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Effect of adipose tissue on the sexual dimorphism in metabolic flexibility

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Abstract

Metabolic flexibility is the ability to transition between fat oxidation (fasting state) and glucose oxidation (fed state). We hypothesized that adipose tissue inflammation and lipid metabolism contribute to sexual dimorphism in metabolic flexibility. Respiratory quotient (ΔRQ, metabolic flexibility) and nonesterified fatty acids (NEFAs) before and during euglycemic-hyperinsulinemic clamp were measured in healthy young women (n = 22) and men (n = 56). Adiponectin levels were measured in plasma. Abdominal subcutaneous adipose tissue gene expression was measured by quantitative reverse transcriptase polymerase chain reaction. As compared with men, women had higher ΔRQ (0.14 ± 0.04 vs 0.09 ± 0.04, $P < .01$). Fasting RQ and fat cell size were not different between sexes. As compared with men, women had lower insulin-suppressed NEFAs ($P < .05$); greater adiponectin levels; and higher expression of adipogenesis, fatty acid storage, and oxidation genes (PPARγ2, PCK1, SCD1, and PPARα; $P < .05$). There were no sex differences in messenger RNA of macrophage markers or chemokines. Stepwise regression analysis revealed that the only adipose tissue characteristics that influenced metabolic flexibility in women were SCD1 and PCK1 messenger RNA (model $R^2 = 0.49$, $P < .05$); in men, these were serum adiponectin and insulin-suppressed NEFAs (model $R^2 = 0.34$, $P < .05$). Healthy young women are more metabolically flexible than men, driven by an increase in insulin-stimulated glucose oxidation rather than differences in fasting fat oxidation. Women have greater capacity for insulin suppression of NEFAs despite similar chemokine and macrophage content in adipose tissue. Combined, these results provide evidence for a role of adipose tissue characteristics in the sexual dimorphism of metabolic flexibility.
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1. Introduction

Metabolic flexibility is the ability to transition between the utilization of fatty acids (FAs) in the fasting state and carbohydrates in the fed, or insulin-stimulated, state. Metabolic flexibility also involves the transition from the release of FA from adipose tissue during fasting to the storage of FA in the fed state [1]. Past studies in metabolic flexibility have focused on skeletal muscle as the primary tissue involved in fuel switching. Using limb balance techniques, Kelley et al [2] found that under fasting conditions glucose oxidation was not different between lean and obese subjects. Fatty acid oxidation (FAO) was lower and FA storage was higher in the obese compared with lean subjects. They also showed that changes in insulin-stimulated substrate utilization differed between lean and obese subjects. In lean subjects, FAO and storage decreased in response to insulin; however, in obese subjects, those same responses to insulin were blunted, demonstrating a reduced capacity for fat oxidation and inflexibility in regulating fat oxidation.

Adipose tissue mass negatively impacts metabolic flexibility [2]. Frayn [3] postulated "that adipose tissue plays a crucial role in buffering the flux of FAs in the circulation during the postprandial period, just as the liver and, to a lesser extent, skeletal muscle buffer postprandial glucose fluxes." Adipose tissue function in response to insulin is disrupted in obesity and metabolic inflexibility. Experiments by Hickner et al [4] and others show a resistance to the insulin suppression of lipolysis in obesity. We have recently shown that the amount of adipose tissue plays a negative role in metabolic flexibility in a population of healthy young men. Impairment of insulin

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suppression of lipolysis was correlated with intraabdominal fat mass. Other abnormalities associated with adipose tissue content included increased expression of genes involved in chemotaxis and markers of macrophage content [5]. Inflammation has been linked to obesity and its associated comorbidities [6,7]. Serum adiponectin has been linked to insulin sensitivity, and levels are low in obesity and type 2 diabetes mellitus [8]. Taken together, these data provide support for the role of adipose tissue in metabolic flexibility in healthy young men. How adipose tissue might influence metabolic flexibility in women is unknown.

Women generally have a higher percentage of body fat than men. Subcutaneous adipose tissue nonesterified fatty acid (NEFA) release is higher (per unit volume) in men upon insulin stimulation, indicating a resistance to the antilipolytic effect of insulin [9,10]. Basal fat oxidation is lower in women compared with men, indicating higher lipid storage in women [11,12]. Women exhibit higher levels of serum adiponectin than men [13]. Men and women with comparable adipocyte size in subcutaneous adipose tissue demonstrate equal basal lipolysis [14]. These data point to a sexual dimorphism in the overall morphologic and functional characteristics of the adipose organ.

Combined, these suggest a sex difference in the contribution of the quantity (body fatness) and the characteristics of the adipose tissue (adipose tissue mass, lipolysis, disordered fat oxidation, inflammation) to metabolic flexibility. To explore this hypothesis, we studied 78 healthy young men and women under carefully controlled conditions, examining sex differences in how adipose tissue mass and characteristics influence metabolic flexibility during a euglycemic-hyperinsulinemic clamp (EHC).

2. Research design and methods

2.1. Study population and design

After providing written informed consent, a cohort of 78 healthy young men (n = 56) and women (n = 22), aged 22.6 ± 3.2 and 22.7 ± 3.4 years with body mass indexes (BMIs) of 26.4 ± 4.1 and 26.1 ± 5.3 kg/m^2, respectively, was enrolled in the study. Participants underwent physical examination, medical laboratory tests, anthropometry, dual-energy x-ray absorptiometry (DEXA), computed tomography (CT), and maximal oxygen uptake test. Abdominal subcutaneous adipose tissue biopsy was obtained during fasting state using a Bergstrom needle. Participants presented to the Pennington inpatient unit and ate a weight-maintaining (35% fat, 16% protein, and 49% carbohydrate) diet for 2 days. An EHC was performed as described below. Earlier studies describe the men in this cohort in terms of skeletal muscle oxidative phosphorylation, glucose metabolism, and metabolic flexibility [15-17]. These investigations involve measures made before diet intervention.

2.2. Euglycemic-hyperinsulinemic clamp

Insulin sensitivity and metabolic flexibility (change in respiratory quotient [ΔRQ] from fasting to insulin stimulated) were measured during an EHC as described previously [18]. Briefly, after an overnight fast, insulin (80 mIU/m^2 body surface unit) was administered intravenously; and glucose was infused to maintain plasma glucose at 90 mg/dL for 2 hours. The glucose disposal rate (GDR, in milligrams per kilogram fat-free mass per minute) was adjusted for kilogram of lean body mass determined by DEXA.

2.3. Maximal aerobic capacity

Maximal oxygen uptake was determined by a progressive treadmill test to exhaustion in accordance to the recommendations described by the American College of Sports Medicine [19]. The volumes of O_2 and CO_2 were measured continuously using a metabolic cart (V-Max29 Series; SensorMedics, Yorba Linda, CA). Heart rate was continuously monitored using a portable heart rate monitor (Polar S-600, Polar Electro, Oulo, Finland).

2.4. Body composition

Body fat mass and lean body mass were measured on a Hologic DEXA in the fan beam mode (QDR 4500; Hologic, Waltham, MA). Coefficient of variation for the measurement of lean body mass, fat mass, and percentage of body fat is 0.8%, 1.6%, and 1.7%, respectively. Visceral fat was measured by CT scanning using a GE HiSpeed CT scanner (Piscataway, NJ).

2.5. Indirect calorimetry

After an overnight fast, fasting RQ and RQ after an insulin infusion were measured for 30 minutes by indirect calorimetry during the EHC using a Deltatrac II indirect calorimeter (DATEX-Ohmeda, Helsinki, Finland). Oxygen consumption and CO_2 production were measured, and values were corrected for nitrogen excretion. Oxidative and nonoxidative glucose disposal was calculated as described by Livesey and Elia [20].

2.6. Fat cell size

Fat cell size (FCS) was determined as previously described [21]. Briefly, adipose tissue was fixed in osmium tetrachloride/collidine-HCl followed by disassociation by urea digestion. Cells were counted on a Multisizer-3 (Beckman Coulter, Fullerton, CA) using a 400-μm aperture (dynamic linear range, 12-320 μm) and reported as the mean of all adipocytes greater than 22.5 μm.

2.7. Laboratory measures

Fasting serum glucose and free FAs were assayed by established enzymatic procedures (Beckman Synchron CX7; Beckman Coulter, Brea, CA) using the Wako (Richmond, VA) free FA reagents. Fasting plasma adiponectin was measured

on an Immulite autoanalyzer (DPC, Los Angeles, CA). After an insulin infusion, serum glucose and insulin were assayed during the EHC in the same manner as the fasting samples. The enzymatic assay was not sensitive enough to reliably measure insulin-suppressed NEFAs, so total NEFAs during the EHC were measured in triplicate by high-performance liquid chromatography as previously described [22].

2.8. RNA and DNA extraction

Human total RNA from approximately 200 mg of adipose tissue was isolated with Trizol reagent (Invitrogen, Carlsbad, CA) followed by column purification (Qiagen, Valencia, CA). The quantity and the integrity of the RNA were confirmed with the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA).

2.9. Real-time quantitative reverse transcriptase polymerase chain reaction for RNA

All primers and probes were designed using Primer Express version 2.1 (Applied Biosystems, Roche, Branchburg, NJ). Sequences of primers and probes are shown in the Supplementary Table. Real-time quantitative reverse transcriptase polymerase chain reactions (qRT-PCRs) [23] were performed as 1-step reactions in ABI PRISM 7900 (Applied Biosystems) using the following parameters: 1 cycle of 48°C for 30 minutes, then 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. 18S was used as the internal control. All expression data were normalized by dividing target gene by the internal control. Serial dilutions of a pooled RNA sample were used as a reference curve as previously described [24]. Using this technique, the cycle threshold value is measured for each unknown sample and referenced back to the standard curve (established on each plate) derived from the relationship between the nanograms of the standard and the cycle threshold value of the unknown.

2.10. Statistical analysis

Population characteristics are represented as means ± SD. All data are a post hoc analysis of data generated from our study of a high-fat diet. Gene expression and clinical data were correlated using linear regressions. Sex differences in biopsy and blood parameters were analyzed using unpaired *t* tests. The change in respiratory quotient (ΔRQ) was divided into quartiles (quartile 1 = ΔRQ <0.06, quartile 2 = 0.06 < ΔRQ ≤ 0.08, quartile 3 = 0.08 < ΔRQ ≤ 0.11, and quartile 4 = ΔRQ >0.11) to illustrate the range in metabolic flexibility within the cohort. A single female subject was in quartile 2 and was thus eliminated from all quartile analyses involving ΔRQ. Analysis of variance was used to test for differences in biopsy and blood parameters across quartiles of metabolic flexibility (ΔRQ), with post hoc testing by mean equality contrast between different groups using the Tukey-Kramer honestly significant difference; $\alpha = 0.05$. In figures, all quartiles not sharing the same letter are significantly different with a $P < .05$. To investigate the influence of sex differences on metabolic flexibility, we performed stepwise linear regression analyses for men and women separately. Type I error rate was set a priori at $P < .05$. All data are represented as means ± SE. Analysis was performed using JMP version 5.0 (SAS, Cary, NC). Graphs were generated using GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA).

3. Results

3.1. Sex differences in population characteristics

Subject characteristics are listed in Table 1. Participants were healthy women (n = 22) and men (n = 56) aged 22.7 ± 3.4 and 22.6 ± 3.2 years, respectively. This investigation focuses on sex differences in metabolic flexibility and FA/adipose tissue metabolism. Body mass index was not significantly different between women and men (26.1 ± 5.3 vs 26.4 ± 4.1 kg/m^2). As compared with men, women had significantly more body fat (32.7% ± 5.4% vs 20.3% ± 6.5%, $P < .01$) and subcutaneous adipose tissue (7.1 ± 0.6 vs 4.9 ± 0.4 kg, $P < .01$) and had less visceral adipose tissue (VAT) (1.3 ± 0.8 vs 2.1 ± 1.3 kg, $P < .01$).

Table 1
Clinical characteristics of the study population

Subject characteristics	Women (n = 22)		Men (n = 56)		*P* value
	Mean ± SD	Range	Mean ± SD	Range	
Age (y)	22.7 ± 3.4	18.0-29.0	22.6 ± 3.2	18.0-29.0	NS
Height (cm)	163.1 ± 7.8	149.6-178.7	176.9 ± 5.8	163.0-189.5	<.01
Weight (kg)	69.0 ± 12.4	52.0-92.4	82.5 ± 13.2	59.2-118.3	<.01
BMI (kg/m^2)	26.1 ± 5.3	19.7-35.7	26.4 ± 4.1	20.1-34.7	NS
WHR	0.78 ± 0.07	0.7-0.9	0.87 ± 0.07	0.7-1.0	<.01
Body fat (%)	32.7 ± 5.4	23.6-42.1	20.3 ± 6.5	8.4-32.3	<.01
VAT mass (kg)	1.3 ± 0.8	0.6-4.1	2.1 ± 1.3	0.5-5.7	<.01
Subcutaneous adipose tissue mass (kg)	7.1 ± 0.6	2.6-14.6	4.9 ± 0.4	1.0-11.6	<.01
VO_2max (mL/[kg min])	31.0 ± 5.7	20.6-42.2	41.2 ± 7.2	23.5-59.2	<.01

WHR indicates waist to hip ratio; VO_2max, maximal oxygen uptake; NS, not significant.

3.2. Sexual dimorphism in metabolic flexibility (ΔRQ) and insulin-suppressed NEFAs

The RQ was measured before and during the EHC to determine metabolic flexibility (ΔRQ). Women had higher metabolic flexibility than men. Change in RQ varied greatly in both women (0.07-0.21) and men (0.03-0.25) (Table 2). Fasting RQ was not different between women and men (0.84 ± 0.04 vs 0.84 ± 0.03, $P > .05$, Table 2, Fig. 1A). However, insulin-stimulated RQ (during the EHC) was higher in women (0.98 ± 0.03 vs 0.93 ± 0.04, $P < .01$, Table 2); and consequently, ΔRQ was also higher in women (0.14 ± 0.04 vs 0.09 ± 0.04, $P < .01$, Fig. 1A) even after adjusting for GDR and NEFAs. We next explored the contribution of insulin-suppressed NEFAs to the observed sex difference in metabolic flexibility. Fasting NEFAs were different between women and men (514 ± 165 vs 397 ± 160 μmol/L, $P < .01$, Table 2, Fig. 1B), as well as insulin-suppressed NEFAs (35 ± 5.9 vs 49.1 ± 24.4 μmol/L, $P < .05$, Table 2, Fig. 1B). It is important to note that not only were insulin-suppressed NEFAs on average lower in women, but the range of insulin-suppressed NEFAs in women was much smaller compared with that in men (20.0-40.0 vs 20.0-160.0 μmol/L, Table 2). We divided male subjects into quartiles of metabolic flexibility (ΔRQ) (quartile 1 = ΔRQ <0.06, quartile 2 = 0.06 < ΔRQ ≤ 0.08, quartile 3 = 0.08 < ΔRQ ≤ 0.11, and quartile 4 = ΔRQ >0.11). All but 1 woman were found in the most flexible quartiles (3 and 4). The ΔRQ was negatively associated with insulin-suppressed NEFAs (analysis of variance $P < .05$, Fig. 1C).

3.3. Adipose tissue contributes to sex differences in metabolic flexibility (ΔRQ)

Although women had more body fat, they were more metabolically flexible than men. Women were a more homogeneous population for the measurement of metabolic flexibility (ΔRQ) compared with men; thus, women were not divided into quartiles and were only divided into 2 groups. Visceral adipose tissue mass was negatively correlated with metabolic flexibility (ΔRQ) in both women ($R^2 = 0.19$, $P = .05$) and men ($R^2 = 0.13$, $P < .01$) (data not shown). We next investigated the role of adipose tissue characteristics in metabolic flexibility. We measured differences in FCS, inflammation, and lipid metabolism gene expression in adipose tissue (lipolysis, lipid synthesis, uptake, oxidation, and storage), as well as serum adiponectin levels.

3.4. Sex differences in FCS

Hypertrophic adipocytes have greater rates of lipolysis, which could influence fuel availability and therefore metabolic flexibility. Our results show that the range of FCS in women (0.30-0.90 μL) was slightly smaller when compared with that in men (0.22-0.95 μL). However, mean FCS was not different between women and men (0.57 ± 0.04 vs 0.60 ± 0.02 μL, $P > .05$, Table 3).

3.5. Sex differences in inflammation markers

Adipose tissue inflammation negatively influences insulin sensitivity [25] and could influence metabolic flexibility. In addition to macrophage number, a recent study showed that the activation pattern of macrophages influences insulin resistance [26]. The classic proinflammatory pattern (M1 activation pattern) contributes to insulin resistance; whereas the alternative anti-inflammatory pathway (M2) protects adipocytes from the detrimental effects of inflammation. Therefore, we measured the expression of chemokines, markers of macrophage content, and M1/M2 activation.

There were no differences between men and women (Table 3) in the expression of chemokines (MCP-1 and MIP-1α messenger RNA [mRNA), macrophage content markers (CD68 and MAC-2 mRNA), or classic inflammatory pathway (M1) (nitric oxide synthase, NOS2 mRNA) marker. Interestingly, the alternative anti-inflammatory pathway (M2) (mannose receptor C type 2, MRC2 mRNA) marker was significantly higher in women when compared with men. This suggests that in women macrophages are predominantly

Table 2
Changes during the EHC

Subject characteristics	Women (n = 22)		Men (n = 56)		*P* value
	Mean ± SD	Range	Mean ± SD	Range	
Fasting RQ	0.84 ± 0.03	0.78-0.90	0.84 ± 0.04	0.74-0.95	NS
Insulin infusion[a] RQ	0.98 ± 0.03	0.92-1.04	0.93 ± 0.04	0.85-1.03	<.01
ΔRQ	0.14 ± 0.04	0.07-0.21	0.09 ± 0.04	0.03-0.25	<.01
Fasting glucose (mg/dL)	76.5 ± 9.1	46.0-92.0	80.5 ± 5.4	66.0-90.0	<.05
Insulin infusion[a] glucose (mg/dL)	89.8 ± 3.6	81.0-97.0	89.0 ± 4.5	78.0-102.0	NS
Fasting insulin (μU/mL)	8.0 ± 5.6	2.1-26.1	8.2 ± 4.6	2.6-22.4	NS
Insulin infusion[a] insulin (μU/mL)	156.3 ± 46.4	100.3-279.3	160.1 ± 35.7	103.9-251.3	NS
GDR (mg/[kg FFM min])	12.8 ± 3.9	6.4-21.7	11.1 ± 4.1	4.0-24.5	NS
Fasting NEFA (μmol/L)	514 ± 165	330-1100	397 ± 160	70.-840	<.01
Insulin infusion[a] NEFA (μmol/L)	35.1 ± 5.9	20-40	49.1 ± 24.4	20-160	<.05

FFM indicates fat-free mass.

[a] During insulin infusion (80 mIU/m^2 body surface unit per minute).

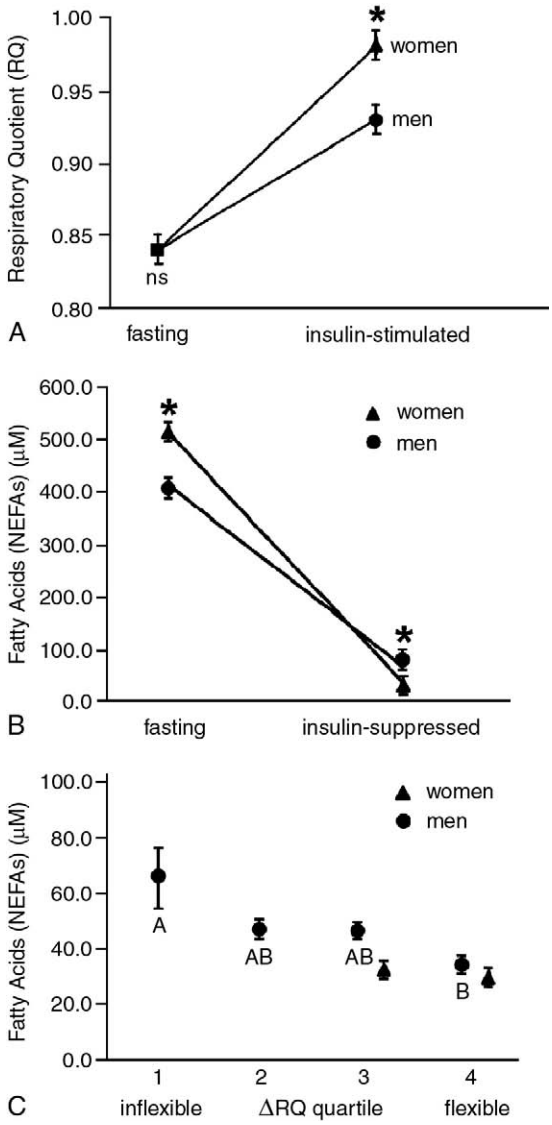

Fig. 1. Metabolic flexibility and insulin-suppressed NEFAs in healthy young men and women. Changes in respiratory quotient (ΔRQ, metabolic flexibility) and NEFAs were measured before and during an EHC in the population of 78 healthy young men (n = 56) and women (n = 22). Fasting RQ was not different between men and women; however, insulin-stimulated RQ (steady state of the EHC) was higher in women compared with men (A). Analysis of variance was used to test for differences in metabolic flexibility (ΔRQ). The change in respiratory quotient was divided into quartiles and correlated with insulin-suppressed NEFAs in both men and women: for men, quartiles 1 to 4 (n = 13, 13, 13, and 17, respectively); for women, quartiles 3 and 4 (n = 4 and 17, respectively). Quartile 2 only had 1 woman; therefore, this subject was excluded from the analysis. Both fasting and insulin-suppressed NEFAs were significantly different between men and women (B). Insulin-suppressed NEFAs were negatively associated with ΔRQ (C). Analysis of variance was used to test for differences in biopsy and blood parameters across quartiles of metabolic flexibility (ΔRQ), with post hoc testing by mean equality contrast between different groups using the Tukey-Kramer honestly significant difference; $\alpha = .05$. Type I error rate was set a priori at $P < .05$. Data are shown as means ± SE. All levels not connected by same letter are significantly different. Type I error rate was set a priori at $P < .05$. *$P < .01$.

Table 3
Sex differences in adipose tissue characteristics

	Women (n = 18)	Men (n = 45)
FCS (μL)	0.57 ± 0.04	0.6 ± 0.02
Adiponectin (μg/mL)	7.8 ± 4.4	5.5 ± 3.3
Inflammation markers (au) (gene expression)		
• CD68	20.6 ± 2.9	16.5 ± 1.9
• MAC-2	10.5 ± 1.4	8.9 ± 0.8
• MCP-1	1.9 ± 0.3	1.4 ± 0.2
• MIP-1α	0.7 ± 0.2	0.6 ± 0.1
• NOS2	20.6 ± 5.3	23.4 ± 2.5
• MRC2	34.1 ± 4.2*	22.1 ± 1.5
Lipid metabolism (au) (gene expression)		
Adipogenesis		
• FAS	82.5 ± 8.9	62.3 ± 5.6
• PPARγ1	2.5 ± 0.9	3.1 ± 0.6
• PPARγ2	27.9 ± 2.3†	14 ± 1.5
Lipid storage		
• PCK1	50.2 ± 6.4*	32.8 ± 4.1
• SCD1	37.3 ± 4.3*	21.8 ± 2.7
Lipid oxidation		
• MCAD	18.1 ± 2.3	14.2 ± 1.4
• PPARα	25.6 ± 2.2*	19 ± 1.4
Lipolysis		
• Perilipin	7.5 ± 0.7	5.9 ± 0.4
• CAP	26.6 ± 3	21.6 ± 1.9
• ATGL	4.5 ± 0.4	3.9 ± 0.2
• HSL	47.5 ± 5.7	44.3 ± 3.6

Fat cell size (mean ± SD) was determined after fixation in osmium tetrachloride/collidine-HCl. Adiponectin was measured on an Immulite autoanalyzer. Gene expression (mean ± SE) was measured by real-time qRT-PCR. Women had similar FCS. Adiponectin (mean ± SD) was significantly greater in women vs men; however, inflammation marker gene expression was similar. Expression of genes involved in adipogenesis (PPARγ2), lipid storage storage (PCK1, SCD1), and lipid oxidation (PPARα) was significantly higher in women compared with men. LPL indicates lipoprotein lipase; FAS, fatty acid synthase; PCK1, phosphoenolpyruvate carboxykinase 1; SCD1, stearoyl–coenzyme A desaturase; MCAD, acyl–coenzyme A dehydrogenase C-4 to C-12 straight chain; CAP, cbl-associated protein; ATGL, adipose triglyceride lipase; HSL, hormone-sensitive lipase; MAC-2, macrophage-associated antigen; MCP-1, monocyte chemoattractant protein–1; MIP-1α, macrophage inflammatory protein 1, α unit; Au, arbitrary units.

* $P < .05$.

† $P < .001$.

activated along the anti-inflammatory pathway, which could contribute to a higher metabolic flexibility.

We believe that CD68 and MAC-2 mRNA expressions are good markers for macrophage content because we previously validated the method against the criterion standard, immunohistochemistry (unpublished data).

3.6. Sex differences in lipid metabolism genes expression in adipose tissue

Metabolic flexibility could be influenced by NEFA supply to skeletal muscle, as the fractional uptake of NEFAs by muscle is high, approaching 40% [1]. Our results showed that women had greater PPARγ2 expression (99% greater expressions when compared with men)(Fig. 2A). In addition, the expression of PCK1 and SCD1, genes required for lipogenesis, was greater in women compared with men

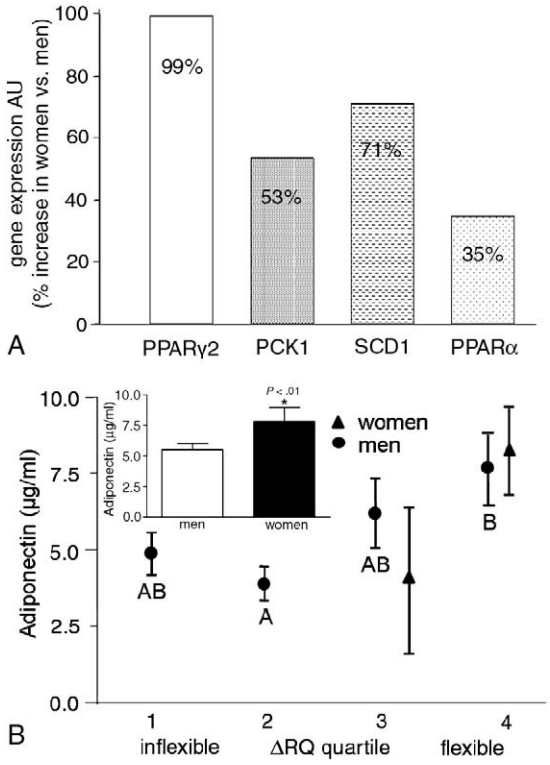

Fig. 2. Adipose tissue FA metabolism gene expression and serum adiponectin levels of healthy young men and women. Messenger RNA gene expression was measured by qRT-PCR. Women have greater expression of genes involved in adipogenesis (PPARγ2), FA storage (PCK1, SCD1), and FAO (PPARα) compared with men (A). Values are expressed as mean ± SE. Serum adiponectin levels were higher in women compared with men (B). Change in RQ was subdivided into quartiles and correlated with serum adiponectin in men and women: for men, quartiles 1 to 4 (n = 13, 13, 13, and 17, respectively); for women, quartiles 3 and 4 (n = 4 and 17, respectively). Quartile 2 only had 1 woman; therefore, this subject was excluded from the analysis. Adiponectin was positively associated with metabolic flexibility in men and women, but was not significant in women (B).

(Fig. 2A). PPARα (gene involved in FAO) mRNA was 35% higher in women when compared with men (Fig. 2A). There were no sex differences for perilipin, CAP, ATGL, and HSL mRNA gene expression.

3.7. Sex differences in adiponectin

Adiponectin has been shown to increase insulin action [8], which could also positively impact metabolic flexibility. Serum adiponectin levels were higher in women compared with men (7.83 ± 1.14 vs 5.51 ± 0.49 μg/mL, $P < .05$, Fig. 2B); and adiponectin was positively associated with metabolic flexibility in women and men, but was not significant in women (Fig. 2B). There was no correlation between adiponectin and ΔRQ in women, possibly because almost all women had both high adiponectin and high metabolic flexibility.

3.8. Sex differences in correlates of metabolic flexibility

To identify the dominant sex differences among factors that contribute to metabolic flexibility, we used a combination of univariate and stepwise linear regression analyses for men and women. We first performed univariate correlations between metabolic flexibility (ΔRQ) and clinical characteristics, as well as adipose tissue gene expression in women and men separately. All factors that were significantly correlated with ΔRQ (insulin-suppressed NEFAs, VAT mass, serum adiponectin, FCS, total adipose tissue mass, subcutaneous adipose tissue mass, PPARγ2, SCD1, PCK1, and CD68) were loaded into the stepwise linear regression analyses within each sex. The only 2 adipose tissue characteristics that correlated with metabolic flexibility in women were SCD1 and PCK1 mRNA (model $R^2 = 0.49$, $P < .05$, data not shown). The only 2 adipose tissue characteristics that correlated with metabolic flexibility in men were insulin-suppressed NEFAs and serum adiponectin (model $R^2 = 0.34$, $P < .05$, data not shown).

4. Discussion

Storlien et al [1] suggested that adipose tissue might be an important determinant of metabolic flexibility, especially by regulating fuel supply. We found that women are more metabolically flexible than men. We explored the possible causes for this observation and excluded differences in FCS and VAT mass as factors in the sexual dimorphism of metabolic flexibility. Women had a greater capacity to suppress NEFAs in the insulin-stimulated state and higher serum adiponectin and expression of genes involved in adipogenesis, fat storage, and fat oxidation in adipose tissue; and most interestingly, adipose tissue macrophages are activated along an anti-inflammatory pathway in women. These findings support a role for adipose tissue characteristics in the sexual dimorphism of metabolic flexibility.

Both fasting and insulin-suppressed NEFAs display sexual dimorphism. Nonesterified fatty acids are higher in women in the fasting state and higher in men in the insulin-stimulated state. Reduced NEFAs during insulin infusion are consistent with a decrease in NEFA release from adipose tissue (lipolysis) and a decrease in NEFA delivery to muscle; this supports fuel availability as an important contributing factor in the sexual dimorphism of metabolic flexibility in healthy young adults. Women are more flexible and were found only in the 2 most flexible quartiles of men. Insulin-suppressed NEFAs were negatively associated with metabolic flexibility in men. This was not the case in women, possibly because all women were flexible (high ΔRQ) and had low insulin-suppressed NEFAs.

Body fat is distributed differently in men and women, with more VAT mass in men [27,28]. Adipose tissue is a mixture of adipocyte size distribution with metabolic properties [29]. In our healthy individuals matched for BMI, there were no sex differences in abdominal FCS, suggesting no influence on the metabolic flexibility sexual dimorphism. However, gluteal-femoral fat was not examined and may play an important role in metabolic flexibility.

Another factor to be considered is adiponectin, an adipokine that increases fat oxidation in muscle and liver [13,30]. Adiponectin levels are higher in women compared with men (Fig. 2B); and metabolic flexibility significantly correlated with adiponectin serum levels, suggesting a role for adiponectin in the sexual dimorphism of metabolic flexibility.

In a pilot study, we showed that macrophage content measured by immunohistochemistry and by RT-PCR expression of CD68 and MAC-2 strongly correlated (R^2 = 0.77, $P < .05$). Therefore, in this study, we measured macrophage content using the more contemporary method of RT-PCR. Adipose tissue macrophage infiltration contributes to metabolic disturbances in both lipolysis and glucose uptake and oxidation [25,31]. More than this, a recent study showed that only "classically activated" macrophages negatively influence the metabolic profile, whereas the anti-inflammatory alternative activated macrophages have a positive effect [26]. We show here that there is no difference in adipose tissue chemokines and macrophage content in healthy young individuals; however, in women, macrophages are predominantly activated along the "alternative" anti-inflammatory pathway, which could contribute to greater metabolic flexibility (Table 3). Therefore, this study identifies a possible role for the adipose tissue macrophage activation pattern in metabolic flexibility.

Lipid uptake and storage capacity contribute to metabolic flexibility, probably by affecting NEFA availability. Genes of lipolytic cascade were not different in men and women. However, PPARγ2, a gene required for adipogenesis [32], was 99% greater in women compared with men. Two genes involved in FA storage were significantly increased in women: PCK1, which contributes to triacylglycerol synthesis [33,34], and SCD1, which is a key controller of FA partitioning between oxidation and storage [35]. Women had 53% and 71% higher PCK1 and SCD1 gene expression in abdominal adipose tissue, respectively. Increased capacity for FA storage in adipose tissue could contribute to a greater capacity to store FA during insulin stimulation in the postprandial state and might permit a greater insulin suppression of NEFAs in women. Further work on this using transgenic overexpression of SCD1 in WAT is warranted. Overexpression of Lipin1 in WAT, a key gene in lipogenesis, produces fat, insulin-sensitive mice [36]; this parallels our results.

To further investigate the influence of sex differences on metabolic flexibility, we performed a combination of univariate and stepwise regression analyses for men and women separately using the significant correlations from each univariate analysis (insulin-suppressed NEFAs, VAT mass, serum adiponectin, FCS, total adipose tissue mass, subcutaneous adipose tissue mass, PPARγ2, SCD1, PCK1, and CD68) to guide the subsequent stepwise linear regression. The 2 major adipose tissue correlates of metabolic flexibility in women were SCD1 and PCK1, explaining 49% of the variance. In men, insulin-suppressed NEFAs and serum adiponectin explained 34% of the variance. In addition to the sexual dimorphism in metabolic flexibility (ΔRQ), there is also a sexual dimorphism for the determinants of metabolic flexibility. This suggests that greater adipose tissue FA storage capacity contributes to metabolic flexibility.

Although sex hormones were not measured in this population, growing evidence suggests that the ovarian hormones have major effects on lipid and carbohydrate metabolism and may also play a major role in upstream molecular signaling mechanisms for regulating substrate metabolism. It has been shown that estrogen increases the metabolic capacity for both carbohydrate and lipid metabolism, perhaps increasing the overall metabolic flexibility of skeletal muscle. Conversely, progesterone negates both these effects and could therefore result in a state of relative metabolic inflexibility, similar to that observed in the metabolic syndrome [37]. All female subjects were tested in the follicular phase of their menstrual cycle; however, future investigations into the contributions of sex hormones to metabolic flexibility are necessary.

In conclusion, adipose tissue macrophage activation pattern, lipid synthesis, storage, oxidation, and a failure of insulin to suppress NEFAs and adiponectin contribute to metabolic flexibility. Sexual dimorphism contributes to the behavior of adipose tissue in metabolic flexibility. It will be interesting in future work to explore disturbances in this system in women and their consequences for metabolic flexibility.

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Appendix A. Supplementary Data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.metabol.2009.05.008.

References

[1] Storlien L, Oakes ND, Kelley DE. Metabolic flexibility. Proc Nutr Soc 2004;63:363-8.
[2] Kelley DE, Goodpaster B, Wing RR, Simoneau JA. Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. Am J Physiol 1999;277:E1130-41.
[3] Frayn KN. Adipose tissue as a buffer for daily lipid flux. Diabetologia 2002;45:1201-10.
[4] Hickner RC, Racette SB, Binder EF, Fisher JS, Kohrt WM. Suppression of whole body and regional lipolysis by insulin: effects of obesity and exercise. J Clin Endocrinol Metab 1999;84:3886-95.
[5] Odegaard JI, Ricardo-Gonzalez RR, Goforth MH, Morel CR, Subramanian V, Mukundan L, et al. Macrophage-specific PPARgamma

controls alternative activation and improves insulin resistance. Nature 2007;447:1116-20.
[6] Clement K, Viguerie N, Poitou C, Carette C, Pelloux V, Curat CA, et al. Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects. FASEB J 2004;18:1657-69.
[7] Cancello R, Clement K. Is obesity an inflammatory illness? Role of low-grade inflammation and macrophage infiltration in human white adipose tissue. BJOG 2006;113:1141-7.
[8] Kern PA, Di Gregorio GB, Lu T, Rassouli N, Ranganathan G. Adiponectin expression from human adipose tissue: relation to obesity, insulin resistance, and tumor necrosis factor–alpha expression. Diabetes 2003;52:1779-85.
[9] Jensen MD. Lipolysis: contribution from regional fat. Annu Rev Nutr 1997;17:127-39.
[10] Wahrenberg H, Lonnqvist F, Arner P. Mechanisms underlying regional differences in lipolysis in human adipose tissue. J Clin Invest 1989;84:458-67.
[11] Levadoux E, Morio B, Montaurier C, Puissant V, Boirie Y, Fellmann N, et al. Reduced whole-body fat oxidation in women and in the elderly. Int J Obes Relat Metab Disord 2001;25:39-44.
[12] Nagy TR, Goran MI, Weinsier RL, Toth MJ, Schutz Y, Poehlman ET. Determinants of basal fat oxidation in healthy Caucasians. J Appl Physiol 1996;80:1743-8.
[13] Bottner A, Kratzsch J, Muller G, Kapellen TM, Bluher S, Keller E, et al. Gender differences of adiponectin levels develop during the progression of puberty and are related to serum androgen levels. J Clin Endocrinol Metab 2004;89:4053-61.
[14] Leibel RL, Hirsch J. Site- and sex-related differences in adrenoreceptor status of human adipose tissue. J Clin Endocrinol Metab 1987;64:1205-10.
[15] Sparks LM, Xie H, Koza RA, Mynatt R, Bray GA, Smith SR. High-fat/low-carbohydrate diets regulate glucose metabolism via a long-term transcriptional loop. Metabolism 2006;55:1457-63.
[16] Sparks LM, Xie H, Koza RA, Mynatt RL, Hulver MW, Bray G, et al. A high-fat diet coordinately downregulates genes required for mitochondrial oxidative phosphorylation in skeletal muscle. Diabetes 2005;54:1926-33.
[17] Ukropcova B, McNeil M, Sereda O, de Jonge L, Xie H, Bray GA, et al. Dynamic changes in fat oxidation in human primary myocytes mirror metabolic characteristics of the donor. J Clin Invest 2005;115:1934-41.
[18] Heilbronn LK, Rood J, Janderova L, Albu JB, Kelley DE, Ravussin E, et al. Relationship between serum resistin concentrations and insulin resistance in nonobese, obese, and obese diabetic subjects. J Clin Endocrinol Metab 2004;89:1844-8.
[19] Carter H, Jones AM, Barstow TJ, Burnley M, Williams CA, Doust JH. Oxygen uptake kinetics in treadmill running and cycle ergometry: a comparison. J Appl Physiol 2000;89:899-907.
[20] Livesey G, Elia M. Food energy values of artificial feeds for man. Clin Nutr 1985;4:99-111.
[21] Smith SR, Xie H, Baghian S, Needham A, McNeil M, Bogacka I, et al. Pioglitazone changes the distribution of adipocyte size in type 2 diabetics. Adipocytes 2006;2:11-22.
[22] Miles JM, Ellman MG, McClean KL, Jensen MD. Validation of a new method for determination of free fatty acid turnover. Am J Physiol 1987;252:E431-8.
[23] Bustin SA. Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. J Mol Endocrinol 2000;25:169-93.
[24] Smith SR, Lovejoy JC, DeLaney JP, Ruyter I, McNeil M. The inhibition of glycogen synthesis by fatty acids is not mediated by inhibition of extracellular signal–regulated kinase 1/2. Diabetologia 1999;42:1031-2.
[25] Ruan H, Lodish HF. Regulation of insulin sensitivity by adipose tissue–derived hormones and inflammatory cytokines. Curr Opin Lipidol 2004;15:297-302.
[26] Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest 2007;117:175-84.
[27] Bouchard C, Despres JP, Mauriege P. Genetic and nongenetic determinants of regional fat distribution. Endocr Rev 1993;14:72-93.
[28] Leibel RL, Edens NK, Fried SK. Physiologic basis for the control of body fat distribution in humans. Annu Rev Nutr 1989;9:417-43.
[29] Jernas M, Palming J, Sjoholm K, Jennische E, Svensson PA, Gabrielsson BG, et al. Separation of human adipocytes by size: hypertrophic fat cells display distinct gene expression. FASEB J 2006;20:1540-2.
[30] Ong KK, Frystyk J, Flyvbjerg A, Petry CJ, Ness A, Dunger DB. Sex-discordant associations with adiponectin levels and lipid profiles in children. Diabetes 2006;55:1337-41.
[31] Lumeng CN, Deyoung SM, Saltiel AR. Macrophages block insulin action in adipocytes by altering expression of signaling and glucose transport proteins. Am J Physiol Endocrinol Metab 2006;292:E166-74.
[32] MacDougald OA, Lane MD. Transcriptional regulation of gene expression during adipocyte differentiation. Annu Rev Biochem 1995;64:345-73.
[33] Beale EG, Hammer RE, Antoine B, Forest C. Glyceroneogenesis comes of age. FASEB J 2002;16:1695-6.
[34] Reshef L, Olswang Y, Cassuto H, Blum B, Croniger CM, Kalhan SC, et al. Glyceroneogenesis and the triglyceride/fatty acid cycle. J Biol Chem 2003;278:30413-6.
[35] Sampath H, Ntambi JM. Stearoyl–coenzyme A desaturase 1, sterol regulatory element binding protein–1c and peroxisome proliferator–activated receptor–alpha: independent and interactive roles in the regulation of lipid metabolism. Curr Opin Clin Nutr Metab Care 2006;9:84-8.
[36] Peterfy M, Phan J, Xu P, Reue K. Lipodystrophy in the fld mouse results from mutation of a new gene encoding a nuclear protein, lipin. Nat Genet 2001;27:121-4.
[37] Campbell SE, Febbraio MA. Effects of ovarian hormones on exercise metabolism. Curr Opin Clin Nutr Metab Care 2001;4:515-20.