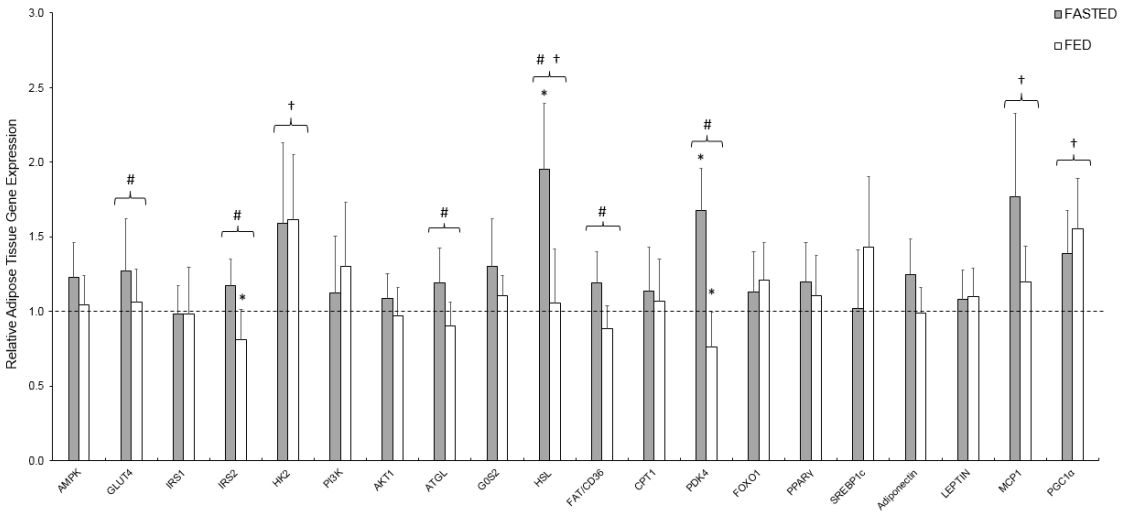
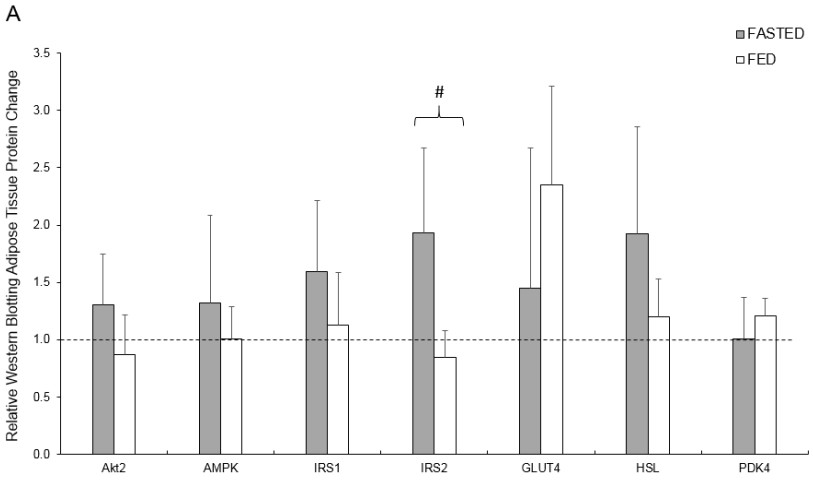
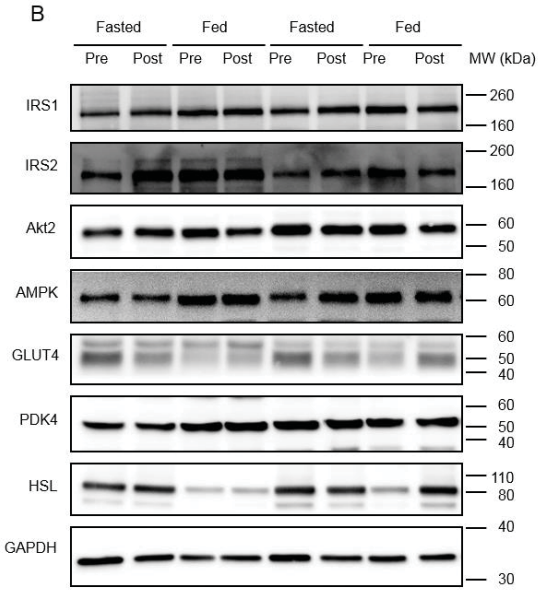

FASTED
FED
Relative Adipose Tissue Gene Expression
3.0
2.5
2.0
1.5
1.0
0.5
0.0
AMPK
GLUT4
IRS1
IRS2
HK2
PI3K
AKT1
ATGL
GIS2
HSL
FAT/CD36
CPT1
PDK4
FOXO1
PPARγ
SREBP1c
Adiponectin
LEPTIN
MCP1
PGC1α

Figure 2


A
FASTED
FED
Relative Western Blotting Adipose Tissue Protein Change
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
#
Akt2
AMPK
IRS1
IRS2
GLUT4
HSL
PDK4


B
Fasted
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Pre
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MW (kDa)
IRS1
260
160
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Akt2
60
50
AMPK
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GLUT4
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HSL
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GAPDH
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Figure 3

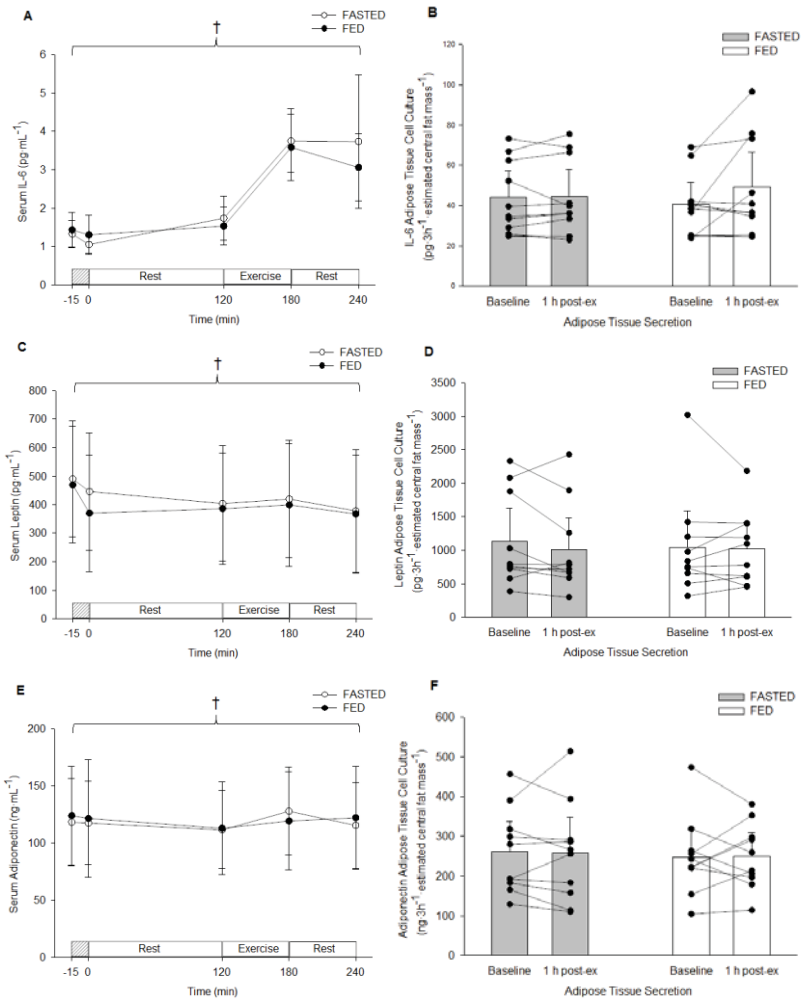
A
FASTED
FED
†
Serum IL-6 (pg mL^{-1})
Rest
Exercise
Rest
-15 0
120
180
240
Time (min)
B
FASTED
FED
IL-6 Adipose Tissue Cell Culture (pg 3n^{-1} estimated central fat mass^{-1})
Baseline
1 h post-ex
Baseline
1 h post-ex
Adipose Tissue Secretion
C
FASTED
FED
†
Serum Leptin (pg mL^{-1})
Rest
Exercise
Rest
-15 0
120
180
240
Time (min)
D
FASTED
FED
Leptin Adipose Tissue Cell Culture (pg 3n^{-1} estimated central fat mass^{-1})
Baseline
1 h post-ex
Baseline
1 h post-ex
Adipose Tissue Secretion
E
FASTED
FED
†
Serum Adiponectin (ng mL^{-1})
Rest
Exercise
Rest
-15 0
120
180
240
Time (min)
F
FASTED
FED
Adiponectin Adipose Tissue Cell Culture (ng 3n^{-1} estimated central fat mass^{-1})
Baseline
1 h post-ex
Baseline
1 h post-ex
Adipose Tissue Secretion

Figure 4