Diabetologia (2012) 55:3029–3037
DOI 10.1007/s00125-012-2676-0

ARTICLE

Marked resistance of femoral adipose tissue blood flow and lipolysis to adrenaline in vivo

K. N. Manolopoulos · F. Karpe · K. N. Frayn

Received: 30 April 2012 / Accepted: 5 July 2012 / Published online: 17 August 2012

Abstract
Aims/hypothesis Fatty acid entrapment in femoral adipose tissue has been proposed to prevent ectopic fat deposition and visceral fat accumulation, resulting in protection from insulin resistance. Our objective was to test the hypothesis of femoral, compared with abdominal, adipose tissue resistance to adrenergic stimulation in vivo as a possible mechanism.
Methods Regional fatty acid trafficking, along with the measurement of adipose tissue blood flow (ATBF) with ^{133}Xe washout, was studied with the arteriovenous difference technique and stable isotope tracers in healthy volunteers. Adrenergic agonists (isoprenaline, adrenaline [epinephrine]) were infused either locally by microinfusion or systemically. Local microinfusion of adrenoceptor antagonists (propranolol, phentolamine) was used to characterise specific adrenoceptor subtype effects in vivo.
Results Femoral adipose tissue NEFA release and ATBF were lower during adrenaline stimulation than in abdominal tissue ($p<0.001$). Mechanistically, femoral adipose tissue displayed a dominant α-adrenergic response during adrenaline stimulation. The α-adrenoceptor blocker, phentolamine, resulted in the 'disinhibition' of the femoral ATBF response to adrenaline ($p<0.001$).
Conclusions/interpretation Fatty acids, once stored in femoral adipose tissue, are not readily released upon adrenergic stimulation. Femoral adipose tissue resistance to adrenaline may contribute to the prevention of ectopic fatty acid deposition.

Keywords Adipose tissue blood flow · Adrenergic regulation · Fatty acid trafficking · Femoral adipose tissue · Lipolysis

Abbreviations

ANCOVA	Analysis of covariance
ATBF	Adipose tissue blood flow
DXA	Dual-energy x-ray absorptiometry
FFM	Fat-free mass
Ra_{NEFA}	NEFA rate of appearance
TG	Triacylglycerol

Introduction

Accumulation of gluteofemoral fat is associated with protection against cardiovascular and diabetes risk [1]. It has been proposed that gluteofemoral adipose tissue acts as a 'metabolic sink', where fatty acids from triacylglycerol (TG)-rich lipoproteins and dietary fats are 'trapped' [2]. Entrapment of fatty acids would prevent ectopic fat deposition in the liver, muscle and pancreas, or reduce the accumulation of visceral adipose tissue, both of which are associated with insulin resistance [3].

Catecholamines (adrenaline [epinephrine], noradrenaline [norepinephrine]) are important regulators of adipose tissue blood flow (ATBF) and lipolysis, as they exert effects on

Electronic supplementary material The online version of this article (doi:10.1007/s00125-012-2676-0) contains peer-reviewed but unedited supplementary material, which is available to authorised users.

K. N. Manolopoulos · F. Karpe · K. N. Frayn
Oxford Centre for Diabetes, Endocrinology and Metabolism,
University of Oxford,
Oxford, UK

K. N. Manolopoulos (✉)
Centre for Endocrinology, Diabetes and Metabolism, College of Medical and Dental Sciences, Institute of Biomedical Research,
University of Birmingham,
Wolfson Drive, Edgbaston,
Birmingham B15 2TT, UK
e-mail: k.manolopoulos@bham.ac.uk

F. Karpe
NIHR Oxford Biomedical Research Centre, ORH Trust, OCDEM,
Churchill Hospital,
Oxford, UK

both adipocytes and the vascular bed by acting through α_1- and α_2-adrenoceptors (promoting vasoconstriction and inhibition of lipolysis) and β_1- and β_2-adrenoceptors (resulting in vasodilatation and stimulation of lipolysis) [4]. Postprandially and during exercise, ATBF increases in a catecholamine-dependent fashion [5–7]. In lipolysis, clear adrenergic response differences between human adipocytes have been described in vitro, depending on the adipose tissue depot of origin. Noradrenaline had a greater lipolytic effect on abdominal than gluteal adipocytes because of a higher expression of β_1- and β_2-adrenoceptors in abdominal adipocytes and a higher sensitivity of gluteal adipocytes to α-adrenoceptor stimulation [8, 9]. Adrenaline induced lipolysis in femoral adipocytes in vitro only when the α-adrenoceptor blocker, phentolamine, was added [10]. Thus there are clear depot-specific differences in the adrenergic response of adipocytes in vitro.

Few studies have compared adrenergic responses in abdominal and femoral adipose tissue in vivo. During adrenaline stimulation, abdominal lipolysis appeared to be higher than femoral lipolysis, measured as glycerol release by adipose tissue microdialysis in lean women [11]. In the same study, femoral ATBF showed an attenuated increase during systemic adrenaline stimulation compared with abdominal. However, this is an indirect measurement only, as NEFA, the product of lipolysis, cannot be recovered by microdialysis, since they are large and not water-soluble molecules. Furthermore, microdialysis is a relative and not direct quantitative measurement of local adipose tissue function, and is insensitive to rapid changes in ATBF [12]. A further approach has been the measurement of whole-leg palmitate release, using blood samples drawn from the femoral vein, which includes efflux from muscle and is not adipose tissue specific [13]. Palmitate release was lower in whole-leg than the abdominal depot during stimulation with isoprenaline, a selective β-adrenoceptor agonist, in women [14]. With respect to sex-specific differences, one study showed a blunted lipolytic response of whole-leg palmitate release in response to adrenaline in women compared with men [15]. To date, direct quantitative measurements of lipolysis, comparing abdominal and femoral fat in vivo, have not been performed.

Recently, we developed a technique to measure fatty acid trafficking across femoral adipose tissue using arteriovenous difference sampling [16]. We have reported the preferential deposition of VLDL-derived fatty acids and NEFA in femoral adipose tissue [17]. Clearly, a lower catecholamine-dependent lipolytic rate in femoral adipose tissue than in abdominal adipose tissue would be an important mechanism in fatty acid entrapment, supporting the metabolic sink hypothesis. Here, we describe a series of studies directly comparing abdominal and femoral ATBF and lipolysis in response to adrenergic stimulation in vivo, using a combination of novel integrative physiology techniques.

Methods

Participants Healthy young male and female individuals with no medical condition and not on any drug therapy were recruited using the Oxford BioBank [18]. The study was approved by Milton Keynes Research Ethics Committee, and all participants gave informed consent in writing. ATBF and/or regional lipolysis were measured in four different study settings (Studies 1–4, see below). Different participants took part in each study, with the exception of Study 4, where all but two participants had taken part in Study 3 previously. Participant numbers and baseline anthropometric characteristics are shown in Table 1. Total and regional fat masses were measured by dual-energy x-ray absorptiometry (DXA), except in Study 1, where total body fat mass was measured using bioelectrical impedance analysis. Participants were asked to consume a corn-free diet for 48 h and to refrain from strenuous exercise and alcohol for 24 h before the study. They attended the Oxford Centre for Diabetes, Endocrinology and Metabolism Clinical Research Unit, having fasted from 22:00 hours the previous night. All studies were performed in the fasting state.

Study design In all studies, abdominal and femoral ATBF were measured using the ^{133}Xe washout technique [19]. Skin temperatures were measured throughout the studies at regular intervals. Abdominal and thigh skin temperatures remained relatively constant, and no differences between sites were noted. In studies 2 and 3, lipolysis (NEFA and glycerol release) was measured across abdominal and femoral adipose tissue with the arteriovenous difference technique [16, 20]. Stable isotope-labelled fatty acids were used to trace lipid pools as described previously [21]. A constant infusion of [$^2H_2$]palmitate (CK Gas Products, Hook, Hampshire, UK) was given intravenously (0.03 μmol min^{-1} [kg body weight]$^{-1}$) in 400 ml human albumin (4.5%) throughout the studies to calculate whole-body and regional NEFA rate of appearance (Ra_{NEFA}). Calculation of whole-body Ra_{NEFA} was as described previously [21]. Regional Ra_{NEFA} calculations are shown in the electronic supplementary material (ESM).

To establish the regional ATBF response to β-adrenoceptor stimulation (Study 1), ATBF was measured during local microinfusion of isoprenaline [5]. Four microinfusion catheters (Medtronic Quick-Set, Northridge, CA, USA) were inserted, two into abdominal adipose tissue, 5 cm on each side of the umbilicus, and one on the anterior aspect of each thigh, half-way between the groin and the knee. A cannula was inserted retrogradely into a vein of the dorsal hand which was placed into a hot box at 60°C for obtaining arterialised blood samples [22]. With a microinfusion pump (CMA-100; CMA Microdialysis, Solna, Sweden), isoprenaline, a β-selective agonist, was infused using one of the microinfusion catheters of each fat depot. As a

Table 1 Baseline anthropometric and metabolic characteristics of participants

Characteristic	Study 1		Study 2		Study 3		Study 4	
	Male (n=8)	Female (n=5)	Male (n=5)	Female (n=2)	Male (n=14)	Female (n=14)	Male (n=7)	Female (n=6)
BMI (kg/m^2)	25.4 (22.7–29.6)	21.5 (17.9–23.1)*	26.3 (25.5–27.0)	22.1 (21.2–23.0)*	24.1 (21.8–30.6)	24.5 (21.5–39.3)	26.2 (22.8–30.6)	26.8 (22.2–39.3)
WHR	0.86 (0.77–0.95)	0.77 (0.72–0.8)*	0.96 (0.92–1.0)	0.75 (0.72–0.79)***	0.94 (0.85–1.05)	0.90 (0.78–0.99)*	0.95 (0.85–1.05)	0.90 (0.88–0.99)
Body fat (%)	16.0 (6.5–23.3)	25.0 (14.0–31.1)*	24.7 (22.8–29.3)	33.6 (29.9–37.4)*	26.6 (17.3–33.3)	35.6 (20.7–50.4)***	29.0 (19.1–33.3)	33.8 (28.2–50.4)*
Fasting TG (μmol/l)	941 (640–1347)	552 (486–1613)	919 (442–1284)	488 (375–601)	1001 (403–1588)	731 (215–1334)	1200 (900–1500)	500 (400–1000)*
Fasting NEFA (μmol/l)	433 (269–628)	569 (495–617)	461 (377–836)	661 (659–664)	514 (348–1084)	672 (417–1012)	410 (330–520)	580 (350–840)
Fasting glucose (mmol/l)	4.9 (4.7–5.2)	5.0 (4.8–5.3)	5.4 (5.3–5.9)	5.3 (5.3–5.3)	5.2 (4.8–6.6)	5.0 (4.4–5.6)*	5.2 (4.8–6.2)	4.7 (4.3–4.9)*
Fasting insulin (pmol/l)	77.8 (41.0–113.2)	106.3 (50.7–119.5)	80.6 (62.5–101.4)	146.5 (103.5–189.6)*	82.0 (49.3–144.5)	69.5 (26.4–116.7)	82.0 (64.6–128.5)	52.8 (16.7–191.0)

Values are median (range)

*p<0.05, ***p≤0.001 comparing women and men

control, a 0.9% NaCl solution (saline) was infused into the remaining catheters. ^{133}Xe was injected through each catheter port into adipose tissue, and ATBF was measured throughout the study. Every 40 min, the concentration of isoprenaline was increased (10^{-8}, 10^{-6}, 10^{-4} mol/l, infusion rate 2 μl/min). At 2 h after the start of microinfusion, a 75 g glucose drink was given as a positive control of endogenous ATBF regulation.

For the study of regional fatty acid trafficking responses to β-adrenoceptor stimulation (Study 2), ATBF and local lipolysis were measured during systemic isoprenaline infusion. An arterial catheter was inserted into the femoral artery, and venous catheters were placed in the superficial epigastric vein and saphenous vein [16, 20]. A cannula was inserted into an antecubital vein for infusion purposes. ^{133}Xe was injected into abdominal and femoral adipose tissue, and ATBF was measured throughout the study. After 60 min of equilibration, a one-step systemic infusion of isoprenaline was started for 60 min (infusion rate 25 ng min^{-1} [kg fat-free mass (FFM)]$^{-1}$). Blood samples were taken simultaneously from all three sites at frequent intervals. After termination of the isoprenaline infusion, blood samples were taken for a further 60 min. Plasma creatinine concentrations were measured as an indication of contamination by muscle venous blood [16].

To study regional fatty acid trafficking in response to mixed α- and β-adrenoceptor stimulation (Study 3), ATBF and local lipolysis were measured during a systemic infusion of adrenaline, using the same design as Study 2. Adrenaline was given as a three-step incremental infusion for 60 min (step 1, 5 ng min^{-1} [kg FFM]$^{-1}$; step 2, 15 ng min^{-1} [kg FFM]$^{-1}$; step 3, 25 ng min^{-1} [kg FFM]$^{-1}$; rate change every 20 min). After termination of the adrenaline infusion, blood sampling was continued for a further 60 min. After the study end, biopsy samples were taken from abdominal and femoral subcutaneous adipose tissue using a 14-gauge needle. The biopsy samples were washed with 0.9% NaCl and then immediately snap-frozen in liquid nitrogen.

To study the role of regional α-adrenoceptors in response to adrenaline stimulation (Study 4), ATBF was measured during a systemic infusion of adrenaline and local microinfusion of phentolamine (α-adrenoceptor blocker) and propranolol (β-adrenoceptor blocker; microinfusion into abdominal adipose tissue only). Three microinfusion probes were placed into the abdominal and one into the femoral adipose tissue of each thigh as above. The abdominal probes were used for microinfusion of propranolol (10^{-4} mol/l at 2 μl/min), phentolamine (10^{-4} mol/l at 2 μl/min) and saline (control). The femoral probes were used for infusing phentolamine (10^{-4} mol/l at 2 μl/min) and saline. After 60 min had been allowed for equilibration and for the locally infused agents to reach an effective concentration, a three-step incremental systemic infusion of adrenaline was given for 60 min as in Study 3. Blood samples were taken from an arterialised dorsal hand vein as above.

Analytical methods Blood samples were drawn into heparinised syringes, and plasma was prepared rapidly at 4°C. Glucose concentrations were measured immediately, and remaining samples were frozen before analysis. Plasma TG, NEFA, glucose and creatinine concentrations were measured enzymatically using commercially available kits on an ILAB600 or ILAB650 clinical analyser (Instrumentation Laboratory UK, Warrington, UK); insulin and glucagon were measured by radioimmunoassay (Millipore, Watford, UK).

Lipid fractions (NEFA and TG) were separated, and fatty acid composition and tracer enrichment were analysed by gas chromatography and mass spectrometry as described previously [21]. Enrichments were converted into tracer-to-tracee ratios. RNA for gene expression analysis was extracted from whole adipose tissue biopsy samples using a standard guanidinium thiocyanate/phenol/chloroform extraction method [23]. Real-time PCR was performed using the TaqMan platform (Applied Biosystems, Carlsbad, CA, USA) according to the manufacturer's instructions. The expression of *ADRA1A*, *ADRA2A*, *ADRB1* and *ADRB2* genes was measured; *PPIA* and *PGK1* expression were used for normalisation.

Calculations and statistics Basal values were the mean of two measurements at −15 and 0 min. AUC was calculated using the trapezoid rule and is presented as a time-averaged value (AUC divided by the relevant time period). Whole-depot lipolysis was calculated by multiplying the depot fat mass from DXA measurements by the regional lipolysis rate per unit fat mass. Statistical tests used were paired *t* test, repeated-measures ANOVA, analysis of covariance (ANCOVA) and Pearson's correlation. Where appropriate, non-parametric tests were used in parallel and did not show different results (not shown). Data were analysed using IBM Statistics for Windows v19 (IBM UK, Portsmouth, UK), and $p<0.05$ was considered significant. All data are presented as mean ± SEM, unless otherwise stated.

Results

Regional ATBF and relationship to anthropometric variables Across all studies ($n=61$), basal ATBF was lower in femoral than abdominal adipose tissue (3.1±0.3 vs 3.8±0.3 ml min^{-1} [100 g tissue]$^{-1}$; $p<0.05$). Abdominal and femoral ATBF correlated positively ($r=0.41$, $p<0.05$), while there was a negative correlation with BMI (abdominal ATBF $r=-0.39$; femoral ATBF $r=-0.29$, $p<0.05$) and

WHR (abdominal ATBF $r=-0.46$; femoral ATBF $r=-0.43$, $p<0.05$). These correlations were independent of sex, with the exception of femoral ATBF and BMI ($r=-0.29$, $p=0.051$).

In the local microinfusion study (Study 1), oral glucose resulted in a marked ATBF increase in both adipose tissue depots in the control sites ($p<0.001$ for variation with time, $p<0.05$ for difference between fat depots; Fig. 1a). The maximum ATBF response to glucose (at 180 min) correlated strongly between abdominal and femoral adipose tissue ($r=0.63$, $p<0.05$). The abdominal ATBF response to glucose correlated negatively with BMI and WHR ($r=-0.64$ and -0.58 respectively, $p<0.05$). The femoral ATBF response to glucose correlated negatively with WHR, independently of sex ($r=-0.69$, $p<0.05$).

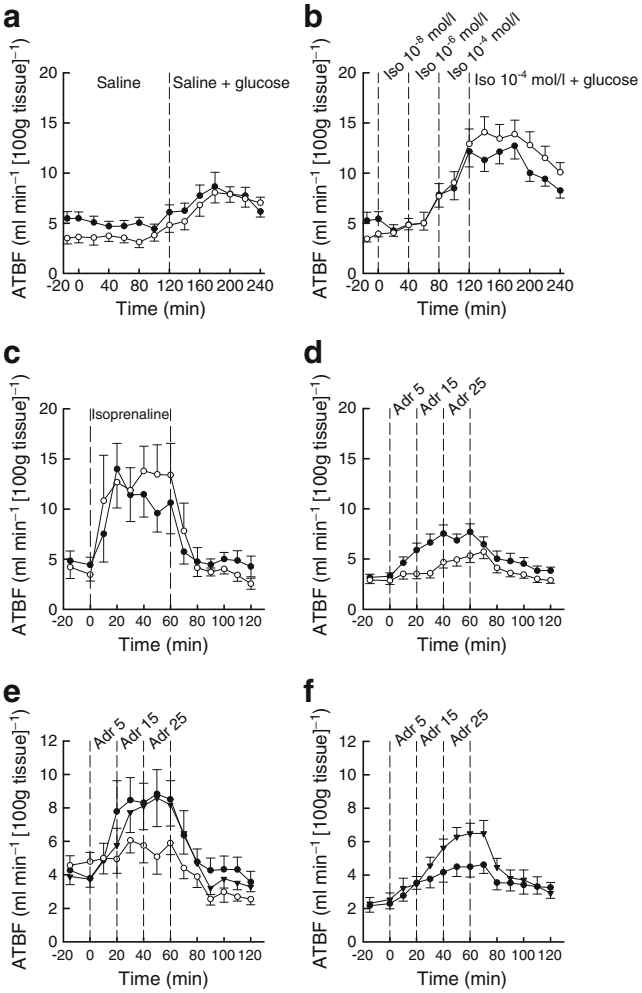

Fig. 1 Abdominal (black circles) and femoral (white circles) ATBF (**a**) during local microinfusion of saline, followed by a 75 g glucose drink at 120 min, (**b**) during incremental local microinfusion of isoprenaline (Iso; given at 2 μl/min), followed by a glucose drink at 120 min, (**c**) during a systemic infusion of isoprenaline at 20 ng min^{-1} (kg FFM)$^{-1}$ and (**d**) during a systemic incremental infusion of adrenaline (Adr 5, 5 ng min^{-1} [kg FFM]$^{-1}$; Adr 15, 15 ng min^{-1} [kg FFM]$^{-1}$; Adr 25, 25 ng min^{-1} [kg FFM]$^{-1}$). (**e**) Abdominal ATBF during a systemic incremental infusion of adrenaline and local microinfusion of saline (black circles), phentolamine (10^{-4} mol/l at 2 μl/min; black upside-down triangles) and propranolol (10^{-4} mol/l at 2 μl/min; white circles). (**f**) Femoral ATBF during a systemic incremental infusion of adrenaline and local microinfusion of saline (black circles) and phentolamine (10^{-4} mol/l at 2 μl/min; black upside-down triangles)

Adrenergic stimulation of regional ATBF Local isoprenaline microinfusion resulted in a similar increase from baseline in abdominal and femoral ATBF (Fig. 1b). Oral glucose did not further affect ATBF. Abdominal and femoral ATBF during isoprenaline microinfusion (at 120 min) correlated positively ($r=0.59$, $p<0.05$). There was a strong negative correlation between BMI and the maximal abdominal and femoral ATBF during isoprenaline ($r=-0.74$, $p<0.05$, independent of sex). Systemic infusion of isoprenaline also resulted in marked ATBF increases which were similar between adipose tissue depots (Fig. 1c).

In contrast, non-selective α- and β-adrenoceptor stimulation with adrenaline resulted in a differential response between abdominal and femoral ATBF (Fig. 1d). Abdominal ATBF increased more than twofold within the first 40 min of infusion ($p<0.001$), while the femoral ATBF response was less pronounced than the abdominal response ($p<0.001$ for variation with time between the two sites). However, the adrenaline-stimulated ATBF at 60 min correlated strongly between abdominal and femoral adipose tissue ($r=0.73$, $p<0.001$, independent of BMI, WHR and sex).

Physiological analysis of regional adrenergic receptor subtype distribution in vivo Having established a differential regional ATBF response to adrenaline, the role of α-adrenoceptors in local ATBF regulation was investigated in vivo by combining the local microinfusion of an α-adrenoceptor blocker (phentolamine) with systemic adrenaline infusion (Fig. 1e, f). Basal ATBF, i.e. before adrenaline infusion but after 60 min of local microinfusion (see Methods section), did not differ significantly between microinfusion sites. In abdominal adipose tissue, local α-adrenergic blockade did not affect the ATBF response to adrenaline, and the response pattern was similar to that of the control site (saline; Fig. 1e). Propranolol microinfusion, given as a negative control in order to exclude any specific α-adrenergic effect on abdominal adipose tissue, blunted the abdominal ATBF response to adrenaline ($p<0.05$ for difference from saline/phentolamine sites; Fig. 1e).

In femoral adipose tissue, only phentolamine was infused locally (Fig. 1f). The local α-adrenergic blockade resulted in the 'disinhibition' of the femoral ATBF response to adrenaline ($p<0.001$ for phentolamine effect compared with femoral control site; Fig. 1f).

Haemodynamic and whole-body metabolic responses to adrenergic stimulation Local microinfusion of isoprenaline, phentolamine and propranolol did not result in measurable haemodynamic or metabolic responses (data not shown). The heart rate and blood pressure responses following ingestion of glucose (Study 1) and systemic isoprenaline (Study 2) or adrenaline infusion (Studies 3 and 4) are shown in Table 2.

Local microinfusion of isoprenaline (Study 1) did not affect plasma glucose, insulin or NEFA concentrations (data not shown). Ingestion of glucose resulted in an increase in plasma glucose and insulin, and a decrease in NEFA concentrations (ESM Fig. 1). Systemic isoprenaline infusion (Study 2) had no effect on plasma glucose concentrations, while there was a transient increase in insulin concentrations (Fig. 2a). Systemic plasma NEFA concentrations rose markedly in the first 20 min of isoprenaline infusion ($p<0.001$) (Fig. 2b). Whole-body Ra_{NEFA} doubled also during this time period ($p=0.001$, ESM Fig. 2). Systemic adrenaline infusion (Studies 3 and 4; data shown are from Study 3) increased plasma glucose concentrations (Fig. 2c). Plasma glucagon (measured in a subgroup of participants of Study 3, $n=15$) increased during adrenaline infusion from 81.1±4.3 to 92.0±5.7 pmol/l ($p=0.001$). There was a small but significant increase in plasma insulin concentration during adrenaline infusion ($p<0.001$, baseline vs 60 min; Fig. 2c). At the end of infusion, insulin increased before returning to basal concentrations ($p=0.002$, 60 vs 75 min; Fig. 2c). Systemic NEFA concentrations rose sharply during the first 40 min of adrenaline infusion ($p<0.001$; Fig. 2d). Whole-body Ra_{NEFA} almost doubled during adrenaline infusion ($p<0.001$; ESM Fig. 3). Plasma TG concentrations did not change during any of the studies (data not shown), which was expected as the studies were carried out in the fasting state (Studies 2–4) or only a pure carbohydrate-containing drink was given (Study 1).

Effect of adrenergic stimulation on regional lipolysis Systemic β-adrenoceptor stimulation with isoprenaline (Study 2) resulted in a significant increase in abdominal and femoral adipose tissue NEFA release ($p<0.001$ for variation with time; Fig. 3a). There were differences in the dynamics of the responses between abdominal and femoral adipose tissue ($p<0.05$ for site difference). However, when the femoral lipolysis response was expressed as a change from baseline, it was comparable with the abdominal adipose tissue response (abdominal 420±92% vs femoral 281±88% after 20 min; p not significant). Adrenaline infusion (Study 3) resulted in a dose-dependent increase in abdominal NEFA release ($p<0.001$ for variation with time; Fig. 3b). In contrast, femoral NEFA release was unresponsive to adrenaline infusion ($p<0.001$ for site difference; Fig. 3b).

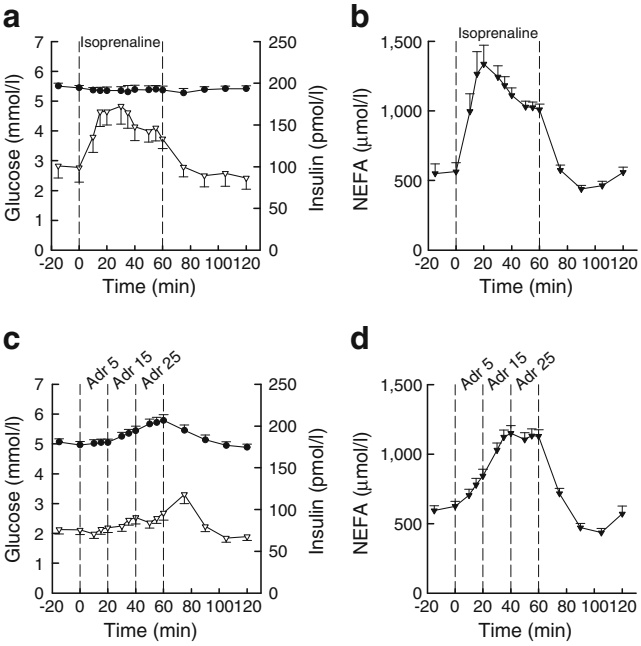

Fig. 2 Plasma metabolite concentrations. (**a**) Glucose (black circles) and insulin (white triangles); (**b**) NEFA during Study 2. A systemic isoprenaline infusion (20 ng min^{-1} [kg FFM]$^{-1}$) was given from 0 to 60 min. (**c**) Glucose (black circles) and insulin (white triangles); (**d**) NEFA during Study 3. An incremental systemic adrenaline infusion (Adr 5, 5 ng min^{-1} [kg FFM]$^{-1}$; Adr 15, 15 ng min^{-1} [kg FFM]$^{-1}$; Adr 25, 25 ng min^{-1} [kg FFM]$^{-1}$) was given from 0 to 60 min

Table 2 Systemic haemodynamic responses

Response	Study 1 (glucose)	Study 2 (isoprenaline)	Study 3 (adrenaline)	Study 4 (adrenaline)
Basal heart rate (bpm)	63±2	62±3	63±2	57±3
Stimulated heart rate (bpm)	72±3*	84±4***	71±2***	67±4***
Basal systolic BP (mmHg)	108±3	118±5	117±2	116±4
Stimulated systolic BP (mmHg)	112±4	138±5*	118±3	117±4
Basal diastolic BP (mmHg)	65±2	75±3	76±2	70±2
Stimulated diastolic BP (mmHg)	61±2	71±4*	69±1*	65±2*

Values are mean ± SD

Study-specific stimuli are shown in parentheses

*$p<0.05$, ***$p<0.001$ compared with basal bpm, beats per minute

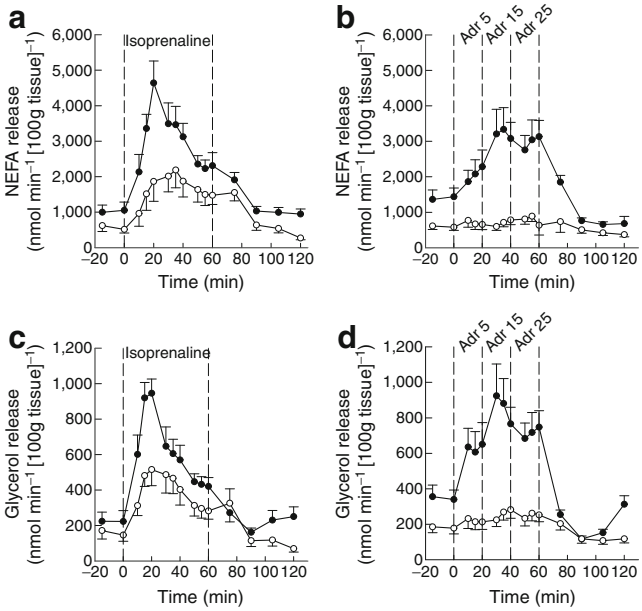

Fig. 3 Abdominal (black circles) and femoral (white circles) NEFA release (**a**) during a systemic isoprenaline infusion (20 ng min^{-1} [kg FFM]$^{-1}$) and (**b**) during an incremental systemic adrenaline infusion (Adr 5, 5 ng min^{-1} [kg FFM]$^{-1}$; Adr 15, 15 ng min^{-1} [kg FFM]$^{-1}$; Adr 25, 25 ng min^{-1} [kg FFM]$^{-1}$). Abdominal (black circles) and femoral (white circles) glycerol release (**c**) during systemic isoprenaline and (**d**) incremental adrenaline infusion

Expressed as change from baseline, abdominal NEFA release increased by 242±61% compared with only 33±68% in femoral adipose tissue after 60 min of infusion ($p<0.05$). These site-specific differences in the lipolytic response were also found when glycerol release was measured (Fig. 3c, d). Abdominal and femoral NEFA release responses at 60 min of adrenaline infusion correlated positively with each other, independent of BMI and sex ($r=0.67$, $p<0.05$). Abdominal adrenaline-stimulated lipolysis showed a negative relationship to measures of abdominal fat mass. The overall abdominal NEFA response to adrenaline (AUC) correlated negatively with waist circumference ($r=-0.61$, $p<0.05$) and WHR ($r=-0.68$, $p=0.005$), independently of BMI and sex. Femoral NEFA release AUC did not correlate significantly with any measure of fat mass. In contrast, femoral NEFA release per 100 g tissue at 60 min was negatively associated with waist circumference ($r=-0.64$, $p=0.01$) and WHR ($r=-0.65$, $p=0.008$) independently of BMI and sex. Abdominal and femoral Ra_{NEFA}, during both isoprenaline and adrenaline infusion, mirrored the total NEFA release data above (ESM Figs 4 and 5).

Male/female differences With the exception of the mixed α- and β-adrenoceptor stimulation study (Study 3), the studies were not powered to allow between-sex comparisons. In Study 3, there were no differences in basal and adrenaline-stimulated abdominal and femoral ATBF between male and female participants (Fig. 4a, b). This was also the case when abdominal lipolysis was compared, whereas women had a higher femoral basal lipolytic rate, both per unit fat mass, as well as per whole depot ($p=0.007$ vs men for both; whole-depot data shown in Fig. 4c, d). They also showed a higher adrenaline-stimulated femoral NEFA release rate than men, both at 40 and 60 min of adrenaline infusion. This difference in femoral lipolysis was true per unit fat mass ($p=0.006$ at 40 min, $p=0.051$ at 60 min), as well as per whole depot ($p<0.001$ for both 40 and 60 min). The basal and stimulated difference in femoral NEFA release rate is a true sex difference independent of femoral fat mass (ANCOVA $p<0.05$ for sex with femoral fat mass as covariate).

Regional adrenoceptor gene expression Distinct differences between abdominal and femoral adipose tissue were found with regard to the mRNA expression of *ADRA2A*, *ADRB1* and *ADRB2* in biopsy samples of whole adipose tissue (ESM Fig. 6). The mRNA of both β-adrenoceptor subtypes was more highly expressed in abdominal adipose tissue ($p<0.001$ for *ADRB1* and $p=0.008$ for *ADRB2*). While there was no difference in regional α_1-adrenoceptor expression, a higher mRNA expression of α_2-adrenoceptors was found in femoral adipose tissue ($p<0.001$ for *ADR2A*). There was no correlation between BMI or WHR and regional adrenoceptor mRNA expression. Women had a higher expression of abdominal β_1-adrenoceptor mRNA and a lower abdominal

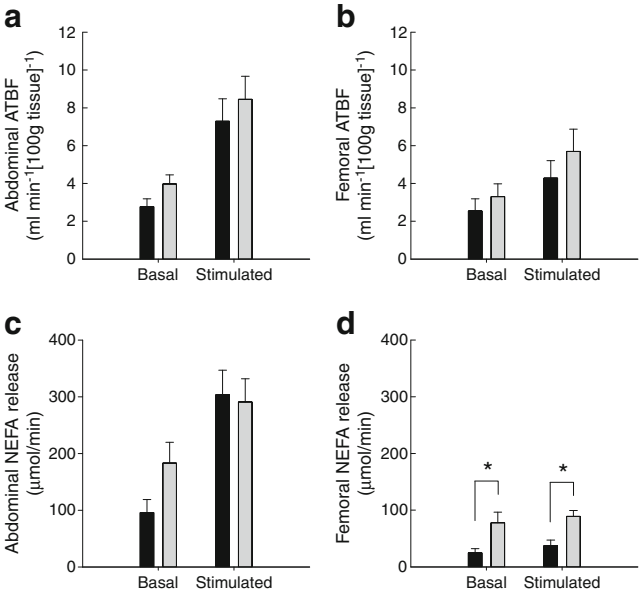

Fig. 4 Abdominal (**a**) and femoral (**b**) ATBF before (basal) and after 60 min of an incremental adrenaline infusion (stimulated) in men (black bars; $n=14$) and women (grey bars; $n=14$). Whole-depot abdominal (**c**) and femoral (**d**) NEFA release before (basal) and after 60 min of an incremental adrenaline infusion (stimulated) in men (black bars; $n=14$) and women (grey bars; $n=14$). *$p<0.05$

and femoral α_1-adrenoceptor mRNA expression than men (ESM Figs 7 and 8).

Discussion

The aim of these studies was to elucidate physiological mechanisms that render gluteofemoral adipose tissue protective against cardiovascular and diabetes risk, by means of fatty acid entrapment in vivo. Here we showed that abdominal and femoral adipose tissues are distinct fat depots with differential catecholamine-dependent regulation, and that femoral adipose tissue is less responsive to physiological adrenaline stimulation. We confirmed that basal ATBF is independent of catecholamine regulation [5] and showed that it is lower in femoral adipose tissue in line with a lower metabolic activity [17, 24]. As found previously, abdominal and femoral ATBF responses to dietary stimuli correlate with each other, and there is a strong inverse relationship with measures of obesity [17, 25]. ATBF and lipolytic responses to isoprenaline were comparable between abdominal and femoral adipose tissue, underlining that these responses are mechanistically mediated via β-adrenoceptors. Interestingly, the ATBF response to isoprenaline was similar regardless of the delivery mode (local microinfusion vs systemic infusion), suggesting that maximal β-adrenoceptor stimulation was reached in both cases and was the limiting factor in ATBF response. This is supported by the decrease in responses seen in both ATBF and lipolysis after the initial infusion periods, suggesting desensitisation, which is known to occur when adrenoceptors are maximally stimulated over prolonged periods of time [26]. Furthermore, the addition of a further sympathetic stimulus in the form of a glucose drink (in Study 1) did not lead to any further ATBF increases. The role of β-adrenoceptors as determinants of regional fatty acid trafficking is supported by a large body of in vitro data [8–10, 27]. However, they are in contrast with the only in vivo study comparing abdominal and femoral adipose tissue lipolysis during isoprenaline infusion [14]. In that study no lipolytic response to selective β-adrenoceptor stimulation was found in leg fat of women. This could be due to the low isoprenaline dose used and the measurement of lipolysis in the whole leg, as opposed to the more specific arteriovenous difference technique used here.

In contrast with selective β-adrenoceptor stimulation with isoprenaline, the physiological stimulus in vivo is adrenaline. Here, we showed that femoral adipose tissue ATBF and lipolysis are unresponsive to adrenaline, in marked contrast with abdominal adipose tissue. Mechanistically, we provide in vivo evidence that this is due to differences in the balance between local α- and β-adrenoceptor function, with femoral adipose tissue displaying a dominant α-adrenergic response during adrenaline stimulation. These findings are again in line with previous in vitro studies showing differential responses to adrenergic stimulation in adipocytes derived from different adipose tissue depots [8–10]. Others, using microdialysis and radioactive tracer techniques, have also shown differential responses to adrenergic stimuli between abdominal and leg adipose tissue in vivo [11, 14, 15]. However, there is not complete consistency in this area [15], which is probably due to methodological differences and limitations. This is the first study to use direct quantitative measurements for the comparison of lipolysis between abdominal and femoral adipose tissue in vivo. Here we showed that women had a higher basal and adrenaline-stimulated femoral lipolysis rate, which may be a sign of increased metabolic activity of female femoral adipose tissue. This is in contrast with findings from others, who found a lower femoral lipolytic rate and postulated this as the mechanism for the typical female fat distribution phenotype [15, 28, 29]. This may be due to methodological differences, such as the use of whole-leg blood sampling and low catecholamine doses. Clearly, male/female differences in regional fatty acid trafficking warrant further investigation.

Entrapment of fatty acids in a dedicated organ, adipose tissue, is thought to prevent ectopic fatty acid deposition, which is associated with insulin resistance and diabetes. Our findings suggest that the ‘metabolic sink’ function of femoral adipose tissue may be based on the finding that, under physiological conditions, fatty acids, once stored in femoral adipose tissue, are not readily released upon adrenergic stimulation. However, it is important to note that an overall lower lipolytic rate must be balanced by an overall lower uptake rate, otherwise femoral fat would increase steadily over time. In contrast, femoral fat mass remains stable over large time periods and changes only in relation to hormonal changes, e.g. during lactation and menopause [30] or, of course, during positive energy balance [31]. Recently, we have reported that femoral adipose tissue may have a special role in the uptake of VLDL-derived TG and NEFA [17]. The decreased responsiveness to lipolytic stimuli shown here may result in the preferential entrapment of fatty acids that have been recycled in the body and were not taken up by other adipose tissue depots when first entering the circulation. Hence, the lower femoral adipose tissue lipolytic rate may ultimately prevent ectopic fatty acid deposition and the subsequent development of insulin resistance and diabetes. Adipose tissue dysfunction, as seen in obesity [25, 32, 33] and in clinical conditions such as Cushing’s syndrome and lipodystrophy [1], is associated with increased cardiovascular disease risk and insulin resistance. Whether femoral adipose tissue dysfunction specifically is a contributing factor, or whether there are other additional determinants conveying a protective role of femoral adipose tissue remains to be investigated.

Acknowledgements Part of the results was presented during the 71st Scientific Sessions of the American Diabetes Association and the 11th International Congress on Obesity. We thank Louise Dennis and Jane Cheeseman for expert nursing support with the studies, and Sandy Humphreys, Marjorie Gilbert, Leanne Hodson, Pauline Sutton, Matthew Neville and Jennifer Collins for expert laboratory support (all University of Oxford, Oxford Centre for Diabetes, Endocrinology and Metabolism).

Funding The studies were funded by the Wellcome Trust.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

Contribution statement KNM researched the data and wrote the manuscript, and FK and KNF helped design the study, discussed the data and reviewed/edited the manuscript. All authors provided final approval of the version to be published.

References

1. Manolopoulos KN, Karpe F, Frayn KN (2010) Gluteofemoral body fat as a determinant of metabolic health. Int J Obes (Lond) 34:949–959
2. Lemieux I (2004) Energy partitioning in gluteal-femoral fat: does the metabolic fate of triglycerides affect coronary heart disease risk? Arterioscler Thromb Vasc Biol 24:795–797
3. Despres JP, Lemieux I (2006) Abdominal obesity and metabolic syndrome. Nature 444:881–887
4. Lafontan M, Langin D (2009) Lipolysis and lipid mobilization in human adipose tissue. Prog Lipid Res 48:275–297
5. Ardilouze JL, Fielding BA, Currie JM, Frayn KN, Karpe F (2004) Nitric oxide and beta-adrenergic stimulation are major regulators of preprandial and postprandial subcutaneous adipose tissue blood flow in humans. Circulation 109:47–52
6. Frayn KN (2010) Fat as a fuel: emerging understanding of the adipose tissue-skeletal muscle axis. Acta Physiol (Oxf) 199:509–518
7. Karpe F, Fielding BA, Ardilouze JL, Ilic V, Macdonald IA, Frayn KN (2002) Effects of insulin on adipose tissue blood flow in man. J Physiol 540:1087–1093
8. Arner P, Hellstrom L, Wahrenberg H, Bronnegard M (1990) Beta-adrenoceptor expression in human fat cells from different regions. J Clin Invest 86:1595–1600
9. Wahrenberg H, Lonnqvist F, Arner P (1989) Mechanisms underlying regional differences in lipolysis in human adipose tissue. J Clin Invest 84:458–467
10. Lafontan M, Dang-Tran L, Berlan M (1979) Alpha-adrenergic antilipolytic effect of adrenaline in human fat cells of the thigh: comparison with adrenaline responsiveness of different fat deposits. Eur J Clin Invest 9:261–266
11. Horowitz JF, Klein S (2000) Whole body and abdominal lipolytic sensitivity to epinephrine is suppressed in upper body obese women. Am J Physiol Endocrinol Metab 278:E1144–E1152
12. Karpe F, Fielding BA, Ilic V, Humphreys SM, Frayn KN (2002) Monitoring adipose tissue blood flow in man: a comparison between the ^{133}xenon washout method and microdialysis. Int J Obes Relat Metab Disord 26:1–5
13. Martin ML, Jensen MD (1991) Effects of body fat distribution on regional lipolysis in obesity. J Clin Invest 88:609–613
14. Guo Z, Johnson CM, Jensen MD (1997) Regional lipolytic responses to isoproterenol in women. Am J Physiol 273:E108–E112
15. Jensen MD, Cryer PE, Johnson CM, Murray MJ (1996) Effects of epinephrine on regional free fatty acid and energy metabolism in men and women. Am J Physiol 270:E259–E264
16. McQuaid SE, Manolopoulos KN, Dennis AL, Cheeseman J, Karpe F, Frayn KN (2010) Development of an arterio-venous difference method to study the metabolic physiology of the femoral adipose tissue depot. Obesity (Silver Spring) 18:1055–1058
17. Le KA, Ventura EE, Fisher JQ et al (2011) Ethnic differences in pancreatic fat accumulation and its relationship with other fat depots and inflammatory markers. Diabetes Care 34:485–490
18. Tan GD, Neville MJ, Liverani E et al (2006) The in vivo effects of the Pro12Ala PPARγ2 polymorphism on adipose tissue NEFA metabolism: the first use of the Oxford Biobank. Diabetologia 49:158–168
19. Larsen OA, Lassen NA, Quaade F (1966) Blood flow through human adipose tissue determined with radioactive xenon. Acta Physiol Scand 66:337–345
20. Frayn KN, Coppack SW, Humphreys SM, Whyte PL (1989) Metabolic characteristics of human adipose tissue in vivo. Clin Sci (Lond) 76:509–516
21. Bickerton AS, Roberts R, Fielding BA et al (2007) Preferential uptake of dietary fatty acids in adipose tissue and muscle in the postprandial period. Diabetes 56:168–176
22. Frayn KN, Macdonald IA (1992) Methodological considerations in arterialization of venous blood. Clin Chem 38:316–317
23. Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159
24. Tan GD, Goossens GH, Humphreys SM, Vidal H, Karpe F (2004) Upper and lower body adipose tissue function: a direct comparison of fat mobilization in humans. Obes Res 12:114–118
25. McQuaid SE, Hodson L, Neville MJ et al (2010) Downregulation of adipose tissue fatty acid trafficking in obesity: a driver for ectopic fat deposition? Diabetes 60:47–55
26. Chuang TT, Iacovelli L, Sallese M, De Blasi A (1996) G protein-coupled receptors: heterologous regulation of homologous desensitization and its implications. Trends Pharmacol Sci 17:416–421
27. Richelsen B (1986) Increased alpha 2- but similar beta-adrenergic receptor activities in subcutaneous gluteal adipocytes from females compared with males. Eur J Clin Invest 16:302–309
28. Rosenstock J, Banarer S, Fonseca VA et al (2010) The 11-beta-hydroxysteroid dehydrogenase type 1 inhibitor INCB13739 improves hyperglycemia in patients with type 2 diabetes inadequately controlled by metformin monotherapy. Diabetes Care 33:1516–1522
29. Romanski SA, Nelson RM, Jensen MD (2000) Meal fatty acid uptake in adipose tissue: gender effects in nonobese humans. Am J Physiol Endocrinol Metab 279:E455–E462
30. Wells JC, Griffin L, Treleaven P (2010) Independent changes in female body shape with parity and age: a life-history approach to female adiposity. Am J Hum Biol 22:456–462
31. Kotelevtsev Y, Holmes MC, Burchell A et al (1997) 11Beta-hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid-inducible responses and resist hyperglycemia on obesity or stress. Proc Natl Acad Sci USA 94:14924–14929
32. Summers LK, Samra JS, Humphreys SM, Morris RJ, Frayn KN (1996) Subcutaneous abdominal adipose tissue blood flow: variation within and between subjects and relationship to obesity. Clin Sci (Lond) 91:679–683
33. Karpe F, Fielding BA, Ilic V, Macdonald IA, Summers LK, Frayn KN (2002) Impaired postprandial adipose tissue blood flow response is related to aspects of insulin sensitivity. Diabetes 51:2467–2473