Adjusted and Unadjusted Energy Usage Rates Both Determine Body Fat and Plasma Leptin in Male Fischer 344 Rats

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Previous studies of the relationship between plasma leptin and energy usage have yielded contradictory findings. The present study was therefore conducted to clearly distinguish and measure the energy usage rate and the energy usage rate adjusted for a surrogate of metabolically active tissue mass. We investigated the simultaneous relationships between these two measures of energy usage, leptin, and body fat in 21-month-old adult male Fischer 344 rats on three different long-term dietary regimens: (1) continuous ad libitum feeding (Ad-lib); (2) ad libitum feeding until early adulthood, and then continuous 60% caloric restriction (CR); and (3) ad libitum feeding until early adulthood, then 60% caloric restriction until 16 months, and then ad libitum feeding for 5 months (CR/Ad-lib). Two versions of the daily usage rate were measured: daily dietary caloric intake (DCI), and daily energy expenditure (EE) based on indirect calorimetry. Two versions of the metabolically active tissue mass were also measured: fat-free mass (FFM), and the sum of the weight of the heart, brain, liver, and kidneys. Energy usage rates were adjusted for these measures of metabolically active tissue mass to yield measures of the energy metabolic rate. Correlation, regression, and path analyses showed that both the energy usage rate and adjusted energy usage rate played important independent roles in determining body fat and plasma leptin, but only after multivariate techniques were used to account for the simultaneous interactions between variables. Increases in the energy usage rate were associated with increases in body fat and the adjusted energy usage rate. Increases in the adjusted energy usage rate were associated with decreases in body fat and plasma leptin. These findings suggest that differences in subjects adjusted energy usage rate could explain some of the apparently contradictory findings concerning the relationship between energy usage and plasma leptin in previously published studies. In conclusion, this appears to be the first study to clearly separate and quantify the effects of the energy usage rate and adjusted energy usage rate on body fat and plasma leptin. The findings suggest that under conditions of long-term stable body weight, both of these measures of energy usage play independent simultaneous roles in determining body fat and plasma leptin.

LEPTIN, a hormone synthesized by the adipocyte, has been shown to play a central role in the regulation of fat mass and energy balance in rodents and humans.[1] A number of studies have investigated the relationships between plasma leptin, body fat, and energy usage under conditions of stable body weight, and have yielded findings that appear contradictory. For instance, two investigators found the relationship between leptin and daily energy expenditure (EE) to be negative,[2,3] while two others found it to be positive.[4,5]

A clearer understanding of the relationships between plasma leptin, body fat, and energy usage under conditions of long-term stable energy intake would help to elucidate the role of leptin in chronic human obesity and long-term weight control. The fact that plasma leptin has been found to be positively and strongly correlated with the level of body fat[1] suggests that a higher energy intake would induce higher levels of plasma leptin. Similarly, evidence that physical activity reduces plasma leptin[6] suggests that higher rates of energy metabolism would induce lower levels of plasma leptin. Together, these findings imply that in humans, food intake and the energy metabolic rate may have separate effects on the plasma leptin level, as well as the level of body fat. In this report, we present the results of a study with male Fischer 344 rats on three different long-term feeding regimens. The purpose of using the three regimens was to yield a wide range of values for the variables under study, ie, energy usage, leptin, and body fat. The aim of the study was to carefully distinguish and measure the energy usage rate and the energy usage rate adjusted for metabolically active tissue mass, and to examine their simultaneous effects on body fat and leptin.

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MATERIALS AND METHODS

Animals and Diets

Three groups of 10 male Fischer 344 rats were purchased from the National Institute on Aging–funded National Center for Toxicological Research (NCTR) facility in Jefferson, Arkansas, where they had been individually housed in shoebox cages. All animals were individually housed and fed NIH-31 feed throughout their lifespan and were killed at 20.5 months of age. The ad libitum (Ad-lib) rats were fed consistently throughout their lifespan. The calorically restricted (CR) rats were fed ad libitum for the first 13 weeks, and then caloric intake was gradually reduced until it reached 60% of the caloric intake of the Ad-lib rats at 16 weeks and was kept at this level until the end of the study. The CR/Ad-lib rats were fed CR at the NCTR facility until 16 months of age, when they were purchased and moved to the St. Luke's-Roosevelt Hospital Center Animal Care Department in New York City, where they were maintained at 24°C with a 7 PM to 7 AM dark cycle. The CR and Ad-lib rats were purchased at 20 months of age and then housed at St. Luke's-Roosevelt Hospital Center for 2 weeks before sacrifice, to allow for recovery from relocation stress. After purchase, CR animals were housed in shoebox cages and fed at 9 AM, and CR/Ad-lib and Ad-lib animals were housed in wire-bottomed metabolic cages to facilitate accurate determination of daily food intake. The research protocol was approved by the St. Luke's-Roosevelt Institute for Health Sciences Institutional Animal Care and Use Committee.

Indirect Calorimetry

Two surrogates for the daily rate of energy usage were used in the study. The first was 24-hour energy expenditure (EE), measured for each rat using indirect calorimetry. The rats were divided into six groups, each containing five similar-diet animals, and each group was placed in the calorimeter on three occasions several days apart to allow for habituation. The readings obtained on the third occasion were used in the analyses reported here. The calorimeter consisted of a Magnos IV oxygen analyzer and a Uras 3G carbon dioxide analyzer (both from Hartmann Braun, Berlin, Germany) and six Plexiglas metabolic chambers maintained in a sound-insulated environmental room separated from the analyzer. The room was supplied with first-pass fresh air at constant temperature. Air was continuously circulated through the chambers, in which the rats were individually housed, at a fixed rate by individual vacuum pumps, each attached to a flow monitor to regulate and record the air flow rate. Temperature probes located at each flow meter measured the temperature of the air. A 100-MHz, 486 personal computer signaled the solenoid switches to sample air from each chamber in sequence at approximately 10-minute intervals. Sampled air was passed through a condenser (Hartmann Braun) to remove humidity before entering the analyzers. The oxygen concentration, carbon dioxide concentration, air flow rate, and air temperature were measured and processed on-line by the computer to calculate the nonprotein respiratory quotient (RQ), oxygen consumption rate, and carbon dioxide production rate for each rat at each sampling time point. At the start and about halfway through the 2-week calorimetry period, the analyzers were calibrated using a Westoff gas-mixing pump (Digimix, type m/300c; Bochin, Germany) and the entire system was tested by burning propane. The RQ estimation error was previously estimated to be less than 1% in the system. EE was calculated from oxygen consumption and carbon dioxide production using the equation of Brouwer.[7]

Daily Caloric Intake

The second surrogate for the daily rate of energy usage was the average net measured daily caloric intake (DCI) during the week prior to death, based on weighed net daily food intake. For the CR rats, this was equal to the daily food ration, because there were no observed leftovers in the cages. For the Ad-lib rats, allotted food, leftover food, and spillage were measured twice weekly in the metabolic wire-bottomed cages in which they were housed. This yielded a value for net daily food intake, which was estimated to be accurate to within 2%. Food intake in grams was converted to caloric intake assuming 4.4 kcal/g[8] and a bioavailability of 97% for the CR rats and 95% for the Ad-lib and CR/Ad-lib rats.[9] DCI was considered a useful measure of whole-body energy usage and resting energy usage, because the rats in the present study were all under conditions of stable body weight prior to sacrifice. Figure 1 shows this for the CR/Ad-lib rats, and the average body weight-versus-age curves available from the NCTR facility show this for the CR and Ad-lib rats.

Organ Weights

The animals were sacrificed between 9 AM and 5 PM during the lighting period. They were decapitated after being anesthetized with carbon dioxide, and then immediately dissected. The adrenals, heart, liver, lungs, kidneys, spleen, testes, and brain were then excised and weighed.

Body Fat

Each carcass was weighed after removal of the trunk blood and contents of the digestive tract and bladder, and then stored at −20°C until analysis for lipid content using the method described by Folch et al.[10] Fat-free mass (FFM) was calculated as the difference between body weight and body fat.

Adjusted Energy Usage Rates

There were two measures of the adjusted energy usage rate. The first, EEadjFFM, used EE as its surrogate for energy expenditure and FFM as its surrogate for metabolically active tissue mass. The second, DCIadjOW, used DCI and organ weight (OW), respectively, as the two surrogates. OW, which included the weight of the heart, brain, kidneys, and liver, was used because it was found that OW was a better correlate of DCI than was FFM in the rats in the present study[11] and has been

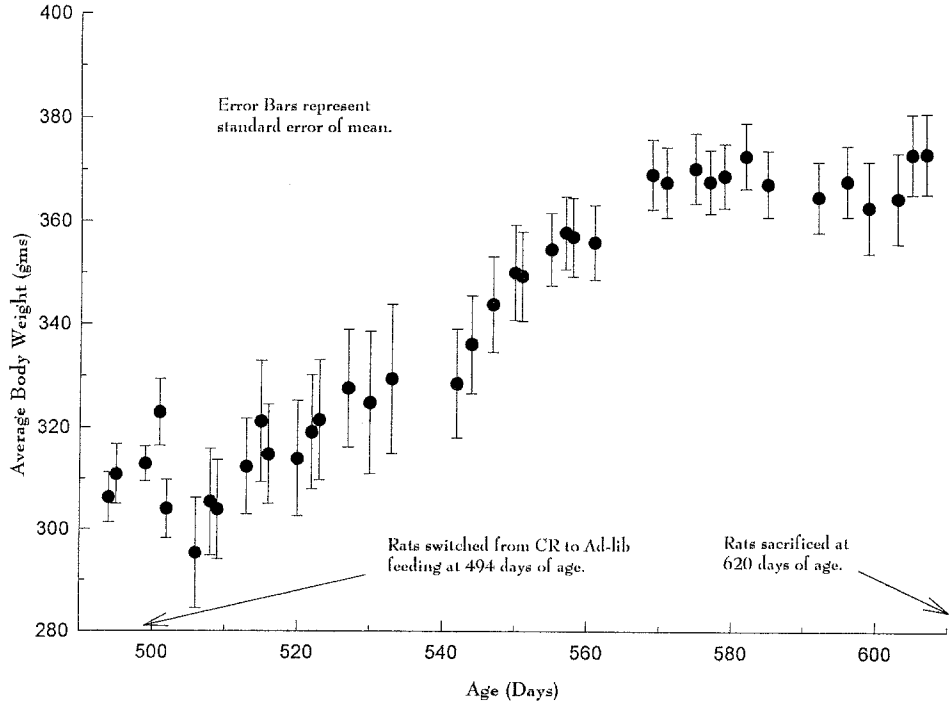

Fig 1. Mean measured body weight versus age (n = 10) in male Fischer 344 rats switched from CR to Ad-lib feeding. Body weight stabilized at ~575 days of age, ~45 days before death.

found to be a good correlate of the resting metabolic rate in humans.[12] The adjustments were performed by first regressing the numerator on the denominator, then subtracting the Y-intercept from the numerator, and then dividing by the denominator to yield the adjusted value of the numerator.[13]

Plasma Leptin

Plasma leptin was measured in trunk blood using a commercially available kit (Linco Research, St. Charles, MO). The intraassay coefficient of variation was 6% to 8%.

Statistical Methods

SPSS for Windows (Version 7.0; SPSS, Chicago, IL) was used to perform correlation/regression analyses and ANOVA. EQS (Version 5.1) structural equation software (Multivariate Software, Encino, CA) was used for a confirmatory path analysis. Statistical significance was set at a P level less than .05.

Pairwise deletion of missing values was used to yield a maximum sample size for each analysis, because there were only, at most, three missing data points in each analysis. There was one Ad-lib rat with a missing body fat datum, one CR rat with a missing leptin datum, and one CR/Ad-lib rat with a missing leptin datum and a high outlier for DCI, identified during tests for univariate outliers.

In testing whether our data satisfied the assumptions of the correlation/regression procedures, it was found that leptin, for all three groups of rats combined, was positively skewed. Hence, both Pearson and Spearman rank correlation coefficients were calculated for this variable. The Spearman coefficients were very similar to the Pearson coefficients in terms of their values and significance levels, and are not reported here. All regression analyses were performed with the linear form and with the square-root transformation of leptin, because this transformation yielded a normal distribution for leptin. The analyses with square-root transformation yielded values and significance levels for the multiple R^2 that were not substantially different from those for the linear form. Also, the significance levels for partial regression coefficients based on the square-root transformation were essentially the same as those based on the linear form. The regression results presented here are based on the linear form of leptin.

In testing whether our data satisfied the assumptions of the ANOVA procedure, it was found that most of the variables had nonhomogeneous variance. Hence, one-way ANOVA with Tamhane's T2 post hoc test[14] was used to account for the nonhomogeneity of variance.

Confirmatory path analysis, which analyzes the simultaneous relationships between interrelated variables,[15] was used to facilitate data interpretation and to illustrate the strength of the combined relationships between DCI, DCIadjOW, body fat, and leptin suggested by the regression findings. The maximum-likelihood solution method was used to estimate the model, and the Sartorra-Bentler scaled chi-square was used to account for the effect of non-normality of leptin.

Results of analyses not reported here are available from the first author.

RESULTS

Table 1 shows that body fat, FFM, OW, and EE were all highest in the Ad-lib group, intermediate in the CR/Ad-lib group, and lowest in the CR group. Leptin and DCI showed the same pattern, except that leptin was not significantly higher in CR/Ad-lib versus CR rats, and DCI was not significantly higher in Ad-lib versus CR/Ad-lib rats. Neither of the two measures of the adjusted energy usage rate, DCIadjOW and EEadjFFM, showed any significant between-group differences.

Correlation and regression analyses were performed, using data for all three rat groups combined to investigate the relationships between leptin, body fat, EE, EEadjFFM, DCI, and DCIadjOW. Table 2, which lists the bivariate correlation coefficients, shows that leptin was highly and significantly correlated with body fat (r = .87), EE (.69), and DCI (.61). Table 3 lists the results of a hierarchical multiple regression analysis[16] with leptin as the dependent variable. Equation 2 shows that EE and EEadjFFM together explained 66% of the variance in leptin (multiple R^2 = 0.66). Similarly, equation 5 shows that DCI and DCIadjOW together explained 67% of the variance in leptin. However, neither EE nor EEadjFFM made a significant independent contribution when body fat was controlled for, as shown by their nonsignificant partial regression co-coefficients when body fat was entered (equations 3 and 4) and by the fact that the multiple R^2 values in equations 3 and 4 were both only 1% larger than the value in equation 1. On the other hand, both DCI and DCIadjOW made independent significant contributions to the variance in leptin over and above that made by body fat. This is shown by the significant partial regression coefficient for DCI in equation 6 (−0.17) and for DCIadjOW in equation 7 (−6.59). It is also shown by the higher multiple R^2s in equation 6 (0.81) and equation 7 (0.86) versus equation 1 (0.75). When DCIadjOW was entered with DCI and body fat (equation 8), the partial regression coefficient for DCI was not significant (−0.04), showing that DCI made no significant independent contribution to the variance in leptin after the effects of DCIadjOW and body fat were accounted for.

No satisfactory explanation could be found for the relatively small differences in the relationships between leptin, EE, and EEadjFFM, on the one hand, and leptin, DCI, and DCIadjOW, on the other, in Tables 2 and 3. As stated earlier, the fact that all animals were weight-stable prior to death suggests that DCI measured over a 1-week period was a good estimate of each animal's energy intake during this period.

Equation 9 in Table 3, with body fat as a dependent variable, shows that EE and EEadjFFM together explained 86% of the variance in body fat and that each made independent significant

Table 1. Plasma Leptin, Body Composition, and Energy Usage in Male Fischer 344 Rats on Three Different Diets

Parameter	CR	CR/Ad-lib	Ad-lib	ANOVA (F)
Plasma leptin (ng/mL)	3.4 ± 1.73	6.4 ± 3.3‡	11.8 ± 4.3§	15.4*
Body fat (g)	20.5 ± 2.2†	50.0 ± 8.7‡	81.0 ± 18.8§	63.5*
FFM (g)	248.2 ± 4.3†	312.7 ± 27.5‡	364.2 ± 19.0§	85.1*
Body fat (%)	7.62 ± .79†	13.74 ± 1.67‡	18.02 ± 2.79§	73.0*
OW (g)‖	12.40 ± .45†	17.49 ± 1.7‡	19.77 ± 1.18§	93.6*
24-hour EE (kcal/d)	47.2 ± 6.3†	60.1 ± 10.2‡	73.1 ± 9.4§	20.6*
EEadjFFM (kcal/d/g)	0.21 ± .02	0.21 ± .03	0.22 ± .03	0.4
DCI (kcal/d)	49.1 ± 0.0†	69.7 ± 8.0	73.0 ± 7.7§	41.4*
DCIadjOW (kcal/d/g)	3.41 ± .13	3.60 ± .25	3.40 ± .28	2.4

NOTE. Data are presented as the mean ± SD. All rats were 21 months of age when measurements were made. All data cells contain 10 data points except as follows: 9 in CR and 9 in CR/Ad-lib for leptin; 9 in Ad-lib for body fat, FFM, and EEadjFFM; 9 in CR/Ad-lib for DCI; and 9 in CR/Ad-lib and Ad-lib for DCIadjOW.

*$P < .001$.

Significant differences on Tamhane's T2 post hoc test ($P < .05$): †CR v CR/Ad-lib, ‡CR/Ad-lib v Ad-lib, §Ad-lib v CR.

‖Organs include the heart, brain, kidneys, and liver.

Table 2. Bivariate Correlation Coefficients (Pearson's *r*)

	Leptin	Body fat	EE	EEadjFFM	DCI	DCIadjOW
Leptin	1.00 (28)					
Body fat	.87* (27)	1.00 (29)				
EE	.69* (28)	.76* (28)	1.00 (30)			
EEadjFFM	.15 (27)	.09 (28)	.65* (28)	1.00 (29)		
DCI	.61* (28)	.85* (28)	.73* (29)	.07 (28)	1.00 (29)	
DCIadjOW	−.27 (27)	.06 (28)	.14 (28)	.18 (28)	.37 (28)	1.00 (28)

NOTE. Units of measure are as follows: leptin, ng/mL; body fat, g; EE, kcal/d; EEadjFFM, kcal/g/d; DCI, kcal/d; DCIadjOW, kcal/g/d. Numbers in parentheses are numbers of rats with data available for the correlation analysis.

*$P < .001$.

contributions, because their partial regression co-coefficients (2.37 and −677.6, respectively) were significant. A similar result was found when DCI and DCIadjOW were entered as independent variables, as shown in equation 10 in Table 3.

The lowest value of the tolerance statistic for any of the independent variables in Table 3 was 0.174, indicating that there were no multicolinearity problems in the regression analyses.

Figure 2 contains the results of the confirmatory path analysis. It shows the hypothesized model with path coefficients in standardized form. Each line connecting two variables represents a hypothesized effect, with the arrowhead indicating the direction of the effect. The numbers on the lines are path coefficients, which are equivalent to standardized partial regression coefficients. For instance, the path coefficient between DCIadjOW and leptin (−0.32) is the same as the beta coefficient for DCIadjOW in equation 7 in Table 3. E_2, E_3, and E_4 represent the errors or residuals in body fat, DCIadjOW, and leptin, respectively, not accounted for by the model. For instance, the path coefficient for E_4, 0.38, can be interpreted as showing that DCI, DCIadjOW, and body fat together explain 86% $(1 - E_4^2)$ of the variance in leptin—the same as the multiple R^2 for equation 8 in Table 3. There was a good fit between the hypothesized model and the relationships between the four measured variables in our sample of rats (Sartorro-Bentler scaled chi-square = 0.76, $df = 1, 27$, $P = .39$, Bentler-Bonett normed fit index = 0.997, Bentler-Bonett non-normed fit index = 1.045, comparative fit index = 1.000).

Table 3. Multiple Regression Results

Equation No.	Variables in Equation	Partial Regression Coefficient	Beta Coefficient	Multiple R^2
With plasma leptin as dependent variable (n = 27)				
1	Body fat	0.15‡	0.87	.75‡
2	EE	0.36‡	1.07	.66‡
	EEadjFFM	−95.1†	−0.57	
3	EE	0.03	0.09	.76‡
	Body fat	0.14‡	0.80	
4	EEadjFFM	10.07	0.06	.76‡
	Body fat	0.15‡	0.86	
5	DCI	0.32‡	0.83	.67‡
	DCIadjOW	−12.11‡	−0.59	
6	DCI	−0.17*	−0.44	.81‡
	Body fat	0.22‡	1.24	
7	DCIadjOW	−6.59‡	−0.32	.86‡
	Body fat	0.16‡	0.89	
8	DCI	−0.04	−0.10	.86‡
	DCIadjOW	−5.88†	−0.28	
	Body fat	0.17‡	0.97	
With body fat as dependent variable (n = 27)				
9	EE	2.37‡	1.21	.86‡
	EEadjFFM	−677.6‡	−0.71	
10	DCI	2.12‡	0.92	.81‡
	DCIadjOW	−35.4†	−0.23	

NOTE. Units of measure are as in Table 2.

*$P < .05$.

†$P < .01$.

‡$P < .001$.

DISCUSSION

The present study suggests that both the energy usage rate (represented by DCI and EE) and the energy usage rate adjusted for metabolically active tissue mass (represented by DCIadjOW and EEadjFFM) played important independent and simultaneous significant roles in determining the level of both body fat and leptin under the conditions of stable body weight in the present study. This is illustrated by several findings. First, DCI and DCIadjOW together explained 81% of the variance in body fat (equation 10, Table 3) and 67% of the variance in leptin

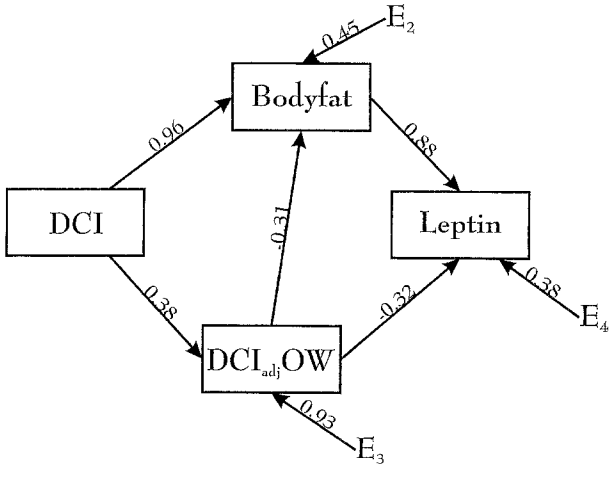

Fig 2. Confirmatory path-analytic model showing quantitative relationships, all significant, between DCI, DCIadjOW, body fat, and plasma leptin. Our data fit the model well: Sartorra-Bentler scaled chi-square = 0.76, $P = .39$; Bentler-Bonnet normed fit index = 0.997; Bentler-Bonnet nonnormed fit index = 1.045; robust comparative fit index = 1.000.

(equation 5, Table 3). Similarly, EE and EEadjFFM together explained 86% of the variance in body fat (equation 9, Table 3) and 66% of the variance in leptin (equation 2, Table 3)—and the partial regression coefficients in all four of these equations were significant. Also, while body fat alone explained 75% of the variance in leptin (equation 1, Table 3), DCI and DCIadjOW independently and significantly explained a further 6% and 11% of this variance, respectively (equations 6 and 7, respectively, Table 3).

Neither EEadjFFM nor DCIadjOW exhibited a significant bivariate correlation with leptin ($r = .15$ and $-.27$, respectively, Table 2). This agrees with other similar previous findings. For instance, Kennedy et al[17] found that leptin was not correlated with the resting metabolic rate normalized per kilogram of metabolically active body mass in both human male and female subjects with a wide range of body weight. However, in the present study, EEadjFFM was found to be significantly correlated with EE ($r = .65$; Table 2), and after leptin was adjusted for variations in EE, EEadjFFM exhibited a significant negative relationship with leptin (partial regression coefficient = -95.1 in equation 2, Table 3). Similarly, DCIadjOW was almost significantly correlated with DCI ($r = .37$, $P = .051$; Table 2), and after leptin was adjusted for variations in DCI, DCIadjOW also exhibited a significant negative relationship with leptin (partial regression coefficient = -12.11 in equation 5, Table 3). DCIadjOW and leptin were also significantly related after leptin was adjusted for variations in body fat and DCI (equation 8, Table 3, and Fig 2). In other words, because the energy usage rate (EE and DCI), energy usage rate adjusted per unit of metabolically active tissue mass (EEadjFFM and DCIadjOW, respectively), and body fat were all correlated, their independent relationships with leptin could be accurately revealed only by using multivariate techniques to analyze their simultaneous relationships with leptin. The same applies to the relationship between DCIadjOW and body fat. These two variables did not exhibit a significant bivariate correlation ($r = .06$; Table 2). However, they were significantly related after body fat was adjusted for variations in DCI, as shown by the significant partial regression coefficient for DCIadjOW in equation 10 (Table 3).

The path-analytic model in Fig 2 depicts the simultaneous quantitative interrelationships (all significant) between DCI, DCIadjOW, body fat, and leptin. Specifically, it shows that increases in DCI were associated with increases in body fat (path coefficient = 0.96) and in DCIadjOW (0.38). Increases in DCIadjOW, in turn, were associated with decreases in body fat (-0.31) and leptin (-0.32), while increases in body fat were associated with increases in leptin (0.88). Overall, DCI was positively associated with leptin (Pearson's $r = 0.61$; Table 2). This is because in our sample of rats, the increases in leptin associated with increases in body fat, associated with increases in DCI, were greater than the decreases in leptin associated with increases in DCIadjOW, associated with increases in DCI. DCI was the exogenous causal factor in the model, because it was the only variable directly manipulated by the experimental regimen.

The following possible physiological explanations are offered for the path model in Fig 2. First, the path coefficient between DCI and body fat was positive (0.96) and that between DCIadjOW and body fat was negative (-0.31). This suggests that body fat was higher in rats that ate more and in rats in which the metabolic rate (DCIadjOW) was lower, a finding which seems physiologically appropriate. Second, the positive path coefficient between body fat and leptin (0.88) is due to the constant ratio of leptin to body fat in rats in the present study, as discussed elsewhere.[18] Physiologically, this reflects the role of leptin as an afferent signal of the level of body fat.[1] Taken together, these data suggest that DCI and DCIadjOW affect leptin via their effects on body fat: as daily food intake (DCI) increases, it increases body fat and hence increases leptin, and as the metabolic rate (DCIadjOW) increases, it decreases body fat and hence decreases leptin. Third, the metabolic rate appears to have an independent direct negative effect on leptin in that increases in DCIadjOW induce decreases in leptin, as shown by the negative path coefficient (-0.32) between DCIadjOW and leptin. This may reflect the fact that higher metabolic activity induces a higher rate of clearance of leptin from the circulation, probably via leptin receptors, which have been found in a number of organs including the small intestine, kidneys, liver, lungs, heart, spleen, and testes.[19,20] Several previously published empirical studies have yielded results that are consonant with the negative path coefficient between DCIadjOW and leptin. For instance, short-term cold immersion of mice increased the metabolic rate and reduced obese mRNA expression,[21] and cold immersion of rats reduced plasma leptin.[22,23] In addition, it has been found that several hours of exercise reduces plasma leptin in humans[6,24,25] and rats,[26] and that ephedrine and caffeine administration increases the metabolic rate and decreases plasma leptin in rhesus monkeys.[27]

Several investigators have found negative correlations between the energy usage rate and plasma leptin in cross-sectional studies involving human subjects,[2,3] or inconsistent results in different groups of subjects.[4,5] The model in Fig 2 suggests that different levels of the adjusted energy usage rate in different groups of subjects could explain such differences. In other words, if the independent effect of the adjusted energy usage rate was accounted for, some of these inconsistencies may have disappeared. The study by Toth et al[5] is instructive in this regard. They found a positive correlation between EE and leptin ($r = .67$) in 17 heart-failure patients with very low levels of physical activity (average, 359 kcal/d) and stable body weight, but not in 46 healthy control subjects with normal levels of physical activity (817 kcal/d) and stable body weight. In the present study, both DCI ($r = .61$) and EE ($r = .69$) were positively correlated with leptin, and the rats undoubtedly engaged in low levels of physical activity due to the small space for exercise in the rat cages. Assuming that the negative path coefficient between DCIadjOW and leptin in the model in Fig 2 applies to the subjects in the study by Toth et al, this suggests that the higher levels of physical activity in their control subjects could have depressed plasma leptin below the level in the heart-failure patients, and thus eroded the significant correlation between EE and leptin observed in the heart-failure patients.

In the present study, EE and EEadjFFM together and DCI and DCIadjOW together (equations 2 and 5, respectively, Table 3)

explained almost as much of the variance in leptin (66% and 67%, respectively) as did body fat (75%) alone (equation 1, Table 3). This could have relevance in both research and clinical settings. For instance, the negative path coefficient between DCIadjOW and leptin in Fig 2 suggests that wheel-running in rodent studies may decrease plasma leptin—and there is some evidence for this.[26] It also suggests that seasonal variations in physical activity levels in free-living humans could result in seasonal variations in plasma leptin levels. In addition, some investigators have found correlations between leptin and body fat that were substantially lower than in the present study. For instance, Ostlund et al[2] found this bivariate correlation coefficient to be .50 in a cross-sectional study involving 204 adult human subjects. It is conceivable that the energy usage rate and adjusted energy usage rate are stronger correlates of leptin than is body fat in such studies. This might be particularly important in certain illnesses, including heart failure, that affect the energy usage rate or in subjects with abnormally high energy usage rates due to high levels of physical activity.

In conclusion, the present study appears to be the first to clearly separate and quantify the independent simultaneous effects of the energy usage rate and energy usage rate adjusted for metabolically active tissue mass on body fat and plasma leptin. The findings suggest that under conditions of long-term stable body weight, both of these energy usage rates play separate important roles in determining the level of body fat and plasma leptin.

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