Gluteofemoral adipose tissue plays a major role in production of the lipokine palmitoleate in humans

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Abstract

The expansion of lower-body adipose tissue (AT) is paradoxically associated with reduced cardiovascular disease and diabetes risk. We examined whether the beneficial metabolic properties of lower-body AT are related to production and release of the insulin-sensitizing lipokine palmitoleate (16:1*n-7*). Using veno-arterial difference sampling we investigated the relative release of 16:1*n-7* from lower-body (gluteofemoral) and upper-body (abdominal subcutaneous) AT depots. Paired gluteofemoral and abdominal subcutaneous AT samples were analysed for triglyceride fatty acid composition and mRNA expression. Finally, the triglyceride fatty acid composition of isolated human pre-adipocytes was determined. Release of 16:1*n-7* was markedly higher from gluteofemoral AT when compared with abdominal subcutaneous AT. Stearoyl-CoA desaturase 1 (*SCD1*), the key enzyme involved in endogenous 16:1*n-7* production, was more highly expressed in gluteofemoral AT and was associated with enrichment of 16:1*n-7* in the gluteofemoral AT. Furthermore, isolated human pre-adipocytes from gluteofemoral AT displayed a higher content of SCD1-derived fatty acids. We demonstrate that human gluteofemoral AT plays a major role in determining systemic concentrations of the lipokine palmitoleate. Moreover, this appears to be an inherent feature of gluteofemoral AT. We propose that the beneficial metabolic properties of lower-body AT may be partly explained by intrinsically greater production and release of palmitoleate.

Body fat distribution is a major determinant of health (1). Abdominal adipose tissue (AT) accumulation increases cardiovascular disease (CVD) and type 2 diabetes risk**,** whereas the expansion of gluteofemoral AT provides protection from metabolic-related diseases (2, 3). The mechanisms surrounding this paradox remain to be elucidated. Evidence from *in vivo* and *in vitro* rodent models suggests that AT-derived circulating palmitoleate (16:1*n*-7) acts as an insulin-sensitizing lipokine which improves insulin signalling in muscle and liver (4, 5). A strong positive relationship has recently been demonstrated between the proportion of palmitoleate in the plasma free fatty acid pool and insulin sensitivity in humans (6). Considering this, we proposed that site-specific differences in the release of 16:1*n-7*, via stearoyl-CoA desaturase 1 (SCD1) activity, may provide an important mechanistic explanation for the differing contributions of distinct AT depots to metabolic health. Using veno-arterial difference sampling we investigated the release of 16:1*n-7* from lower-body (gluteofemoral) and upper-body (abdominal subcutaneous) AT depots. AT fatty acid (FA) and mRNA profiling were combined with *in vitro* studies to investigate tissue-specific FA metabolism in humans.

Research Design and Methods

All studies were approved by the Oxfordshire Clinical Research Ethics Committee and all volunteers gave written, informed consent.

In vivo measurements of fatty acid release from human adipose tissue

Following an overnight fast, FA release was measured in 48 healthy individuals (28 males, BMI 20-31 kg/m^2, and 20 females, BMI 18.8-39.3 kg/m^2) recruited for previous and on-going studies of AT veno-arterial difference measurements (7). For metabolic characteristics refer to online supplementary Table 1. Non-esterified fatty acids (NEFA) were extracted from plasma and analysed by gas chromatography (GC) as described below.

Adipose tissue fatty acid composition and transcriptional profiling

Thirty six healthy individuals, BMI 24-46 kg/m^2 (18 males) and 21-40 kg/m^2 (18 females) were recruited from the Oxford Biobank (8). For metabolic characteristics refer to online supplementary Table 1. Paired AT samples were taken by needle biopsy (8) from the periumbilical and upper buttock areas. mRNA was extracted from biopsies, reversed transcribed and qPCR assays performed (9). Expression values were calculated by the ΔCT transformation method (ΔCT = efficiency^[calibrator Ct−sample Ct]) and normalised to *PPIA* and *PGK1* (9). For FA profiling, total lipids were extracted from the lipid layer obtained during RNA extraction (10). The triglyceride (TG) fraction was separated by solid-phase extraction and fatty acid methyl esters (FAME) prepared and analysed by GC (10). FA were identified by comparing retention times to those of a known FAMEs standard (Sigma-Aldrich Company Ltd, UK) and analytical accuracy was assessed using external and in-house quality control samples (AOCS std#6, Thames Restek UK Ltd; Seven Seas Ltd, Hull, UK). GC results were converted to mol%. The between-assay precision (CV(%), n=33) for TG was: 16:0(4.0), 16:1*n*-7(5.7), 18:0(6.2) and 18:1*n*-9(4.2). The mean (mol %) and within-assay precision (CV(%), n=22) was calculated on replicate measurements of human AT as follows: 16:0, 22.5(1.6); 16:1*n*-7, 5.3(1.4); 18:0, 4.4(2.3); and 18:1*n*-9, 46.0(1.03).

Isolation and culture of human preadipocytes

Paired gluteal and abdominal subcutaneous AT biopsies were obtained as described above from five healthy females (BMI 20–24 kg/m^2). Stromal-vascular cells were isolated as previously described (11). Fully confluent cells were stimulated for 14 days with an adipogenic cocktail (11). 0.25mM 3-isobutyl-1-methylxanthine and 2µM troglitazone were added to the adipogenic medium for the first 4 days. Differentiated cells were harvested for FA composition analysis.

Calculations

Release of 16:1*n*-7 from abdominal and gluteofemoral AT was calculated relative to 16:0 and the essential FA 18:2*n*-6. 18:2*n*-6 is not endogenously synthesised and displayed no significant difference in abundance (mol%) between AT depots. Relative release of 16:1*n*-7 was calculated as: veno-arterial difference of 16:1*n*-7 (µmol/l)/veno-arterial difference of 16:0 or 18:2*n*-6 (µmol/l). Insulin resistance was calculated using the homeostatic model assessment of insulin resistance (HOMA-IR) (12). Product-to-precursor ratios were used as measures of enzyme activity. For SCD1 activity the desaturation indices of 16:0 and 18:0 were calculated from relative abundance (mol%) in the particular lipid fraction (ratio of 16:1*n*-7/16:0 and 18:1*n*-9/18:0). For elongase activity the ratios of 18:0/16:0 and 18:1*n*-7/16:1*n*-7 were calculated.

Statistics

Data were analysed using SPSS for Windows v15 (SPSS, UK, Chertsey, UK). All data are presented as means±SEM unless otherwise stated. All data sets were tested for normality

according to the Shapiro-Wilk test, and log transformed where appropriate. Comparisons were made between AT depots using a paired t-test or a Wilcoxon signed rank test. Gender analysis was performed using independent t-test. Correlations were assessed with Pearson's correlation coefficient.

Results

A major role for gluteofemoral AT in the release of 16:1n-7 in humans

To investigate regional variation in the release of 16:1*n-7* from AT, FA release from the gluteofemoral (GSAT) and abdominal subcutaneous (ASAT) depots was directly assessed using a veno-arterial difference sampling technique. When calculated relative to its precursor palmitate (16:0), the release of 16:1*n-7* was significantly higher from GSAT than ASAT in both sexes (Figure 1A). To confirm this was not driven by regional differences in 16:0 abundance, 16:1*n-7* release was also calculated relative to the essential FA linoleate (18:2*n-6*). Relative to 18:2*n-6,* 16:1*n-7* release was significantly higher from GSAT compared to ASAT in both males (0.47±0.04 vs. 0.36±0.03; $P<0.001$) and females (0.54±0.04 vs. 0.41±0.02; $P<0.001$). To our knowledge this is the first report that individual AT depots differ in their contribution to circulating 16:1*n-7* levels.

An inverse association was observed between insulin resistance, as defined by HOMA-IR, and both the proportion of 16:1*n-7* (mol%) in the plasma NEFA pool (r= -0.50, $P=0.0003$) and the plasma NEFA 16:1*n-7* concentration (r=-0.40, $P=0.006$). Using the group median, subjects were classified by HOMA-IR (<2.4 or ≥2.4). Relative to 16:0 the release of 16:1*n*-7 from GSAT

was higher in the more insulin-sensitive (HOMA-IR<2.4) compared to insulin-resistant individuals (Figure 1B). Release of 16:1*n*-7 relative to 16:0 from ASAT was not different between HOMA-IR groups. Similarly, when expressed relative to 18:2*n-6,* 16:1*n*-7 release from GSAT was higher in insulin-sensitive compared to insulin-resistant individuals (0.46±0.03 vs. 0.38±0.04; P=0.015), suggesting a link between gluteofemoral release of 16:1*n*-7 and insulin sensitivity.

Depot-specific production of 16:1n-7 is an inherent feature of AT

Regional variation in the release of 16:1*n-7* from AT may reflect differences in endogenous production. By analyzing AT TG fatty acid composition, we found the proportion of 16:1*n*-7 and its elongation product 18:1*n*-7 to be significantly higher in GSAT in both sexes (Table 1). This is consistent with depot-specific differences in AT fatty acid composition which have previously been described (13). To explore whether this tissue phenotype is a consequence of the local milieu or an inherent feature, human primary preadipocytes were isolated from both depots and differentiated *in vitro*. In support of intrinsic differences between the tissues, there was a tendency for higher 16:1*n*-7 and a significant enrichment of its elongation product 18:1*n*-7 (P<0.05) in cells derived from GSAT compared to ASAT (Figure 2).

ASAT and GSAT are exposed to the same circulating dietary FA *in vivo*. Therefore it can be assumed that differences in tissue 16:1*n-7* enrichment are determined by 16:0 desaturation via the enzyme SCD1. *SCD1* mRNA expression significantly between depots and sexes (P<0.001). Within-person *SCD1* expression was consistently higher ($P=1.6 \times 10^{-7}$) in GSAT than ASAT in both sexes and higher in females than males in both AT depots (Table 1). This sexual dimorphism for *SCD1* mRNA expression has not previously been reported. Using product-to-

precursor ratios (desaturation indices) there was evidence for significantly higher desaturation in GSAT compared to ASAT in both sexes (Table 1). We also investigated whether differences in the SCD1 desaturation index (16:1*n*-7/16:0) reflect depot-specific *SCD1* mRNA expression. In ASAT there was a strong (r=0.64, *P*=0.005) association between *SCD1* mRNA expression and the 16:1*n*-7/16:0 ratio (Figure 3A), but the corresponding association was not found in GSAT (data not shown).

Significantly greater elongation of 16:0 to 18:0 was observed in ASAT compared to GSAT as determined by the 18:0/16:0 ratio (Table 1). Overall, the mRNA expression of several *ELOVL* genes which may act at this step varied significantly between depot and sex (Table 1). There was a striking association between *ELOVL5* mRNA expression and the 18:0/16:0 ratio in GSAT in both sexes (r=0.64 *P*=0.004 females and r=0.48, *P*=0.045 males) (Figure 3B). There was also a strong (r=0.50, *P*=0.03) association between *ELOVL5* mRNA expression and the 18:0/16:0 ratio in ASAT in females but not in males (Figure 3C). Elongases play a critical role in FA modification but their regulation remains unclear (14). These findings suggest a significant role for *ELOVLs* and *SCD* in depot-specific AT FA metabolism which may influence the FA composition of stored TG.

Discussion

Gluteofemoral AT is sometimes regarded as a quiescent ‘metabolic sink’ which retains excess FA (1), but we provide evidence that it may play a more active role. This study has revealed AT depot-specific and sexual dimorphic intrinsic desaturation and elongation of FA. This enables tissue-specific release of palmitoleate which may constitute an insulin-sensitizing lipokine signal (4, 5). Indeed, we have previously reported a case of human partial lipodystrophy

resulting from a mutation (P467L) in the *PPARG* gene which was characterized by insulin resistance and extremely low plasma NEFA 16:1*n*-7. Upon insulin-sensitization with rosiglitazone a marked increase in the 16:1*n*-7/16:0 ratio was observed in this individual (10).

Previous reports of an association between plasma palmitoleate in the NEFA fraction and insulin sensitivity are conflicting (6, 15). In agreement with Stefan *et al*. we demonstrate a strong positive relationship between the proportion of 16:1*n-7* in the plasma NEFA pool and insulin sensitivity (6). The 16:1*n-7* content in plasma lipid fractions other than NEFA are difficult to assess in this context (16, 17) as they do not reflect AT well (18). The plasma NEFA pool is strongly determined by FA released from subcutaneous AT, however in obesity we believe fat mobilisation becomes impaired. We have recently discussed growing evidence that lipolysis per kg fat mass is reduced in obesity (19), a situation associated with the clear down-regulation of key enzymes involved in fat mobilisation (HSL and ATGL) (8). Once fat mobilisation becomes disturbed the association between AT-derived 16:1*n-7* and insulin sensitivity may be lost, this would provide one explanation for the absence of such an association in obese cohorts (15).

Lower-body fat predominantly consists of the gluteal and femoral depots. There is evidence to suggest that these AT depots possess distinct characteristics, (20, 21). In *vivo* human studies have shown that both gluteal (22) and femoral (7) AT depots are metabolically less active than ASAT. Although a direct within-subject comparison of 16:1*n-7* release from the two lower-body depots has yet to be undertaken, tissue enrichment of 16:1*n*-7 in these depots is similar (23). When examining regional differences in FA release, depot size and sexual dichotomy of body fat distribution should be given careful consideration. Identifying ASAT and visceral AT is challenging when conventional methods such as dual energy X-ray absorptiometry (DEXA) are utilized since discrimination between these depots is not possible. The more routine use of

imaging modalities such as computed tomography in combination with DEXA (24), to dissect anatomically distinct regions will enable more comprehensive studies on regional fatty acid trafficking. We speculate that individuals who preferentially accumulate lower-body fat will have a greater net release of 16:1*n-7* from this depot relative to other fat depots which may contribute to the beneficial properties of the lower-body fat depot.

Visceral fat accumulation confers increased metabolic risk which may be explained by an increased flux of NEFA from this depot into the portal vein having direct effects on liver metabolism (25). The 16:1*n-7* enrichment in upper-body subcutaneous and visceral AT is similar (13, 23, 26) and it would be of interest to determine the effect of 16:1*n-7* from the visceral depot on intra-hepatic metabolism.

The finding that gluteofemoral AT plays an important role in 16:1*n-7* production *in vivo* in humans provides further mechanistic understanding of the metabolically-protective properties of this AT depot. We anticipate that this work will be the starting point for more complex *in vivo* human studies to gain further insight into the role and function of the lipokine palmitoleate.

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Table 1: Triglyceride fatty acid composition (mol%) and mRNA expression of lipid metabolism genes in gluteofemoral and abdominal subcutaneous adipose tissue

	Abdominal		Gluteofemoral	
	Male (n=18)	Female (n=18)	Male (n=18)	Female (n=18)
Fatty acid (mol %)				
14:0	4.5 ± 0.2	4.9 ± 0.3	3.9 ± 0.2*	4.2 ± 0.3*
14:1*n*-5	0.5 ± 0.0	0.7 ± 0.0†	0.7 ± 0.0*	0.8 ± 0.1*
16:0	27.9 ± 0.3	26.3 ± 0.4‡	24.6 ± 0.5*	23.3 ± 0.4*
16:1*n*-7	5.9 ± 0.4	6.5 ± 0.3	8.6 ± 0.5*	8.8 ± 0.4*
18:0	3.7 ± 0.3	3.5 ± 0.3	2.7 ± 0.2*	2.5 ± 0.2*
18:1*n*-9	42.4 ± 0.4	42.9 ± 0.6	44.3 ± 0.4*	44.8 ± 0.6*
18:1*n*-7	2.0 ± 0.1	2.1 ± 0.1	2.2 ± 0.1*	2.2 ± 0.1*
18:2*n*-6	10.8 ± 0.3	11.1 ± 0.5	10.8 ± 0.3	11.5 ± 0.5*
20:4*n*-6	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0*	0.2 ± 0.0†
Fatty acid ratios				
16:1*n*-7/16:0	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.0*	0.4 ± 0.0*
16:1*n*-7/18:2*n*-6	0.5 ± 0.0	0.8 ± 0.0	0.6 ± 0.0*	0.8 ± 0.1*
18:1*n*-9/18:0	12.4 ± 1.0	13.3 ± 1.0	18.1 ± 1.5*	20.1 ± 1.6*
18:0/16:0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0*	0.1 ± 0.0*
18:1 *n*-7/16:1*n*-7	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0*	0.3 ± 0.0*
mRNA expression				
FASN	0.5 ± 0.0	0.5 ± 0.1	0.5 ± 0.0	0.4 ± 0.0*
ELOVL3	0.1 ± 0.0	0.3 ± 0.1§	0.2 ± 0.0*	0.7 ± 0.1*,§
ELOVL5	0.8 ± 0.0	1.0 ± 0.1†	0.9 ± 0.0*	1.0 ± 0.1
ELOVL6	0.2 ± 0.0	0.4 ± 0.1‡	0.2 ± 0.0	0.3 ± 0.0$^{\|}$
ELOVL7	0.6 ± 0.1	0.3 ± 0.1§	0.7 ± 0.1	0.3 ± 0.0§
SCD	0.4 ± 0.1	0.7 ± 0.0‡	0.6 ± 0.1*	0.8 ± 0.1*,‡

Data presented as mean ± sem. $^{\|}$*P*<0.05, **P*<0.001 abdominal vs. gluteofemoral; †*P*<0.05, ‡*P*<0.01, §*P*<0.001 male vs. female for the same depot

Figure Legends

Figure 1 Depot-specific release of 16:1*n*-7 from lower- and upper-body subcutaneous AT depots. (**A**) The release of 16:1*n*-7 relative to 16:0 was greater from gluteofemoral AT compared to abdominal AT in both sexes, $P \leq 0.001$. (**B**) When grouped according to insulin resistance status (as calculated by HOMA-IR) the release of 16:1*n*-7 relative to 16:0 from the gluteofemoral depot was higher in individuals with a HOMA-IR<2.4 ($P<0.05$). Release of 16:1*n*-7 relative to 16:0 from abdominal AT was not different between HOMA-IR groups. All data are represented as mean ± SEM.

Figure 2 Fatty acid profiling of isolated human pre-adipocytes derived from gluteofemoral and abdominal subcutaneous AT. Following a 14 day adipogenic time-course triglyceride fatty acid composition (mol %) of gluteofemoral-derived pre-adipocytes displayed enrichment in 18:1*n*-7, the elongation product of 16:1*n*-7. $P<0.05$ gluteofemoral compared to abdominal pre-adipocytes. Data are represented as mean ± SEM.

Figure 3 Associations between mRNA expression of lipid metabolism genes and triglyceride fatty acid product-to-precursor ratios. (**A**) *SCD1* mRNA expression in abdominal AT was strongly associated with the 16:1*n*-7/16:0 ratio in males (r=0.64, $P=0.005$) but not females (r=0.23, NS). (**B**) *ELOVL5* mRNA expression in gluteofemoral AT was significantly associated with the 18:0/16:0 ratio in both sexes (r=0.64, $P=0.004$ females and r=0.48, $P=0.045$ males). (**C**) *ELOVL5* mRNA expression in abdominal AT was significantly associated with the 18:0/16:0 ratio in females (r=0.50, $P=0.03$).