

Spillover of Fatty Acids During Dietary Fat Storage in Type 2 Diabetes

Relationship to Body Fat Depots and Effects of Weight Loss

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Spillover of lipoprotein lipase-generated fatty acids from chylomicrons into the plasma free fatty acid (FFA) pool is an important source of FFA and reflects inefficiency in dietary fat storage. We measured spillover in 13 people with type 2 diabetes using infusions of a [³H]triolein-labeled lipid emulsion and [U-¹³C]oleate during continuous feeding, before and after weight loss. Body fat was measured with dual energy X-ray absorptiometry and computed tomography. Participants lost ~14% of body weight. There was an ~38% decrease in meal-suppressed FFA concentration ($P < 0.0001$) and an ~23% decrease in oleate flux ($P = 0.007$). Fractional spillover did not change ($P = \text{NS}$). At baseline, there was a strong negative correlation between spillover and leg fat ($r = -0.79$, $P = 0.001$) and a positive correlation with the trunk-to-leg fat ratio ($R = 0.56$, $P = 0.047$). These correlations disappeared after weight loss. Baseline leg fat ($R = -0.61$, $P = 0.027$) but not trunk fat ($R = -0.27$, $P = 0.38$) negatively predicted decreases in spillover with weight loss. These results indicate that spillover, a measure of inefficiency in dietary fat storage, is inversely associated with lower body fat in type 2 diabetes. *Diabetes* 62:1897–1903, 2013

Free fatty acids (FFAs) mediate insulin resistance (1,2), drive VLDL triglyceride synthesis in the liver (3), and play an important role in the pathogenesis of hypertension (4,5) and diabetes (6). Spillover of lipoprotein lipase (LPL)-generated fatty acids from chylomicrons into the plasma FFA pool is an important source of FFA (7–10) and reflects inefficiency in dietary fat storage. Previous work has shown that the amount of fat taken up in leg fat per gram of tissue increases as a function of leg fat mass, whereas it actually decreases as a function of visceral fat mass and does not change in upper body subcutaneous fat (11). However, it is not clear whether these findings reflect changes in rates of LPL-mediated meal fat hydrolysis, changes in fractional

spillover, or both. We therefore undertook a study in people with type 2 diabetes to determine the effects of weight loss on spillover and to investigate potential associations between spillover and body fat depots.

RESEARCH DESIGN AND METHODS

Subjects. Written, informed consent was obtained from 13 overweight and obese volunteers with type 2 diabetes and dyslipidemia who were studied according to a protocol approved by the Mayo Institutional Review Board. Enrollment was open to subjects aged between 35 and 60 years who were not receiving insulin therapy or glucagon-like peptide 1 analogs and had suboptimal glycemic control (HbA_{1c} 7–12%). Participants were required to have elevated triglycerides (150–400 mg/dL) and a BMI between 25 and 40 kg/m². All had a history and physical examination together with screening laboratory testing before participation.

Protocol. At study entry, participants underwent a 7-point oral glucose tolerance test (OGTT) to determine insulin sensitivity (12). Body composition, including total, trunk, and leg fat mass, was determined by dual energy X-ray absorptiometry (13), and single-slice computed tomography was used to measure visceral and subcutaneous abdominal fat (14).

At baseline, participants were admitted to the Clinical Research Unit for an acute study to measure spillover. The study was preceded by a 5-day isoenergetic controlled diet (15% protein, 35% fat, and 50% carbohydrate) prepared by the Clinical Research Unit metabolic kitchen. On the morning of the study, an infusion catheter was placed in a forearm vein and a second catheter was placed in a contralateral hand vein for sampling of arterialized venous blood (15). At 0800 h (0 min), participants drank a priming dose of a liquid meal, followed by an aliquot every 15 min until 390 min, as previously described (16,17). The liquid meal was made from Ensure Plus to which canola oil was added to achieve the macronutrient distribution described above. The feeding regimen provided ~6% of calculated daily basal energy requirements per hour as estimated by the Harris-Benedict equation.

After 270 min of continuous feeding, intravenous infusions of [U-¹³C]oleate (~0.5 nmol · kg⁻¹ · min⁻¹) and a lipid emulsion labeled with [9,10-³H(N)oleyl] triolein (18) (~0.4 μCi · min⁻¹) were started and continued to the end of the study (390 min). Blood samples were taken at 330, 345, 360, 375, and 390 min for concentrations of FFA, triglycerides, glucose, insulin, [U-¹³C]oleate enrichment, and [³H]oleate-specific activity.

Participants then participated in a multidisciplinary 5-month weight loss intervention that included group cognitive behavioral therapy, regular nutritional counseling, use of an online food journal, and a physical activity monitor. They were encouraged to maintain a daily energy deficit to facilitate weight loss. Participants met with a study physician every 2 weeks to be weighed, download their activity monitor data, and troubleshoot if weight loss goals were not met. At 5 months, the study was repeated in its entirety, including the OGTT and body composition measurements.

Analyses. Blood samples were collected in chilled 5-mL EDTA tubes containing 2.0 mg paraoxon to inhibit LPL (19) and kept on ice until centrifugation at 4°C. Chylomicrons were isolated using a triple-spin ultracentrifugation method (18). Plasma FFA concentration and specific activity were determined by high-performance liquid chromatography (20), using [²H₃₁]palmitate as an internal standard (21). The same method was used to determine the meal's fatty acid content. Plasma triglyceride concentrations were measured using a commercial enzymatic method (COBAS Integra Autoanalyzer). [U-¹³C]oleate atoms percent enrichment (APE) was determined by liquid chromatography-mass spectrometry (22).

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Calculations. Mean values from the 300- to 390-min sampling interval were used to calculate kinetic data. Rate of appearance (R_a) of oleate was determined using Steele's equation for steady-state conditions, as previously described (20,21). FFA clearance was calculated as the FFA tracer infusion rate divided by plasma tracer concentration (23); this calculation assumes that at steady state, the rate of disappearance of the tracer equals the infusion rate. The R_a of [^3H]oleate was calculated from the following formula:

$$R_a [^3\text{H}]oleate = \frac{[U-^{13}\text{C}]oleate \text{ infusion rate}}{[U-^{13}\text{C}] \text{ enrichment}/[^3\text{H}] \text{ specific activity}}$$

Fractional spillover (%) is then derived from the formula:

$$\text{Spillover} = \frac{R_a [^3\text{H}]oleate}{R_d [^3\text{H}]triolein} \times 100,$$

where the rate of disappearance (R_d) of [^3H]triolein equals the infusion rate of the triglyceride tracer (7).

The fractional contribution of meal fat to total oleate R_a was calculated from the formula:

$$\% \text{ oleate } R_a \text{ from meal} = \frac{\text{spillover} \times \text{oleate ingestion rate}}{\text{oleate } R_a}$$

Statistical methods. Results are presented as mean \pm SEM. Data from the two study days were compared using paired t tests to determine significance ($\alpha < 0.05$). Correlations were determined by regression analysis. Trunk fat-to-leg fat ratio (analogous to waist-to-hip ratio and hereafter referred to as trunk-to-leg ratio) was calculated for each subject.

RESULTS

Clinical characteristics of the participants at baseline and at 5 months are summarized in Table 1. Sulfonylurea therapy was discontinued in four of five participants during the weight loss intervention, whereas all remained on metformin. There was a marked decrease in weight, averaging $13.9 \pm 2.0\%$. Significant improvements were observed in hemoglobin A_{1c}, insulin sensitivity index, and fasting triglyceride levels. Changes in body composition are reported in Table 2. There was a small but significant decrease in lean body mass at 5 months. Highly significant decreases in total, trunk, leg, visceral, and abdominal subcutaneous fat were observed. The trunk-to-leg ratio also decreased significantly. Fractional loss of fat was greater from the trunk depot than from the leg ($30 \pm 5\%$ vs. $24 \pm 3\%$, $P < 0.01$).

Figure 1 shows plasma triglyceride (*upper panel*) and total FFA (*lower panel*) concentrations during continuous feeding. Triglyceride and FFA concentrations both decreased after weight loss (366 ± 35 to 232 ± 30 mg/dL and 186 ± 17 to 115 ± 10 $\mu\text{mol/L}$, respectively; both $P < 0.001$). There were also significant decreases in plasma glucose and insulin levels during continuous feeding (206 ± 11 to 155 ± 12 mg/dL and 51 ± 4 to 33 ± 4 $\mu\text{U/mL}$, respectively; both $P < 0.005$; Fig. 2).

TABLE 1
Subject characteristics at baseline and at 5 months

	Baseline	5 months
Age (y)	52 ± 2	—
Sex (n)	—	—
Male	9	
Female	4	
Weight (kg)	104.8 ± 4.3	$90.5 \pm 4.7^{**}$
BMI (kg/m^2)	33.8 ± 1.0	$29.2 \pm 1.1^{**}$
HbA _{1c} (%)	7.9 ± 0.3	$6.3 \pm 0.2^{**}$
Triglycerides (mg/dL)	230 ± 21	$134 \pm 20^{**}$
Insulin sensitivity index	2.0 ± 0.3	$5.7 \pm 0.8^{**}$

** $P < 0.001$ vs. baseline.

TABLE 2
Body composition at baseline and at 5 months

	Baseline Mean \pm SEM	5 months Mean \pm SEM
Lean body mass (kg)	60.7 ± 3.2	$57.3 \pm 3.1^{**}$
TBF (kg)	40.1 ± 2.1	$29.2 \pm 2.6^{**}$
Trunk fat (kg)	25.4 ± 1.2	$17.8 \pm 1.6^{**}$
Leg fat (kg)	10.0 ± 0.9	$7.7 \pm 0.9^{**}$
Trunk fat (% TBF)	64 ± 1	$61 \pm 1^\dagger$
Leg fat (% TBF)	24 ± 1	$26 \pm 1^\dagger$
Trunk-to-leg ratio	2.7 ± 0.2	$2.5 \pm 0.2^\dagger$
Abdominal SQ fat (cm^2)	264 ± 31	$187 \pm 25^{**}$
Visceral fat (cm^2)	328 ± 18	$208 \pm 22^{**}$

SQ, subcutaneous; TBF, total body fat. ** $P < 0.001$ vs. baseline; $^\dagger P < 0.05$ vs. baseline.

Table 3 reports plasma [$U-^{13}\text{C}$]oleate APE and [^3H]oleate-specific activities during the 330- to 390-min sampling interval. These data show that steady-state conditions were achieved with respect to both tracers. There was

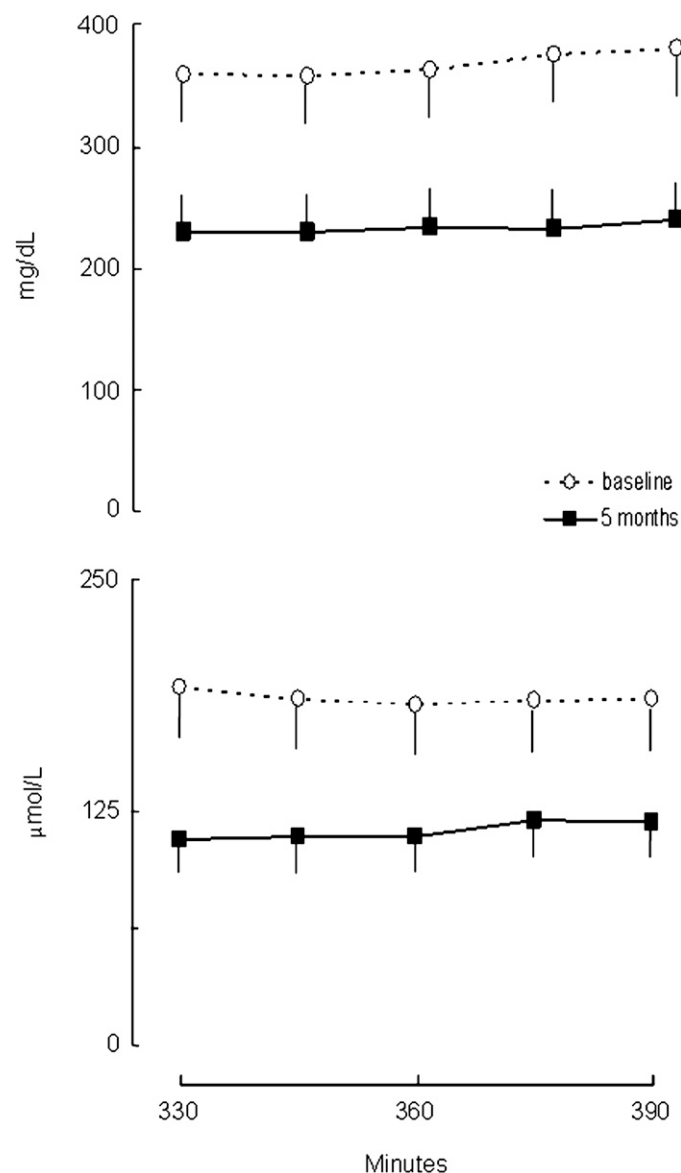


FIG. 1. Plasma triglyceride (*upper panel*) and total FFA (*lower panel*) concentrations during continuous feeding, before and after weight loss.

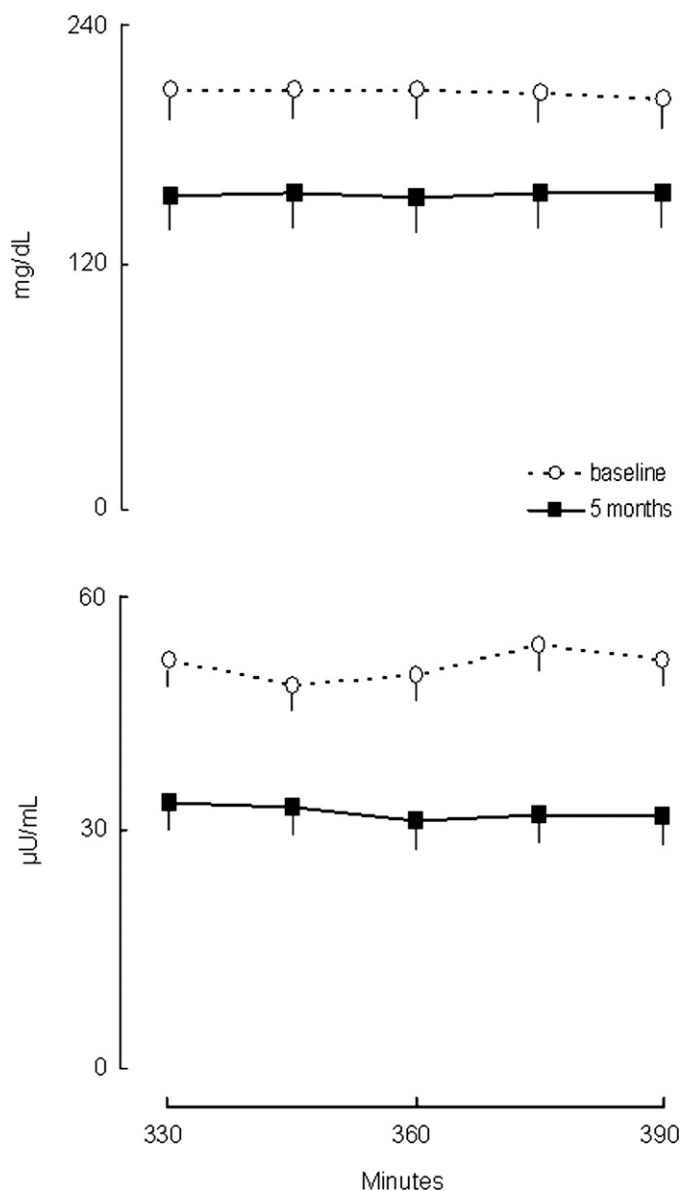


FIG. 2. Plasma glucose (upper panel) and insulin (lower panel) concentrations during continuous feeding, before and after weight loss.

a significant decrease in oleate R_a and a borderline significant increase in oleate clearance after weight loss, whereas there was no change in fractional spillover (Table 4). The contribution of spillover to total oleate R_a increased after weight loss from $63 \pm 6\%$ to $83 \pm 5\%$ ($P < 0.02$).

TABLE 3
Plasma [U- 13 C]oleate APE and [3 H]oleate SA during continuous feeding at baseline and at 5 months

	Time (min)				
	330	345	360	375	390
[U- 13 C]oleate APE (%)					
Baseline	0.079 \pm 0.007	0.080 \pm 0.006	0.080 \pm 0.007	0.074 \pm 0.005	0.077 \pm 0.006
5 months	0.098 \pm 0.007	0.091 \pm 0.008	0.090 \pm 0.007	0.090 \pm 0.007	0.089 \pm 0.007
[3 H]oleate-specific activity (dpm/nmol)					
Baseline	2.96 \pm 0.34	3.30 \pm 0.35	3.24 \pm 0.41	3.31 \pm 0.41	3.17 \pm 0.39
5 months	5.33 \pm 0.39	5.12 \pm 0.38	5.10 \pm 0.40	5.02 \pm 0.39	5.18 \pm 0.47

The relationships between body fat depots and spillover at baseline are shown in Fig. 3. There was a strong negative correlation between leg fat and spillover ($r = -0.79$, $P = 0.001$). There was a lesser but still significant negative correlation between trunk fat and spillover ($r = -0.66$, $P = 0.015$), and a positive correlation between trunk-to-leg ratio and spillover ($r = 0.56$, $P = 0.047$). Total body fat mass correlated negatively with spillover (Supplementary Fig. 1), but there was no relationship between visceral fat or abdominal subcutaneous fat and spillover (data not shown). Multivariate analysis revealed that leg fat was a stronger negative predictor of spillover than truncal fat ($\beta = -0.8$ vs. -0.3). The significant correlations between body fat depots and spillover shown in Fig. 3 disappeared after weight loss (Supplementary Fig. 2).

A significant positive correlation was found between Δ spillover and leg fat, both at baseline and at 5 months (Fig. 4). There were no significant correlations among trunk fat, the trunk-to-leg ratio, or visceral fat, and Δ spillover, before or after weight loss. No significant correlations were found between Δ leg fat or Δ trunk fat and Δ spillover. There was a borderline positive correlation between Δ visceral fat and Δ spillover ($P = 0.07$). The proportion of total body fat loss that was due to leg fat loss was calculated, dividing the subjects arbitrarily into a group with baseline leg fat <10 kg (6.9 ± 0.4 kg, $n = 5$) and a group with baseline leg fat >10 kg (11.9 ± 0.9 kg, $n = 8$). The individuals with higher baseline leg fat lost more leg fat as a percentage of total fat loss than those with low baseline leg fat ($24 \pm 2\%$ vs. $17 \pm 1\%$, $P < 0.005$). There was a borderline significant correlation between the percentage of fat loss represented by leg fat and Δ spillover ($R = 0.54$, $P = 0.057$; data not shown).

DISCUSSION

In our study, a 5-month lifestyle intervention in obese individuals with type 2 diabetes led to an average weight loss of almost 14%. The change in body weight was accompanied by improvements in glycemic control, insulin sensitivity, triglyceride concentrations, and meal-related suppression of adipose tissue lipolysis. However, fractional spillover, a reflection of the efficiency of dietary fat storage, did not change. At baseline, there was a significant negative correlation between spillover and body fat mass, especially leg fat. Spillover correlated positively with the trunk-to-leg ratio. These correlations disappeared after weight loss, but baseline leg fat was a significant positive predictor of the change in spillover occurring during weight loss.

The continuous feeding paradigm used in our study was successful in achieving steady-state concentrations of glucose, insulin, FFA, and triglycerides, as previously

TABLE 4

Oleate R_a , clearance, and spillover during continuous feeding at baseline and at 5 months

	Baseline	5 months
R_a ($\mu\text{mol}/\text{min}$)	64.7 ± 8.9	$49.7 \pm 6.7^*$
Clearance (mL/min)	948 ± 68	$1,032 \pm 81^\dagger$
Fractional spillover (%)	28.2 ± 2.9	31.4 ± 3.7

* $P < 0.01$ vs. baseline; $^\dagger P = 0.07$ vs. baseline.

described (16,17). We also achieved steady-state conditions with respect to $[U-^{13}\text{C}]$ oleate enrichment and $[^3\text{H}]$ oleate-specific activity, fulfilling the requirements for calculation of precursor-product relationships (24). The improvement in glucose concentrations after weight loss, occurring despite lower insulin concentrations in our subjects, was due to improved whole-body insulin sensitivity, as indicated by the higher insulin sensitivity index. These changes reflect the effects of both negative energy balance and weight loss (25). The improvement in insulin sensitivity was also apparent at the level of adipose tissue lipolysis; there was a decrease in FFA concentration and R_a after weight loss despite a decrease in plasma insulin concentrations, consistent with previous studies (26). Triglyceride concentrations also decreased, likely because of a decrease in VLDL production (27).

Fractional spillover represents the portion of LPL-generated fatty acids that are released into the plasma FFA pool rather than transported locally into tissues. Spillover can be determined by labeling endogenous chylomicrons (8,9,28) or with the use of a labeled lipid emulsion (7), as was done in the current study. The labeled lipid emulsion has proven to be an excellent surrogate for the study of chylomicron triglyceride metabolism at the level of LPL action. We have previously validated the method in humans (18,29) and have shown that the tissue distribution and spillover of the lipid emulsion (30) is similar to results obtained when chylomicrons are labeled and infused in animals (31).

Despite marked weight loss, there was no change in spillover in our subjects. Most systemic spillover in humans occurs in the splanchnic bed, at least in people who are overweight and obese (32). Spillover in visceral adipose tissue, in turn, correlates strongly with intracellular lipolysis (30). We recently reported that insulin infusion suppressed plasma FFA by $\sim 40\%$ during meal absorption but did not reduce spillover in overweight and obese subjects (16). Assuming that suppression of lipolysis in visceral fat is required to decrease the high rates of spillover in that tissue, and considering 1) the large contribution of the splanchnic bed to systemic spillover (32) and 2) the well-known resistance to the antilipolytic effect of insulin in that tissue (33), the failure of weight loss to reduce systemic spillover may reflect dysregulation of visceral lipolysis that persists after weight loss. The amount of visceral fat in our diabetic subjects after weight loss remained markedly higher than that in healthy lean volunteers and nearly fourfold greater than we have reported in obese individuals after successful bariatric surgery (34). We have recently found that nicotinic acid infusion, which caused a reduction in plasma FFA similar to that induced by insulin infusion (16), resulted in a significant decrease in systemic spillover in lean and obese volunteers (17). Considering that visceral fat contains

nicotinic acid receptors (35), these observations are consistent with the idea that visceral fat is a major site of spillover and that suppression of intracellular lipolysis is required to reduce spillover in that tissue.

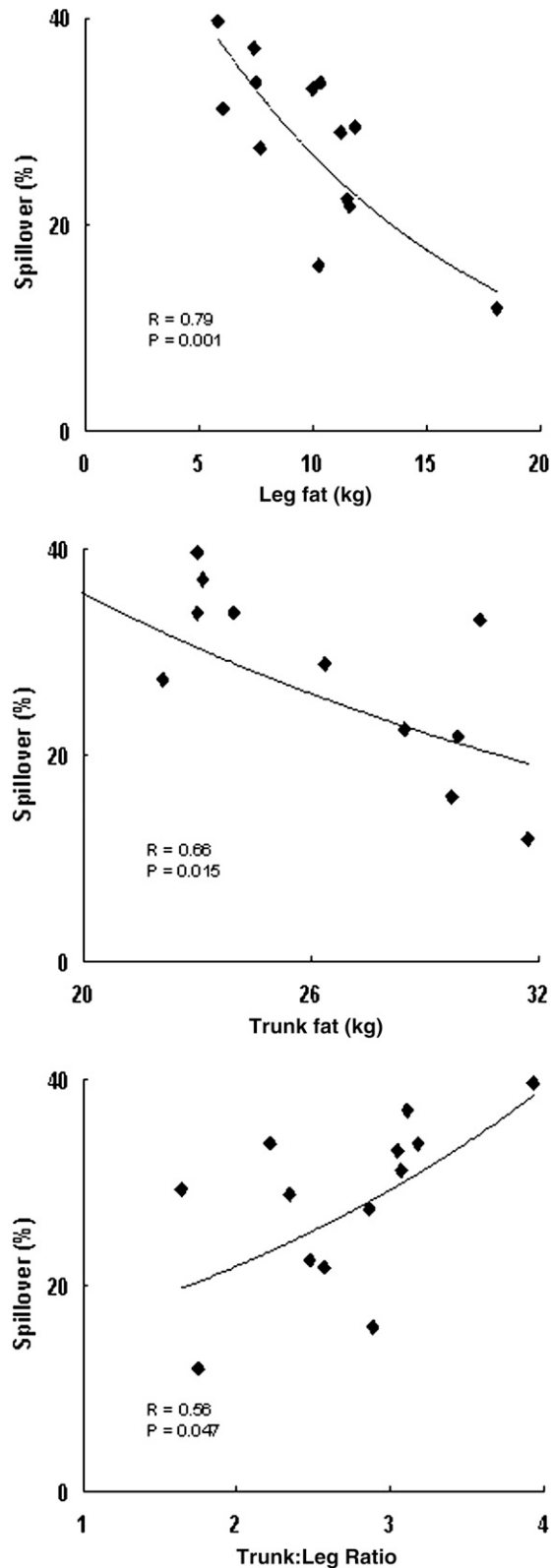


FIG. 3. Relationship among systemic fractional spillover (y axis) and leg fat (upper panel), trunk fat (middle panel), and trunk-to-leg ratio (lower panel) at baseline.

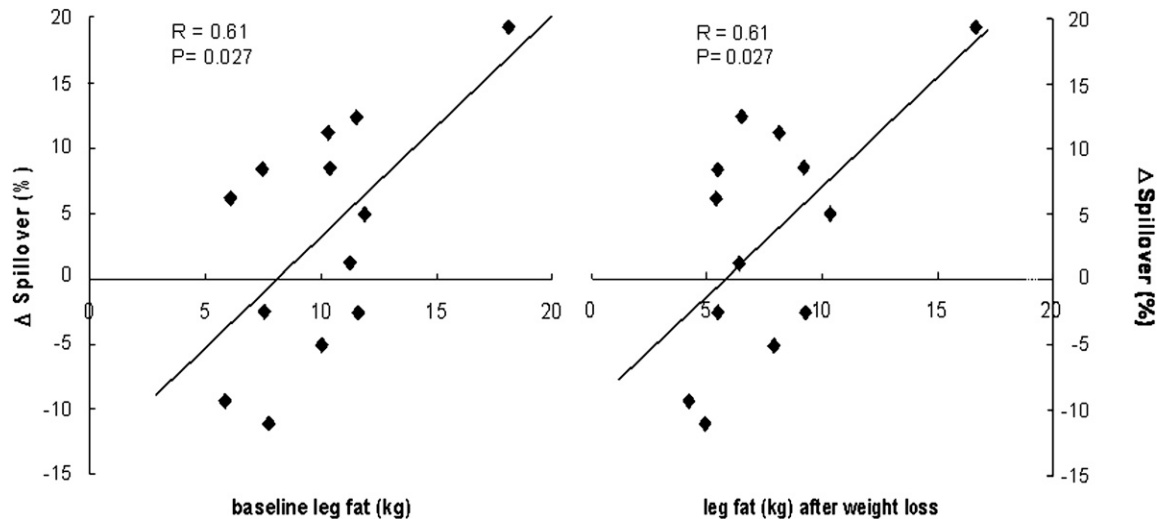


FIG. 4. The relationship between Δ spillover and leg fat at baseline (*left*) and at 5 months (*right*).

Abnormalities in lipid metabolism tend to be associated with increases in upper body but not lower body fat (36). Multivariate analysis revealed that leg fat was a stronger negative predictor of spillover than trunk fat in our study, although this finding should be interpreted cautiously in view of the small number of participants. The strong negative correlation between leg fat and systemic fractional spillover in our subjects indicates that systemic fat storage is more efficient in individuals with larger amounts of leg fat. This suggests that spillover in leg fat is lower than in upper body fat. Votruba et al. (11) found that the amount of dietary fat taken up in leg fat per gram of tissue increases as a function of leg fat mass in nondiabetic individuals, consistent with the idea that accumulation of lower body fat is associated with a shift away from fat storage in the upper body, where fractional spillover is high (32), and toward fat storage in the lower body. If this is the case, systemic fractional spillover could be relatively low in an individual with high splanchnic fractional spillover, provided that the contribution of the splanchnic bed to systemic uptake of meal fat was low. The explanation for the disappearance of the correlations between fat depots and spillover after weight loss is not apparent. It is noteworthy that baseline fractional spillover in our diabetic subjects is similar to that in nondiabetic people (16,17). This is consistent with a complex role for the relative size of body fat depots in determining the overall efficiency of meal fat uptake in adipose tissue. We analyzed data from a number of studies in aggregate ($n = 64$) and found a positive correlation between systemic fractional spillover and plasma FFA concentration ($P < 0.0001$; unpublished results).

The trunk-to-leg ratio in this group of people with type 2 diabetes is nearly double that of obese nondiabetic individuals (unpublished results). Others have found that people with type 2 diabetes have higher waist-to-hip ratios (37) and more visceral fat (38) than nondiabetic control subjects at the same BMI. Whether there is an association between leg fat and spillover in obese nondiabetic people or in lean individuals is not known.

We found that baseline leg fat was a significant predictor of changes in spillover with weight loss; that is, spillover tended to increase in individuals with high amounts of leg fat and tended to decrease in those with small leg fat

depots. This is somewhat difficult to reconcile with the association between a large leg fat depot and low rates of spillover before weight loss. We found that the relative contribution of leg fat to total fat loss was lower in individuals with a small leg fat depot at baseline than in people who had larger amounts of leg fat and that there was a borderline correlation between the percentage of fat loss represented by leg fat and Δ spillover. This may indicate that the leg assumes a greater role in meal fat disposal as the amount of leg fat in relation to trunk fat increases. If fat storage is inherently more efficient (i.e., lower fractional spillover) in the leg than in the trunk, then systemic spillover would decrease as the contribution of the leg to whole-body meal fat disposal increased, even if fractional spillover in visceral and abdominal subcutaneous fat did not change. It is possible that weight loss produces a shift in the distribution of fat uptake such that a reduction in the size of the leg fat depot reduces lower body fat uptake. The reciprocal appears to hold true, at least in nondiabetic men: with short-term supervised overfeeding, weight gain was associated with an increase in lower body fat uptake (39). In a recent study where healthy men were overfed, an increase in the concentration of dietary fatty acids in FFA was positively correlated with Δ visceral fat (40). The investigators did not use an FFA tracer in that study, and the relationship between dietary fatty acids in FFA and leg fat was not reported. Nonetheless, the findings are consistent with the observation in the current study of a borderline correlation between Δ spillover and Δ visceral fat resulting from weight loss and are also consistent with a role for visceral fat in systemic spillover, as we reported previously (32).

During positive energy balance, accumulation of additional fat in adipose tissue requires transport of fatty acids from the circulation into that tissue. High rates of spillover, such as appear to prevail in visceral fat, could represent a mechanism for limiting gain of body fat. The concept that human adipose tissue might also have limited storage capacity is not new (41), but evidence for this is lacking. An overfeeding study (42) would be an ideal way to address the question of limited fat storage but would be difficult to justify on ethical grounds in people with diabetes. Tchoukalova et al. (43) found that overfeeding in lean individuals resulted in increased abdominal fat cell size but not

number but that fat cell number but not size increased in leg fat. This suggests that lower body fat has greater plasticity than upper body fat.

It has been estimated that anywhere from 20–25% (7) to 50% (10) of circulating FFAs derive from spillover, a contribution that would be expected to increase with increased intake of dietary fat. Although the mechanisms that regulate spillover are poorly understood (44), the current study suggests that body fat distribution and/or the size of body fat depots is a determining factor. Increased FFA availability plays a role in ectopic fat accumulation (45), insulin resistance (2), hypertension (4), and dyslipidemia (3). It may also exert effects on energy expenditure via sympathetic nervous system (5,46,47), uncoupling protein-3 (48), or peroxisome proliferator-activated receptor- δ (49) activation.

In summary, our study demonstrates that weight loss in people with type 2 diabetes improves insulin sensitivity but does not change mean systemic fractional FFA spillover. At baseline, the size of the leg fat depot was associated negatively with spillover and positively with Δ spillover resulting from weight loss. Additional studies of regional spillover in adipose tissue are needed to clarify these results.

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No potential conflicts of interest relevant to this article were reported.

J.P.A. was involved in every aspect of the project, conducted the studies, analyzed data, and cowrote the manuscript. E.S. assisted in the conduct of the studies and edited the manuscript. L.A.H. and K.G. participated in study design, were centrally involved in the weight loss intervention, and edited the manuscript. D.T.V. and A.S. helped with study planning, execution, and data analysis, edited the manuscript, and contributed to discussion. B.A.I. participated in data analysis and edited the manuscript. R.H.N. participated in study design, assisted with the conduct of the study, analyzed data, and edited the manuscript. J.M.M. was involved in study design and data analysis and cowrote the manuscript. J.M.M. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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