HHS Public Access

Author manuscript

Int J Obes (Lond). Author manuscript; available in PMC 2015 December 01.

Published in final edited form as:

Int J Obes (Lond). 2015 June ; 39(6): 884–887. doi:10.1038/ijo.2015.10.

Adipose Tissue Fatty Acid Storage Factors: Effects of Depot, Sex and Fat Cell Size

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Abstract

Background/Objectives—Patterns of postabsorptive adipose tissue fatty acid storage correlate with sex-specific body fat distribution. Some proteins and enzymes participating in this pathway include CD36 (facilitated transport), acyl-CoA synthetases (ACS; the first step in fat metabolism), and diacylglycerol acetyl-transferase (DGAT; the final step of triglyceride synthesis). Our goal was to better define CD36, ACS and DGAT in relation to sex, subcutaneous fat depots, and adipocyte size.

Subjects/Methods—Data was collected from studies conducted at Mayo Clinic between 2004 and 2012. Abdominal and femoral subcutaneous fat biopsy samples must have been collected in the postabsorptive state from healthy males and premenopausal females. Body composition was measured with DXA and abdominal CT scans. Adipocyte size (microscopy), CD36 protein content (ELISA), and ACS and DGAT enzyme activities were measured. Data are presented as medians; 25^{th}:75^{th} quartiles.

Results—Males (n=60) and females (n=78) did not differ by age (37;28:46 yr), BMI (28.4;24.6:32.1 kg/m^2), or abdominal (0.60;0.45:0.83 μg/cell) and femoral adipocyte size (0.76;0.60:0.94 μg/cell). Femoral ACS and DGAT were greater in females than males when expressed per mg lipid (ACS: 73 vs. 55 pmol/mg lipid/min; DGAT: 5.5 vs. 4.0 pmol/ mg lipid/ min; $p<0.0001$ for both) and per 1000 adipocytes (ACS: 59 vs. 39 pmol/1000adipocytes/min; DGAT: 4.3 vs. 3.1 pmol/1000adipocytes/min; $p\leq0.0003$ for both). There were no differences in abdominal fat storage factors between sexes. ACS and DGAT decreased as a function of adipocyte size ($p<0.0001$ for both). The decrease in ACS was greater in males and abdominal subcutaneous fat. There were no sex differences in CD36 in either fat depot, nor did it vary across adipocyte size.

Conclusions—Facilitated transport of fatty acids by CD36 under postabsorptive conditions would not be different in those with large vs. small adipocytes in either depot of both sexes. However, intracellular trafficking of fatty acids to triglyceride storage by ACS and DGAT may be less efficient in larger adipocytes.

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Conflict of Interest Statement: The authors have no conflict of interest to disclose.

Keywords

DGAT; acyl-CoA synthetase; CD36; fat biopsy

INTRODUCTION

Adipose tissue is essential to maintaining whole body energy balance. In addition to releasing relatively large amounts of free fatty acids (FFA) via lipolysis under postabsorptive and exercise conditions, the direct FFA storage pathway in adipose tissue also takes up and stores circulating FFA in the fasting state [1–5]. The cellular proteins and enzymes that traffic FFA to adipocyte triglycerides also come into play during the storage of meal fatty acids, which additionally requires lipoprotein lipase [6]. We have noted that the patterns of direct adipose tissue FFA storage are consistent with sex differences in body fat and body fat distribution [2]. Consistent with their greater subcutaneous fat mass, the FFA storage efficiency is greater in subcutaneous adipose tissue of females than males [1, 2, 7]. Furthermore, there is preferential fatty acid storage in lower body compared with upper body subcutaneous adipose tissue of females and upper body vs. lower body subcutaneous adipose tissue of males [2]. We estimate that there is a redistribution of ~ 800 g/year of fatty acids via this pathway from upper body to lower body subcutaneous adipose tissue in women [1], but none in men. This fatty acid storage process is independent of lipoprotein lipase and must in part be determined by transmembrane transport of fatty acids and the enzymatic machinery required to process non-esterified fatty acids along the pathway towards triglycerides.

Some of the key adipocyte factors in the trafficking of fatty acids to storage as a triglyceride are: 1) CD36, a protein that facilitates transmembrane fatty acid transport; 2) acyl-CoA synthetase (ACS), the enzyme that activates/traps fatty acids inside the cell and is necessary for subsequent metabolism; and 3) diacylglycerol transferase (DGAT), the enzyme involved in the last step of triglyceride synthesis. CD36 appears to be most important in adipocyte fatty acid transport under conditions of low extracellular fatty acid concentrations [8]. Under these conditions there is more than sufficient ACS and DGAT to promote triglyceride synthesis. In contrast, ACS and DGAT might become rate-limiting for fatty acid storage under conditions of high fatty acid availability, when CD36 does not seem to be important [8].

Adipose tissue can adjust to changes in energy balance by recruiting new adipocytes [9] (e.g. hyperplasia) and/or increasing adipocyte size (e.g. hypertrophy) [9, 10]. These two fundamentally different processes for adipose expansion could result in different tendencies for fatty acid storage. Whether differences in fatty acid storage properties of adipocytes relate to adipocyte size across different fat depots in males and females is unknown. To better understand these relationships we pooled data from over 100 male and female participants with a range of body fat. Our goal was to gather sufficient data to test whether sex, depot and adipocyte size relate to differences in CD36, ACS and DGAT from subcutaneous adipose tissue depots.

METHODS

We used data generated from IRB approved studies conducted by our laboratory at the Mayo Clinic, Rochester, MN between 2004 and 2012. Informed, written consent was obtained from all volunteers. Only males and premenopausal females were included in this database. Each participant underwent a whole body dual energy x-ray absorptiometry (DXA) scan and single slice computerized tomography (CT) scan at the L_{2-3} interspace to determine visceral, upper body and lower body subcutaneous adipose tissue mass as previously described [11]. Only those subcutaneous adipose tissue depot samples collected by needle biopsy [2] in the postabsorptive state prior to any intervention were included. Samples were processed for measurements of adipocyte size [12] on the day of the biopsy and a portion of the sample was flash frozen in liquid nitrogen and stored at −80 C for analysis of CD36 protein content [13], ACS [14] and DGAT enzyme activities [15]. Part of this process includes thoroughly washing fresh tissue sample to remove blood contaminates. We tested six adipose tissue samples to determine the proportion of CD36, ACS and DGAT present in the stromovascular fraction vs. the adipocyte fraction. On average, < 5% of the ACS and DGAT activity was found in the stromovascular faction, and we cannot exclude contamination of that fraction with adipocytes of sufficiently small size to physically separate with the stromovascular fraction. Likewise, we found very little CD36 protein in the stromovascular fraction (data not shown). Given these results, we treated the whole tissue data as being largely, if not solely, representative of adipocyte properties. As there is no commercially available standard for the CD36, ACS and DGAT assays, we used an in house control sample of human adipose tissue from surgical waste to control for intra- and inter-assay variability. A fresh aliquot of the control sample was included in duplicate at regular intervals within each assay. An average of the control sample from multiple assays was used to determine its target value. When a new lot of control sample was needed, that sample was run as a secondary control and calibrated to the initial control sample over the course of multiple assays to determine the crossover adjusted target value. Both intra- and inter-assay coefficient of variation were accepted if ≤ 15%. The results of the samples were normalized to the target value of the control sample. The results of the assays were expressed per mg adipose tissue lipid and per 1000 adipocytes.

Statistical analysis

Non-normally distributed data are presented as medians; 25th, 75th quartiles. Spearman's correlations tested the strength of the relationship between the three fatty acid storage factors (e.g. CD36, ACS and DGAT) within each subcutaneous adipose tissue depot. Wilcoxon Rank Sums were used to compare fatty acid storage factor across variables of interest. Regression models tested the relationships between each fatty acid storage factor and adipocyte size while taking into consideration subcutaneous adipose tissue depot and sex. All statistical analyses performed with JMP 9.0.1 (SAS Institute Inc.).

RESULTS

The subject characteristics are provided in Table 1. Females had more subcutaneous fat than males, but the groups were otherwise well-matched.

We sought to determine if subcutaneous adipose tissue CD36 content and ACS and DGAT activity were interrelated. ACS and DGAT activities were strongly related in abdominal (ρ=0.71, P<0.0001) and femoral subcutaneous adipose tissue depots (ρ=0.77, P<0.0001). ACS and DGAT activities were less well correlated with CD36 in abdominal (ρ=0.42, P<0.0001 and ρ=0.21 P=0.03, respectively) and femoral subcutaneous adipose tissue depots (ρ=0.48, P<0.001 and ρ=0.43 P<0.0001, respectively).

Femoral subcutaneous adipose tissue ACS and DGAT activities were greater in females than males, whereas CD36 content was similar between sexes. Abdominal subcutaneous CD36, ACS and DGAT were similar in females and males (Table 2). We also examined whether these factors differed between depots within each sex. When expressed per 1000 adipocytes, all three factors were greater in femoral than abdominal subcutaneous adipose tissue in females (CD36: p<0.0001; ACS: p<0.0001; DGAT: p=0.0009). When expressed per mg adipose tissue lipid, only CD36 and ACS were greater in femoral than abdominal subcutaneous adipose tissue in females (p=0.004 and p=0.0003, respectively). In males, none of the fatty acid storage factors per 1000 adipocytes were different between depots, but DGAT activity per mg adipose tissue lipid was greater in abdominal than femoral subcutaneous adipose tissue (p=0.02).

Figure 1 depicts the relationships between abdominal CD36, ACS and DGAT per amount of adipose tissue lipid as a function of adipocyte size for abdominal adipose tissue in females and males, and Figure 2 depicts these relationships for femoral adipose tissue. We used regression models to assess how CD36, ACS and DGAT per mg adipose tissue lipid vary as a function of adipocyte size taking into consideration adipose tissue depot and sex. ACS activity per mg adipose tissue lipid decreased as a function of adipocyte size (β=−28.5, p<0.0001), and was less in abdominal subcutaneous adipose tissue (β= −4.7, p=0.007) and males (β= −4.4, p=0.01). DGAT activity per mg adipose tissue lipid also decreased as a function of adipocyte size (β= −3.0, p<0.0001), but was not different between adipose tissue depots (p=0.39) or sexes (p=0.48). CD36 content per mg adipose tissue lipid did not vary as a function of adipocyte size, adipose tissue depots or sex (p=0.08).

DISCUSSION

In humans, the redistribution of FFA between adipose tissue depots via the direct storage pathway that occurs in the postabsorptive condition may contribute to patterns of body fat distribution [1, 2]. We have reported that inter-individual differences in adipose tissue CD36, ACS and DGAT relate to differences in direct FFA storage [2, 7, 16, 17]. However, because of the limited numbers of observations in any individual study, drawing conclusions as to how these fatty acid storage factors vary across a heterogeneous population of adults is difficult. We therefore collated data from a series of studies to allow us to test how sex, adipose tissue depot and adipocyte size relate to CD36, ACS and DGAT. Our key findings are that subcutaneous adipose tissue ACS and DGAT activity per mg adipose tissue lipid decreases as adipocyte size increases. In contrast, CD36 content per mg adipose tissue lipid is not different between depots, between depots with larger and smaller adipocytes, or between males and females.

CD36 is a transmembrane protein that facilitates inward FFA transport, especially when extracellular fatty acid conditions are low [8, 18]. Protein expression of CD36 in abdominal subcutaneous and visceral adipose tissue did not vary across BMI levels of adult humans [19]. Our results indicate that CD36 content per mg adipose tissue lipid does not systematically vary as a function of fat cell size, suggesting that CD36 content of abdominal and femoral subcutaneous adipocytes increases in direct proportion to adipocyte size. Given the purported function of CD36, under conditions of low extracellular fatty acid concentrations, FFA uptake would be no greater in depots with large vs. small adipocytes.

We found that ACS and DGAT activities per mg adipose tissue lipid decrease as a function of adipocyte size, indicating that, unlike CD36, ACS and DGAT activities do not increase in proportion to adipocyte size. This is consistent with the report of reduced expression of lipogenic enzyme genes, including isoforms of DGAT, in those with larger adipocytes [20]. These findings suggest that intracellular factors in the fatty acid storage pathway could limit the maximum storage rates in humans with hypertrophic, as opposed to hyperplastic, adipose tissue. The importance of this observation relates to the extreme ranges of adipocyte size in persons with similar amounts of fat in a depot; we found a 7-fold range in fat cell size of individuals with the same CT-measured abdominal subcutaneous fat or DXA-measured leg fat [21].

There have been a number of studies examining sex differences in the biochemical pathways of adipose tissue fatty acid storage in humans. Comparisons between males and females have been made by measuring gene expression of proteins of the fatty acid storage pathway [1, 22], but given that mRNA only explains ~40% of the variation in protein concentration [23], the measures of protein abundance and enzyme activity rates provide a more direct analyses of proteins involved in fatty acid storage. Results of the current analysis expand upon our observation that CD36 in abdominal and femoral subcutaneous adipose tissue depots is similar in males and females [2]. We also used direct measures of ACS and DGAT activity to confirm that these factors are comparable between the sexes in the abdominal subcutaneous adipose tissue depot, but are greater in the femoral depot of females than males [2]. Together these findings indicate that protein-facilitated transport of fatty acids is seldom a regulating factor of adipocyte fatty acid storage in subcutaneous adipose tissue depots for either sex, whereas ACS and DGAT in femoral subcutaneous adipose tissue may give premenopausal females a competitive advantage for femoral adipocyte FFA storage in the postabsorptive state.

One limitation to our study is that the findings can only be applied to healthy, males and premenopausal females. Following menopause, females experience changes in body fat distribution [24, 25] and also have increased DGAT activity in abdominal and femoral adipose tissue [17]. Furthermore, most of our volunteers were Caucasian, and thus these results cannot be extrapolated to African ancestry, Asian or Latino populations without further study.

In summary, ACS and DGAT activity in the femoral subcutaneous adipose tissue is substantially greater in females than males, which could explain why females store more fatty acids in leg fat than males. Using this larger dataset we found that under postabsorptive conditions that ACS and DGAT activity per unit subcutaneous adipose tissue lipid mass

decrease as adipocyte size increases, which could potentially decrease the efficiency of the fatty acid storage pathway in those with hypertrophic vs. hyperplastic obesity. Our results indicate that CD36 is seldom a limiting factor in subcutaneous adipose tissue storage of fatty acids, regardless of sex, fat depot or adipocyte size.

Acknowledgments

Support: This work was supported by grant NCRR UL1 TR000135, National Institutes of Health grants DK-45343, DK-40484 and DK-50456.

KCH and MDJ designed the research. KCH, CK, SS and NCB performed the research. KCH and MDJ analyzed the data and wrote the paper. Dr. Michael Jensen is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

The authors are grateful to the participants for playing an integral role in making this research possible. In addition, thanks goes to the Mayo Clinic CRU staff, and especially Barbara Norby, Carley Vrieze, Christy Allred, Lendia Zhou, Debra Harteneck and Darlene Lucas (all from Mayo Clinic), for their technical assistance and help with data collection.

This work was supported by grant NCRR UL1 TR000135, National Institutes of Health grants DK-45343, DK-40484 and DK-50456.

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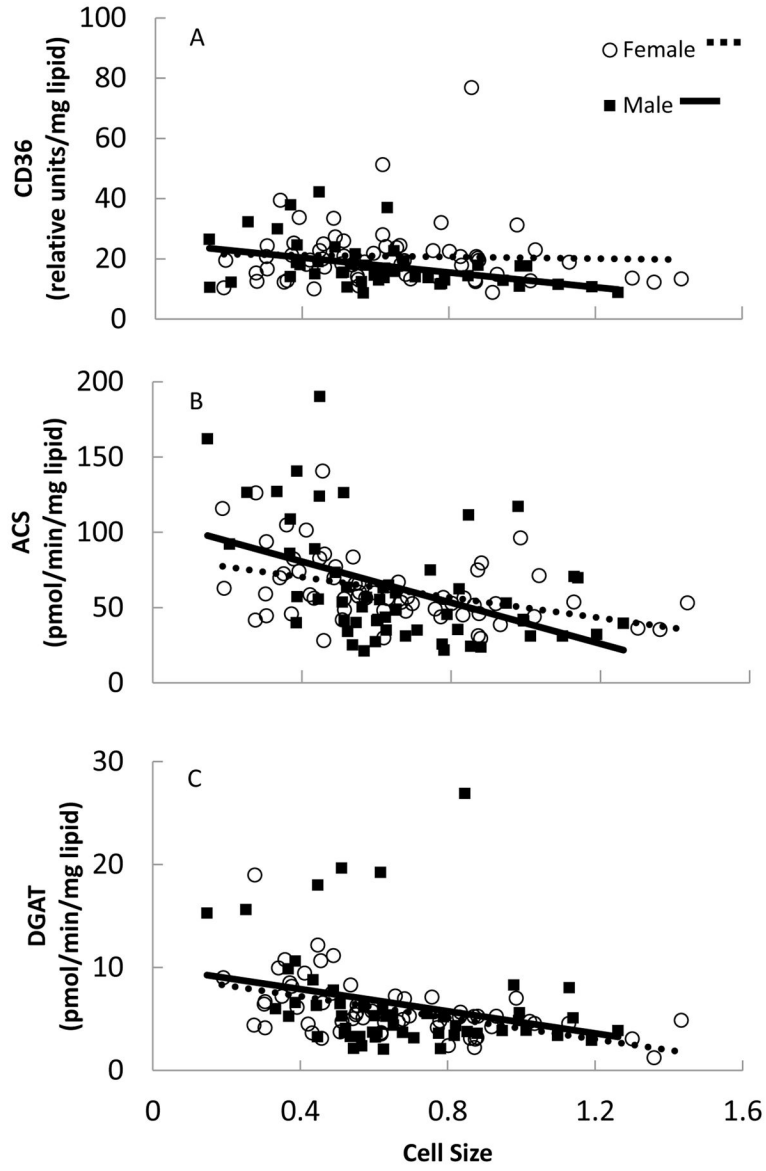

Figure 1.

Abdominal subcutaneous fat storage factors per mg lipid as a function of cell size (μg lipid/cell) in females (○) and males (■). A: CD36 versus adipocyte size (females: *n*=62; males: *n*=47). B: Acyl Co-A Synthetase (ACS) versus adipocyte size (females: *n*=61; males: *n*=56). C: Diacylglycerol Acyl Transferase (DGAT) versus adipocyte size (females: *n*=60; males: *n*=54).

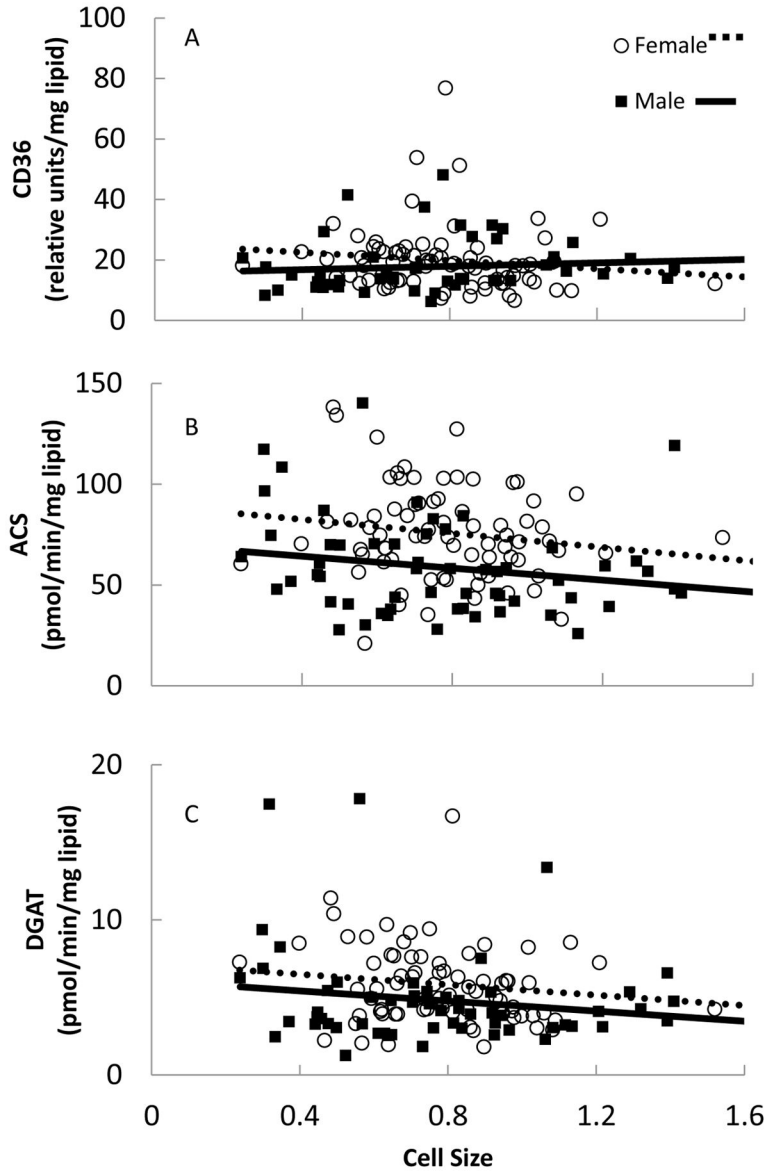

Figure 2.

Femoral subcutaneous fat storage factors per mg lipid as a function of cell size (μg lipid/cell) in females (○) and males (■). A: CD36 versus adipocyte size (females: *n*=75; males: *n*=49). B: Acyl Co-A Synthetase (ACS) versus adipocyte size (females: *n*=76; males: *n*=58). C: Diacylglycerol Acyl Transferase (DGAT) versus adipocyte size (females: *n*=75; males: *n*=58).

Table 1

Subject and adipocyte characteristics by sex.

	Male (n=60)	Female (n=78)	p-value
Age (years)	32 (23,47)	39 (29,46)	0.33
BMI (kg/m^2)	28.3 (25.2,32.3)	28.5 (24.1,32.1)	0.98
Glucose (mg/dl)	91 (84,95)	90 (85,95) (n=76)	0.78
Insulin (μIU/ml)	4.9 (3.2,7.5)	5.3 (3.5,7.2)	0.74
Abdominal			
Subcutaneous Fat (kg)	13.1 (9.8,19.8)	17.4 (12.5,20.2)	0.01
Cell Size (μg lipid/cell)	0.61 (0.45,0.82)	0.58 (0.43,0.84)	0.77
Femoral			
Subcutaneous Fat (kg)	7.9 (6.3,10.3)	11.8 (9.3,15.5)	<0.0001
Cell Size (μg lipid/cell)	0.74 (0.48,0.94)	0.78 (0.64,0.95)	0.23

Values represent the median and 25th:75th percentiles.

Table 2

Fatty acid storage factors in each depot by sex.

	Male	Female	p-value	Male	Female	p-value
Abdominal	**Per 1000 adipocytes**			**Per mg lipid**		
CD36 (relative units)	9.9 (7.9,12.2)	8.0 (5.2,11.9)	0.12	15.7 (12.9,19.3)	14.2 (10.4,18.8)	0.10
ACS (pmol/min)	32 (22,41)	36 (27,44)	0.38	53 (35,80)	57 (47,73)	0.23
DGAT (pmol/min)	3.3 (2.2,4.0)	3.2 (2.4,4.6)	0.92	5.2 (3.6,6.5)	5.2 (4.3,7.0)	0.41
Femoral						
CD36 (relative units)	11.5 (5.7,22.3)	14.5 (9.4,17.5)	0.10	14.3 (12.5,20.8)	18.2 (12.8,22.7)	0.18
ACS (pmol/min)	39 (26,56)	60(43,72)	<0.0001	55 (41,91)	73 (61,90)	<0.0001
DGAT (pmol /min)	3.1 (1.8,4.0)	4.3 (3.1,5.5)	0.0003	4.0 (3.1,5.3)	5.5 (4.0,7.2)	0.0005

Values represent the median and 25th:75th percentiles.