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In Vitro Lipolysis is Associated with Whole Body Lipid Oxidation and Weight Gain in Humans

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Abstract

Objectives—To assess the association of adipocyte size with cellular lipolysis, and between cellular lipolysis and whole body lipid oxidation. Then, to assess the association between adipocyte size and cellular lipolysis with weight and fat mass gain.

Methods—Subjects had assessment of percent body fat (%fat) and adipose tissue biopsy for *in vitro* lipolysis (n=325), and a subset of subjects had measurement of whole body lipid oxidation (n=112). A subset of subjects (n=243) returned for repeat measurements of body weight and composition (mean follow-up 8.2 ± 5.5 years).

Results—*In vitro* lipolysis (r=0.47, $p<0.0001$) and adipocyte size (r=0.49, $p<0.0001$) were strongly associated with %fat. *In vitro* lipolysis ($p=0.04$) but not adipocyte size ($p=0.44$) was associated with whole body fat oxidation. Adipocyte size was not associated with rate of percent weight gain ($p=0.20$), but was negatively associated with rate of percent fat mass gain ($p=0.01$). *In vitro* lipolysis was negatively associated with rate of percent weight gain ($p=0.02$) and had a marginal negative association with rate of percent fat mass gain ($p=0.08$).

Conclusions—These results indicate inherent characteristics of adipocytes, including size and lipolytic activity, may be important determinants of whole body lipid oxidation and subsequent weight gain.

Keywords

Pima Indian; weight gain; lipid metabolism; adipocytes; lipolysis

Introduction

Positive energy balance results in greater triglyceride storage in adipose tissue and resultant accumulation of body fat. Increased fat causes adipocytes to increase in size and release

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more lipids[1,2]. Genetic polymorphisms at several loci have been associated with both adipocyte lipolysis and obesity, indicating a possible connection between adipocyte function and weight. However, direct association between basal adipocyte lipolysis and weight has not been studied in humans.

Adipocyte lipolysis may contribute to the rate of whole body lipid oxidation[3]. This may occur by the liberation of free fatty acids and glycerol, which may provide increased substrate for mitochondrial beta-oxidation. Evidence indicates a role for whole body lipid oxidation in long-term weight regulation. Elevated respiratory quotient (RQ), which indicates greater relative carbohydrate to fat oxidation, predicts weight gain in various populations[4,5,6]. Whole body lipid oxidation is negatively associated with weight gain in Native American men, but not women[4]. However, Pima Indians and Caucasians with high levels of adiposity have higher lipid oxidation rates than their lean counterparts[7]. Nevertheless, positive cross-sectional associations do not preclude these variables as negative determinants of future weight gain[8]. Thus if adipocyte lipolysis is associated with whole body lipid oxidation this would provide a mechanism for any association with weight change.

The purpose of this study was to first to confirm the association of adipocyte size with cellular lipolysis in Pima Indians, and to investigate the association between adipocyte size and cellular lipolysis with whole body lipid oxidation. We then investigated whether adipocyte size and *in vitro* cellular lipolysis were determinants of weight and in particular fat mass gain.

Methods

Subjects and Protocol

Volunteers (n = 325) participated in an inpatient study investigating risk factors for type 2 diabetes at the Obesity and Diabetes Clinical Research Section of the National Institutes of Diabetes and Digestive and Kidney Diseases (NIDDK) in Phoenix, AZ. Volunteers were told about the purpose of the study and written informed consent was obtained before participation. The Institutional Review Board of the NIDDK approved all protocols.

Volunteers were admitted to the clinical research unit and fed a weight maintaining diet. On day 2 of admission, participants underwent measurement of body composition using under water weighing[9]. On day 4 a 75-gram oral glucose tolerance test was administered, and subjects with diabetes mellitus were excluded from the study. Glucose concentrations were measured using the glucose oxidase method (Beckman Instruments, Fullerton CA). A subset of subjects (n = 112) spent 23 hours in a metabolic chamber on day 7 to determine respiratory quotient and 24-hour energy expenditure (see below). Abdominal subcutaneous adipose tissue was removed from the periumbilical region by percutaneous needle biopsy under local anesthesia (1% lidocaine) on day 9 as previously described[10] for *in vitro* cell lipolysis. A total of 243 subjects returned to our research unit for follow-up visits at which body weight and composition were assessed (mean follow-up 8.2 ± 5.5 years; minimum 6 months).

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Measurement of Whole Body Substrate Oxidation and Energy Expenditure

Subjects entered the calorimeter after an overnight fast and received meals at 7:00 am, 11:00 am, 4:00 pm, and 7:00 pm that they were instructed to finish. The diet was calculated to be eucaloric at sedentary conditions for each subject based on their weight[11]. Energy balance was calculated as energy expenditure-energy intake after accounting for any returned food. The macronutrient content of the chamber diet was 20% protein, 50% carbohydrate, 30% fat. Differences between outgoing and ingoing air concentrations of CO_2 and O_2 were measured continuously and mean values recorded every 15 minutes over 23 hours. Average calculated CO_2 production and O_2 consumption was extrapolated to 24 hours. The extrapolated averages were used to calculate substrate oxidation and energy expenditure as previously described[12]. Subject movement while in the chamber was continuously measured by a radar system.

In Vitro Methods

Osmium tetroxide fixed adipocytes' sizes were determined with a Coulter Electronics counter with a 400-μm aperture equipped with a logarithmic range-expander and channelyzer (Coulter Electronics, Irvine, CA).

To determine basal and isoproterenol stimulated lipolysis, isolated adipocytes were incubated in a 500 μL 5% albumin-HEPES buffer at 37 °C for two hours with continuous shaking at 40 cycles/min. The buffer contained no isoproterenol (basal) or 25 nmol/L isoproterenol and 0, 12.5, 25, 50, 100, 200, or 8,000 pmol/L insulin. The incubation period was terminated by separating the cells from the medium by the oil method[13]. Glycerol in the incubation medium under the oil was determined by an enzymatic assay as previously described[14].

Statistics

Baseline demographics were described using means and standard deviations for data that were normally distributed. Skewed data were reported as medians and interquartile ranges in the baseline demographics. Means from different subgroups were compared with ANOVA and Tukey's HSD post-hoc test, skewed data from different subgroups with the Kruskal-Wallis test, and proportions from different subgroups with chi-square and Fisher's exact test. Univariate associations between variables were calculated with Pearson product-moment correlations. Univariate associations between follow-up weight, fat mass, and fat free mass gain with average adipocyte size and *in vitro* basal and isoproterenol stimulated lipolysis used rate of percent gain, which was calculated with the following equation: $[(\Delta kg/kg_{bl})*100]/\Delta yrs$, where: Δkg = kg measurement change since baseline, kg_{bl} = kg measurement at baseline, and Δyrs = years since the baseline measurement. Significant univariate associations were confirmed with multivariate general linear models. Multivariate models for whole body substrate oxidation in the metabolic chamber included fat mass, fat free mass, age, sex, and energy balance, and models for respiratory quotient included % body fat rather than fat mass and fat free mass[15]. Multivariate models for rate of weight, fat mass, or fat free mass gain during follow-up used rate of absolute change and were adjusted for age, sex, ethnicity, and initial weight, fat mass, or fat free mass, respectively. Basal lipolysis per cell and rate of fat mass change were log transformed to meet the assumptions

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of linear regression. Weight-change was assessed between sexes and ethnicities using t-tests. Analyses were performed using SAS 9.2 and SAS Enterprise Guide 4.2 (Cary, NC).

Results

Demographics and Subject Characteristics

Demographic and metabolic data for all analyses are shown in table 1. The majority of subjects (84%) in all analyses were Native American, and the average body composition (32.7% body fat) was considered overweight[16]. *In vitro* lipolysis measurements did not differ significantly between the various subgroups.

In Vitro Lipolysis is Related to Baseline Body Composition

There was a strong positive relationship between average adipocyte size (r=0.49, $p<0.0001$), basal (r=0.47, $p<0.0001$) and isoproterenol-stimulated (r=0.25, $p<0.0001$) lipolysis per cell with % body fat. Multivariate analysis showed that basal ($p<0.0001$), but not isoproterenol stimulated ($p=0.80$) lipolysis per cell, was associated with % body fat after accounting for adipocyte size as measured in μg lipid/cell (table 2).

There was no difference in average adipocyte size, or basal or isoproterenol stimulated lipolysis between ethnicities (all $p>0.05$). Age was positively associated with average adipocyte size (r=0.18, $p=0.0008$), and this remained true after adjusting for % body fat ($p=0.02$). Age was not associated with basal ($p=0.12$) or isoproterenol stimulated ($p=0.07$) lipolysis. There was increased basal lipolysis per cell ($p<0.0001$; difference between least mean square means=0.03 fmoles/cell*s) in men only after adjusting for their comparatively lower % body fat (without adjustment for body fat females had higher basal lipolysis by 1.19 fmoles/cell*s). There was no interaction between sex and % body fat. Addition of adipocyte size to the model did not diminish the association between sex and basal lipolysis per cell ($p<0.0001$; table 2). Average adipocyte size and isoproterenol stimulated lipolysis did not differ between genders (both $p>0.05$).

In Vitro Lipolysis is Related to Adipocyte Size

Average adipocyte size was strongly associated with basal lipolysis per cell (r=0.46, $p<0.0001$, figure 1a) and remained significant in multivariate models adjusted for age and sex ($p<0.0001$; β=0.56; table 2). Average adipocyte size was also associated with isoproterenol stimulated lipolysis per cell (r=0.55, $p<0.0001$, figure 1b), again remaining significant in multivariate models adjusted for age and sex ($p<0.0001$; β=0.45). Basal lipolysis was strongly associated with ED50 for insulin's antilipolytic action (r=0.33, $p<0.001$).

Whole body Substrate Oxidation is Associated with *In Vitro* Lipolysis

Average adipocyte size was negatively associated with 24-hour RQ (24hRQ; r=−0.22, $p=0.01$), although this was no longer significant after adjustment for co-variates ($p=0.37$; table 2). Its association with whole body lipid oxidation (r=0.34, $p=0.0001$) was also significantly reduced in multivariate models ($p=0.44$; table 2). Average adipocyte size was not associated with whole body carbohydrate oxidation (r=0.09, $p=0.30$).

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Basal lipolysis per cell was negatively associated with 24hRQ (r=−0.30, p=0.001) and positively correlated with whole body lipid oxidation (r=0.36, p<0.0001; figure 2). These associations remained significant after adjustment for co-variates (p<0.01 and p=0.04, respectively; table 2). Including average adipocyte size in the models did not reduce the association between basal lipolysis per cell and 24hRQ (p<0.01) or whole body lipid oxidation (p=0.04; table 2). Basal lipolysis per cell was not associated with carbohydrate oxidation (r=0.04, p=0.62).

There was no association between isoproterenol stimulated lipolysis and whole body lipid (r=0.01, p=0.22) or carbohydrate (r=−0.05, p=0.62) oxidation, or 24hRQ (r=−0.08, p=0.17). Neither average adipocyte size nor any measurement of *in vitro* lipolysis was associated with 24-hour energy expenditure (all p>0.05). ED50 for insulin's antilipolytic activity was not associated with any measurement of substrate oxidation.

Relationship Between *In Vitro* Lipolysis and Future Weight Gain

Subjects gained 8.5 ± 15.0 kg during the follow-up period (p<0.0001), or 9.3 ± 15.5% of their original body weight. Neither weight change nor rate of weight gain varied by gender. An average of 54% of the weight gained during follow-up among all subjects was fat mass. This did not differ between ethnicities or genders.

Average adipocyte size was not associated with rate of percent weight (r=−0.08, p=0.20) or fat free mass (r=−0.04, p=0.76) gain. It was, though, negatively associated with rate of percent fat mass gain (r=−0.16, p=0.02) and remained significant after adjustment for co-variates (p=0.01; table 2).

Basal lipolysis per cell was negatively associated with rate of percent weight gain (r=−0.15, p=0.02, figure 3a). This association remained significant in multivariate models adjusting for age, sex, and ethnicity (p=0.02; β=−1.49; table 2). There was no significant interaction between sex and basal lipolysis in this association (p=0.33). Addition of average adipocyte size to the multivariate model slightly attenuated the negative association between basal lipolysis per cell and rate of percent weight gain (p<0.05; β=−1.53; table 2), indicating that this association may be due in part to larger adipocytes releasing more lipid. However, average adipocyte size itself was not significantly associated with weight gain in this model (p=0.63). There was a negative association between basal lipolysis per cell and rate of percent fat mass gain (r=−0.19, p=0.002, figure 3b), which was attenuated in the multivariate models (p=0.08; β=−0.97) and after added adjustment for average adipocyte size (p=0.06; β=−1.11; table 2). Basal lipolysis measurements were not associated with rate of fat free mass change during the follow-up period (r=0.00, p=0.96). Additionally, there was no association between isoproterenol stimulated lipolysis per cell and rate of percent weight, fat mass, or fat free mass change during follow-up (all p>0.05). ED50 for insulin's antilipolytic action was not an independent predictor of future rate of percent weight, fat mass, or fat free mass change during follow-up after adjusting for basal lipolysis (all p>0.05). To normalize for glycerol release, we expressed basal lipolysis as fmol/s and included cell size (measured in μg lipid/cell) in the models, which did not alter the results (data not shown).

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Discussion

We found that percent body fat was positively associated with average adipocyte size and *in vitro* basal lipolysis. *In vitro* basal lipolysis was positively associated with whole body lipid oxidation and negatively with 24-hour respiratory quotient. Larger adipocyte size and higher basal lipolysis predicted lower fat mass gain in univariate models. However, the association between basal lipolysis and fat mass gain was marginal after accounting for known predictors of fat mass gain. In multivariate models higher basal lipolysis but not adipocyte size was associated with lower total weight gain. Neither adipocyte size nor basal lipolysis predicted fat free mass gain. Maximal isoproterenol stimulated lipolysis was not associated with weight, fat mass, or fat free mass gain.

Weyer and colleagues previously observed a positive association between average adipocyte size and basal lipolysis per cell in a smaller sample of Pima Indians[17]. Larger adipocytes have been shown to have enhanced lipolytic capacity compared to smaller adipocytes in healthy human subject of European ancestry[18]. Elevated basal lipolysis has also been seen in large compared to small adipocytes from C57BL/6J mice fed a high fat diet[19]. In our large cohort, we confirmed an association between adipocyte size and both basal and isoproterenol stimulated *in vitro* lipolysis in a large cohort.

We investigated whether *in vitro* cellular lipolysis was associated with whole body substrate oxidation to establish a possible mechanistic link through which adipocyte lipolysis could affect body weight. Imbeult and colleagues found that *in vitro* isoproterenol stimulated lipolysis was associated with whole body oxidation in a study of 20 obese Caucasian males[20]. However, substrate oxidation in that study was measured for 15 minutes in an exclusively fasting state, which may not reflect free-living substrate oxidation. Moro and colleagues performed *in vitro* microdialysis measurement lipolysis in 8 endurance-trained male cyclists while measuring substrate oxidation during exercise[21]. Like the previous study, this method may not produce findings generalizable to the public in typical conditions. Studies have also shown that *in vitro* cellular lipolysis, whole body lipolysis, and whole body lipid oxidation shift similarly after a high fat diet. Kather and colleagues performed *in vitro* cell lipolysis on adipose tissue after feeding subjects different diets and found that basal lipolysis was increased after a high fat diet or an energy restricted diet[22] and Calles-Escandón and Driscoll found that fat intake was positively correlated with *in vivo* basal lipolysis in humans[23]. These observed associations indicate a link between *in vitro* measurements of lipolysis and whole body substrate oxidation. The setting of our study was unique as subjects were fed a weight-maintaining diet with standardized macronutrient composition throughout their inpatient stay providing time prior to any studies to allow cellular and whole body measurements for fat oxidation to adapt from free-living conditions[24]. Under these conditions, we found *in vitro* basal lipolysis per cell was associated with 24-hour RQ and lipid oxidation, but not carbohydrate oxidation. Thus, we were able to provide evidence that an in vitro cellular measurement may reflect whole body lipid metabolism. Average adipocyte size was also negatively associated with RQ and positively with lipid oxidation, although these associations were not statistically significant when assessed in multivariate models.

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We then investigated whether average adipocyte size and *in vitro* lipolysis were associated with weight gain. Landerholm and Stern had found a negative association between baseline epinephrine stimulated lipolysis and weight gain in Sprague-Dawley rats fed a high-fat diet[25]. We demonstrated that in humans *in vitro* basal lipolysis independent of adipocyte cell size was negatively associated with total weight gain. Andersson, Wahrenberg, and Löfgren found an association between CGP 12177, a β_3-adrenoreceptor agonist, stimulated lipolysis and weight gain in female humans[26]. However, we did not find an association between lipolysis stimulated by isoproterenol, which has similar β-selectivity to epinephrine, and weight gain in our mixed sex cohort. We found that adipocyte size and *in vitro* basal lipolysis were negatively associated with fat mass gain, although the association between *in vitro* basal lipolysis and fat mass gain was attenuated when assessed in a multivariate model. Our *in vitro* results (given the associations with RQ and lipid oxidation) are consistent with previous studies. We have shown that higher respiratory quotient, and, in men, lower lipid oxidation predicted weight gain[4,27]. Froidevaux et al also demonstrated that an elevated RQ following a dietary intervention increased risk of regaining weight in women[5]. Our results indicate a link between cellular lipolysis and weight gain via whole body lipid oxidation.

Stored triglycerides must go through lipolysis before the body can utilize them for energy. Lipolysis increases with fasting and calorie deficit, conditions that cause weight loss and utilization of energy stores[22,28,29]. We demonstrated that even during a macronutrient stable weight maintaining diet prior to adipose tissue biopsy and calorimetry measurements, that higher *in vitro* lipolytic rates are associated with whole body lipid oxidation and lower rates of weight gain. Individuals whose adipocytes have increased basal lipolysis in the eucaloric state may preferentially use lipids for energy rather than store them allowing for increased lipid oxidation rates and hence lower rates of weight and perhaps fat mass gain. Additionally, increased lipid oxidation may divert carbohydrates to glycogen, which, in the free-living environment, may reduce food intake[15,30].

Several limitations of this study must be acknowledged. First, a majority of subjects were Pima Indian, which may limit the generalizability of the findings. However, there was no observed difference in cellular basal lipolysis between ethnicities. Second, not all subjects completed measurements of substrate oxidation and follow-up body composition. Analysis of these variables, though, was done independently and differences in the demographics of the subgroups were accounted for. Additionally, a determination of causality in the relationship between *in vitro* cellular basal lipolysis and weight change cannot be made with these data.

Increased adipocyte cell size and *in vitro* lipolysis were positively associated. We further demonstrated that *in vitro* lipolysis independent of co-variates including adipocyte cell size was associated with RQ and whole body lipid oxidation, both previously identified metabolic predictors of weight gain. Higher basal lipolysis was also associated with less weight gain, again independent of cell size. Adipocyte size and basal lipolysis may be inherent characteristics of individual adipose cells that have whole body effects on both lipid oxidation and eventual weight gain.

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Study Importance

What is Already Known About This Subject

- *In vitro* cellular lipolysis and whole body lipid oxidation shift similarly after a change in diet, and the two measures have been found to be associated in a small study of obese men
- Whole body lipid oxidation has been shown to be associated with future weight gain in Native American men and Caucasian women, suggesting *in vitro* lipolysis may be associated with weight gain

What This Study Adds

- We found that *in vitro* basal lipolysis had a positive association with whole body lipid oxidation in a large, mixed-weight sample of men and women
- We found that *in vitro* basal lipolysis had a negative association with weight gain and a marginal negative association with fat mass gain in a mixed sex and race cohort

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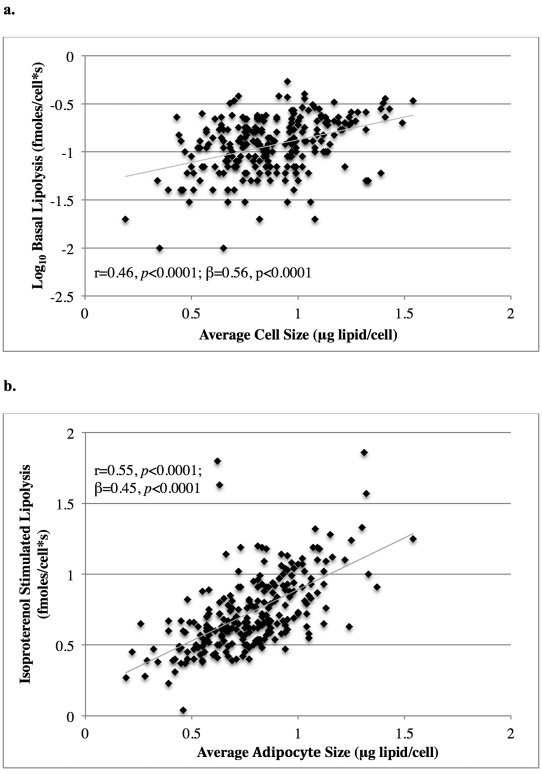

Figure 1. Association Between Adipocyte Size and Lipolysis

The association between average adipocyte size and basal lipolysis per cell (**a.**) and isoproterenol stimulated lipolysis per cell (**b.**). Basal lipolysis values are shown on a logarithmic scale. Unadjusted Pearson product-moment correlations (r) and adjusted parameter estimates (β) are reported with their associated p values.

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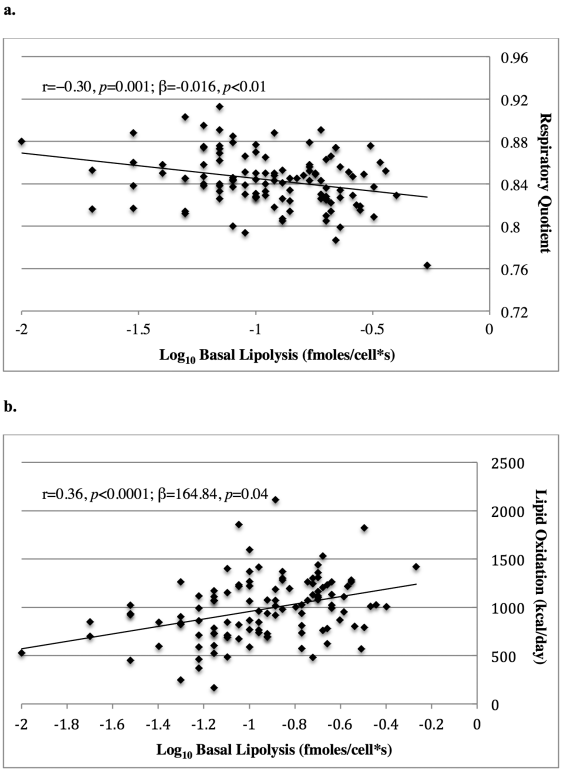

Figure 2. Association Between *In Vitro* Lipolysis and Whole Body Substrate Oxidation

The association between basal lipolysis per cell with respiratory quotient (**a.**) and lipid oxidation (**b.**) during a 24-hour stay in a calorimeter. Basal lipolysis values are shown on a logarithmic scale. Unadjusted Pearson product-moment correlations (r) and adjusted parameter estimates (β) are reported with their associated p values.

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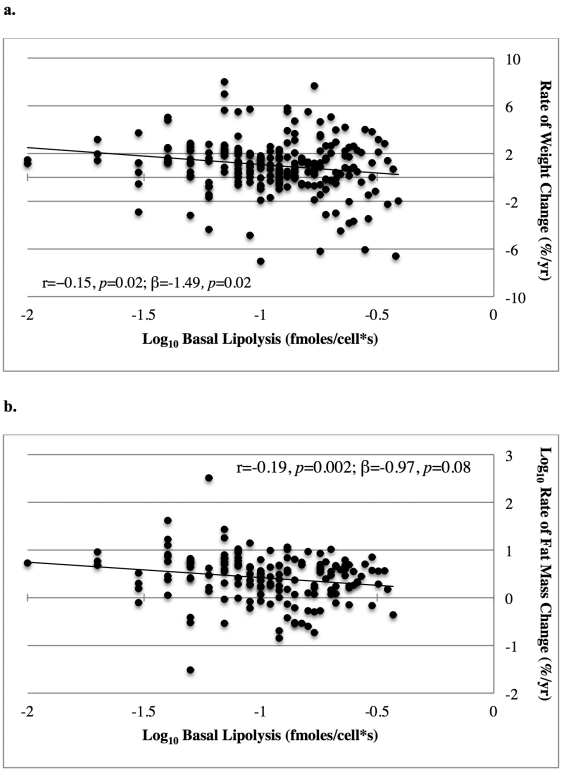

Figure 3. Association Between *In Vitro* Lipolysis and Weight Gain

The association between basal lipolysis per cell with rate of percent weight change (a.) and rate of percent fat mass change (b.) Lipolysis values and rate of percent fat mass change are shown on logarithmic scales. Unadjusted Pearson product-moment correlations (r) and adjusted parameter estimates (β) are reported with their associated p values.

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Table 1

Demographic Information Table 1 compares the characteristics of all subjects who received an adipose tissue biopsy to the subgroups who also had follow-up data available and who had measurement of energy expenditure and substrate oxidation in a metabolic chamber

Analysis	Units	Cross-Sectional		Follow-Up		Chamber	
Gender	-	Male	Female	Male	Female	Male	Female
Race	NA/C/H[a]	142/36/1[f]	130/16/0[g]	115/11/1[f]	114/2/0[g]	33/26/0[f]	46/7/0[g]
Age	Years (Mean±SD[j])	26.8±5.9	26.7±6.2	26.0±5.6	25.9±5.6	28.1±5.8	27.0±6.3
Weight (kg)	Kg (Mean±SD[j])	100.3±27.8	90.5±21.6	102.3±28.4	91.3±20.8	106.0±29.9	93.6±22.7
%Fat	% (Mean±SD[j])	28.0±8.4	38.5±6.6	29.8±8.2	39.2±6.2	28.2±9.2	39.0±6.8
Fx Glucose[b]	mg/dl (Mean±SD[j])	95.0±12.0	102.5±26.8	93.9±14.2	101.6±25.7	97.6±7.9	99.2±10.9
2H Glucose[b]	mg/dl (Mean±SDj)	126.8±39.9	156.0±57.0	126.2±44.7	156.6±58.1	128.7±30.7	154.4±40.9
BasLip[c]	fmoles/cell*s (Median[95% CI[k]])	.11[.07-.17]	.12[.08-.19]	.11[.08-.16]	.14[.08-.20]	.10[.07-.16]	.17[.08-.22]
BasLipSA[d]	10^{-21} moles/μm2*s (Median[95% CI[k]])	2.5[1.7-3.7]	2.8[1.8-4.0]	2.7[1.8-3.7]	3.0[1.9-4.3]	2.4[1.8-3.4]	2.9[1.4-4.2]
AVCS[e]	μg lipid/cell (Mean±SD[j])	0.73±0.23[h]	0.88±0.24[i]	0.70±0.19[h]	0.85±0.24[i]	0.87±0.30[h]	1.02±0.27[i]

[a] NA=Native American, C=Caucasian, H=Hispanic

[b] Fx=Fasting and 2h=2 hour glucose (mg/dl)

[c] BasLip=Basal lipolysis per cell

[d] BasLipSA=Basal lipolysis per μm^2

[e] AVCS=Average cell size (μg lipid/cell)

[f] Significant differences between all groups, $p<0.001$

[g] Subjects with follow-up data different from all other groups, $p=0.005$

[h] Subjects with data from the metabolic chamber different from all other groups, $p<0.001$

[i] Subjects with data from the metabolic chamber different from all other groups, $p<0.001$

[j] SD=Standard deviation

[k] CI=Confidence interval

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Table 2

Summary of Major Multivariate Analyses Models were assessed with and without average adipocyte cell size. Parameter estimates for sex are the differences associated with male vs. female sex and parameter estimates for ethnicity are the differences associated with Caucasian vs. Native American ethnicity

		Without adipocyte cell size		With adipocyte cell size	
Independent variable	**Dependent variable**	***p* value**	**Parameter estimate [95% CI[e]]**	***p* value**	**Parameter estimate [95% CI[e]]**
BasLip[a]	%Fat	<0.0001	12.50 [10.4, 14.7]	<0.0001	7.9 [5.7, 10.2]
IpLip[a]	%Fat	<0.0001	8.70 [6.3, 11.0]	0.80	0.3 [−2.3, 3.0]
Sex	BasLip[a]	<0.0001	0.11 [0.06, 0.17]	<0.0001	0.12 [0.07, 0.18]
%Fat		<0.0001	0.02 [0.02, 0.02]	<0.0001	0.01 [0.01, 0.02]
AVCS[b]	BasLip[a]	<0.001	0.56 [0.46, 0.66]	-	-
Age		0.30	0.01 [−0.00, 0.01]	-	-
Sex		0.63	0.01 [−0.04, 0.06]	-	-
AVCS[b]	24hRQ	0.37	−0.010 [−0.031, 0.012]	-	-
Age		0.71	0.000 [−0.001, 0.001]	-	-
EnBal[c]		0.03	0.000 [0.000, 0.000]	-	-
Sex		0.43	0.005 [−0.008, 0.018]	-	-
%Fat		0.29	0.000 [−0.001, 0.000]	-	-
AVCS[b]	Lipid oxidation	0.44	72.1 [−112.6, 256.7]	-	-
Fat mass		0.05	4.67 [−0.06, 9.40]	-	-
FF mass[d]		0.09	5.30 [−0.92, 11.51]	-	-
Age		0.82	0.81 [−6.07, 7.70]	-	-
Sex		0.76	25.0 [−135.7, 185.8]	-	-
EnBal[c]		<0.0001	−0.54 [−0.74, −0.35]	-	-
BasLip[a]	24hRQ	<0.01	−0.016 [−0.024, −0.008]	<0.01	−0.017 [−0.025, −0.009]
Age		0.56	0.000 [−0.001, 0.001]	0.56	0.000 [−0.001, 0.001]
EnBal[c]		0.07	0.000 [−0.000, 0.000]	0.07	0.000 [−0.000, 0.000]
Sex		0.50	−0.005 [−0.018, 0.009]	0.50	−0.005 [−0.018, 0.009]
%Fat		0.33	0.000 [−0.001, 0.000]	0.38	0.000 [−0.001, 0.000]
BasLip[a]	Lipid oxidation	0.04	164.8 [5.7, 323.9]	0.04	186.1 [3.0, 369.1]
Fat mass		0.06	4.61 [−0.24, 9.47]	0.06	4.85 [−0.12, 9.83]
FF mass[d]		0.17	4.52 [−1.90, 10.94]	0.16	4.67 [−1.80, 11.15]
Age		0.52	2.22 [−4.68, 9.12]	0.47	2.62 [−4.50, 9.74]
Sex		0.56	47.8 [−113.4, 208.9]	0.63	39.9 [−125.2, 205.0]
EnBal[c]		<0.0001	−0.52 [−0.72, −0.32]	<0.0001	−0.52 [−0.73, −0.32]

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		Without adipocyte cell size		With adipocyte cell size	
Independent variable	**Dependent variable**	***p* value**	**Parameter estimate [95% CI[e]]**	***p* value**	**Parameter estimate [95% CI[e]]**
AVCS[b]	Rate of fat mass change	0.01	−0.95 [−1.28, −0.62]	-	-
Fat mass		0.53	−0.01 [−0.03, 0.01]	-	-
Ethnicity		<0.01	2.96 [1.41, 4.51]	-	-
Sex		0.62	0.08 [−1.54, 1.70]	-	-
Age		0.37	0.28 [−0.08, 0.64]	-	-
BasLip[a]	Rate of weight change	0.02	−1.49 [−2.85, −0.15]	0.03	−1.53 [−2.95, −0.11]
Weight		0.01	0.02 [0.00, 0.04]	0.01	0.02 [0.00, 0.04]
Ethnicity		0.53	0.57 [−1.25, 2.39]	0.53	0.57 [−1.25, 2.40]
Sex		0.78	−0.11 [−0.84, 0.63]	0.82	−0.09 [−0.88, 0.70]
Age		0.39	−0.03 [−0.09, 0.04]	0.39	−0.03 [−0.09, 0.04]
BasLip[a]	Rate of fat mass change	0.08	−0.97 [−2.04, 0.10]	0.06	−1.11 [−2.24, 0.01]
Fat mass		0.97	0.00 [−0.02, 0.02]	0.87	0.00 [−0.02, 0.02]
Ethnicity		0.47	0.58 [−0.85, 2.01]	0.44	0.56 [−0.87, 1.99]
Sex		0.49	0.25 [−0.81, 0.31]	0.55	−0.18 [−0.76, 0.41]
Age		0.26	−0.03 [−0.08, 0.03]	0.32	−0.03 [−0.08, 0.03]

[a]BasLip=Basal lipolysis and IpLip=isoproterenol stimulated lipolysis

[b]AVCS=Average cell size

[c]EnBal=Energy balance

[d]FF mass=Fat free mass

[e]95% CI=95% confidence interval