Metabolic Changes Following a One-year Diet and Exercise Intervention in Patients with Type 2 Diabetes

Running title: Weight loss in type 2 diabetes

Jeanine B. Albu¹, Leonie K. Heilbronn², David E. Kelley³, Steven R. Smith⁴, Koichiro Azuma³, Evan Berk¹, F. Xavier Pi-Sunyer¹, Eric Ravussin⁴ and the Look AHEAD Adipose Research Group

¹ Department of Medicine, St. Luke’s –Roosevelt Hospital Center, Columbia University, New York, NY, 10025; ² Garvan Institute, Sydney, Australia; ³ Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA, 15213; and ⁴ Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA, 70808.

Address correspondence:
Jeanine Albu MD
Email: jbal@columbia.edu

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**Objective:** To characterize the relationships between long-term improvements in peripheral insulin sensitivity (glucose disposal rate=GDR), fasting glucose (FG) and free fatty acids (FFA-s), and concomitant changes in weight and adipose tissue (AT) mass and distribution induced by lifestyle-intervention in obese individuals with type 2 diabetes.

**Research Design and Methods:** We measured GDR, FG and FFA-s during a euglycemic clamp, and AT mass/distribution, organ fat and adipocyte size by DXA, CT scan and AT biopsy in 26 men and 32 women in the Look-AHEAD trial before and after 1 year of diet and exercise aimed at weight loss.

**Results:** Weight and FG decreased significantly (P<0.0001) and significantly more in men than in women (-12% vs. -8% and -16% vs. -7%, P<0.05) while FFA-s during hyperinsulinemia decreased and GDR increased significantly (P<0.00001), similarly in both sexes (-53% vs. -41% and +63% vs. +43%, P=NS). Men achieved a more favorable fat distribution by losing more from upper compared to lower and from deeper compared to superficial AT depots (P<0.01). Decreases in weight and AT mass predicted improvements in GDR, but not in FG or fasting FFA-s; however decreases in FFA-s during hyperinsulinemia significantly determined GDR improvements. Hepatic fat was the only regional fat measure whose change contributed independently to changes in metabolic variables.

**Conclusions:** Patients with type 2 diabetes undergoing a one-year lifestyle-intervention had significant improvements in GDR, FG, FFA-s and AT distribution. However, changes in overall weight (AT mass) and hepatic fat were the most important determinants of metabolic improvements.
Most obese patients with type 2 diabetes have an unfavorable adipose tissue (AT) distribution compared to similarly obese men and women without type 2 diabetes (1-2). We have shown they manifest proportionally less “metabolically protective” AT (gluteo-femoral) and more “metabolically adverse” fat depots such as abdominal AT or hepatic fat (2). Such patterns correlate with increased fasting glucose (FG) and decreased insulin sensitivity (3-5) in cross-sectional studies. From the perspective of intervention, in type 2 diabetes, both caloric restriction and relatively modest weight reduction result in FG (6-10) as well as hepatic (7, 9, 11-13) and peripheral insulin sensitivity (8-10, 12-13) improvements. However, not all studies reporting significant weight loss or favorable fat distribution changes have observed a concomitant improvement in peripheral insulin sensitivity (11, 14). Furthermore, there is a surprising paucity of data regarding the relationship between sustained lifestyle intervention-induced changes in fat mass and regional AT distribution and parallel metabolic improvements. In several weight loss studies conducted up to 6 months, in type 2 diabetes, favorable changes in fat distribution and organ fat did not correlate with improved peripheral insulin sensitivity independent of the changes in body weight (11-14). Even fewer studies reported on longer-term (of up to one year) effects of weight loss on fat distribution and metabolic variables in type 2 diabetes (10, 15-16). In one study, while parallel one-year improvements were observed both in the fat distribution (measured by the waist-to-hip ratio, WHR) and in FG and fasting insulin (15), the metabolic improvements did not relate to the WHR change but rather to the overall amount of weight loss (15). One interpretation is that loss of AT, regardless of depot, is the predominant factor related to the metabolic improvement in obese patients with type 2 diabetes, challenging the tenet, built mostly from cross-sectional studies, that AT distribution is a crucial and interactive determinant of the improvement. Yet it is not clear from these studies whether the variability of the weight loss, the sometime limited number of subjects or incomplete AT distribution measurements permitted robust evaluation of the role of specific fat depot in the improvements in metabolic control. In addition, changes in other AT characteristics, such as fat cell sizes or circulating FFA-s, have not been accounted for in previous studies. Larger subcutaneous abdominal fat cells predict insulin resistance and the development of type 2 diabetes (17-19) while increased circulating FFA-s play an important role in the etiology of insulin resistance and hyperglycemia in type 2 diabetes (12, 20-21). Whether regional fat loss contributes to improvements in FFA-s during weight loss in type 2 diabetes has not been previously reported.

The current study was therefore undertaken to examine the importance of changes in AT distribution and other closely related characteristics as determinants of the improvements in “metabolic fitness” in response to weight loss in type 2 diabetes. We tested the hypothesis that simple measures of weight loss rather than various relative changes and permutations of AT distribution are the predominant determinant of metabolic improvement induced by a one-year lifestyle intervention in obese patients with type 2 diabetes. Multiple aspects of AT mass and its distribution were assessed, including upper and lower AT mass (using DXA), AT sub-divisions in the abdomen and lower extremity (using CT imaging) and estimations of fat content in liver and muscle (using CT imaging). This was performed along with an AT biopsy in order to measure mean fat cell size within the abdominal
subcutaneous depot, at both the baseline and following the one-year of lifestyle intervention.

**RESEARCH DESIGN and METHODS**

This was an ancillary study of the Look AHEAD (Action For Health in Diabetes) trial at three of the sixteen participating sites (Pennington Biomedical Research Center, Baton Rouge, LA; the University of Pittsburgh, Pittsburgh, PA; and St. Luke’s – Roosevelt Hospital Center, New York, NY). The primary goal of the Look AHEAD trial is to investigate the effects of a lifestyle intervention of weight loss and physical activity (ILI) versus that of diabetes support and education on cardio-vascular morbidity and mortality (22-23). One-year results from the Look AHEAD trial and other results of this ancillary study have been previously published (24-28).

**Research Volunteers:** Inclusion and exclusion criteria for Look AHEAD, which include a confirmed diagnosis of type 2 diabetes, have been previously described (22-23). This ancillary study included only participants randomized to the ILI arm of the study (24-25). In order to simplify the potential impact of changes in anti-diabetic medications during intervention, those with fasting plasma glucose (FG) ≥ 180 mg/dl and those on insulin or thiazolidinediones (TZD) treatment were excluded from the sub-study. Fifty-eight volunteers with type 2 diabetes (43 non-Hispanic whites, 12 African Americans and 3 Hispanics) were studied at baseline (pre-intervention) and after one-year of ILI. Twenty-six men (age, mean ± SD, 61.6 ± 1.5) and 32 women (age, 58.9±1.3) completed baseline and one year measurements. The sex distribution of volunteers at the three sites was 12F/13M at Pittsburgh, 13F/5M at St Luke’s-Roosevelt, and 7F/8M at Pennington. At baseline, 6 women were pre or perimenopausal and 26 were post-menopausal (8 on hormone replacement therapy, HRT). All participants signed informed consent, the project was approved by each institution’s Institutional Review Board and by the Look-AHEAD Steering Committee.

**Lifestyle Intervention and Study Protocol:**

As described elsewhere (22-25), ILI was designed to achieve weight loss through decreased caloric intake (~ 500 Kcal/d) and increased physical activity (≥ 175 min/wk) with an expected 1-year weight loss of ≥ 7% of initial value. Before and after one-year of ILI our participants were admitted to clinical research facilities on the afternoon preceding the metabolic studies and underwent DXA and CT imaging. After a standardized dinner (50% carbohydrate, 30% fat, and 20% protein), participants were fasted overnight. The next morning, a metabolic weight and a percutaneous adipose tissue biopsy were obtained; one hour later a hyperinsulinemic euglycemic clamp was performed.

Addition or discontinuation of anti-hyperglycemic medications at the one-year testing compared to baseline was noted. Medications added were TZD and Metformin (one man each). Medications discontinued were alpha-glucosidase inhibitors (one woman), meglitinides/repaglinides (2 men and 2 women) and sulphonylureas (11 men, 3 women). Metformin was re-introduced for a week prior to the one-year testing at the same dose as before the study if the patients were on it at baseline and were discontinued during the intervention (6 men, 2 women).

**Body Composition:** Fat-mass (FM) and fat-free mass (FFM, which included all non-fat tissue i.e. lean body mass and bone mineral content) were measured using dual-energy X-ray absorptiometry (DXA, Hologic QDR 4500A, Waltham, MA) according to the Manual of Procedures of the Look AHEAD trial. All DXA scans were analyzed using QDR for Windows V11.1 software. FM and FFM, gluteo-femoral fat (GF-Fat) as well as trunk and arms fat mass (upper body fat, UB-Fat) were measured by the standard default
analysis, in which the commercial computer based algorithm separates the mass of GF-Fat and UB-Fat by two oblique lines that pass through the femoral necks (2). The coefficients of variation (CV) for repeated measures (n=38; unpublished data) of FFM, FM and percentage of body fat were 0.6%, 1.1% and 1.1%, respectively. Three cross-sectional CT scans, 1 cm in width, centered respectively on the T12-L1 and the L4-L5 disc space and at the mid-thigh, were obtained to assess hepatic fat as well as abdominal and thigh AT composition. All CT images were analyzed at the University of Pittsburgh using image analysis software (SliceOmatic, Tomovision, Montreal, Canada). To assess hepatic fat, CT liver and spleen attenuations (Hounsfield units) were determined and to assess AT composition the abdominal and thigh areas for bone, AT and skeletal muscle were measured, as previously described (29). To determine visceral AT (VAT) and abdominal subcutaneous AT (SAT) areas a separation line was drawn manually on the abdominal CT images along abdominal wall musculature in continuity with the fascia of the paraspinal muscles. Abdominal SAT was further divided into superficial and deep SAT by manually tracing the circumferential superficial fascia, as previously described (30). On thigh CT images, fascia lata was used to subdivide mid-thigh AT into SAT and sub-fascial AT (3).

**Abdominal Subcutaneous AT Biopsy and Adipocyte Size:** A percutaneous biopsy of superficial abdominal SAT (approximately 500 mg) was performed approximately 10 cm lateral to the umbilicus using a Bergstrom needle with suction. Adipocyte size and number were determined at the Pennington Biomedical Research Center (PBRC) using a Coulter Counter (Multisizer-3 Beckman Coulter, Fullerton, CA, USA) as previously described (26, 28). Cell size is presented as the geometric mean.

**Hyperinsulinemic Euglycemic Clamp:** A primed-continuous infusion of insulin (80 mU/m²/min) was used for at least 3 hours, with the stipulation that insulin be infused for at least one hour after reaching a plasma glucose concentration of 100 mg/dl, as previously described (27). The mean rate of exogenous glucose infusion during steady-state insulin infusion (last 30 minutes), glucose disposal rate, GDR, was used to assess peripheral insulin sensitivity (31). Oxygen consumption and CO₂ production were measured using metabolic carts (Sensor Medics Corporation, Anaheim CA) over the 40 minutes just preceding and during the last 40 minutes of the insulin infusion; fuel oxidation (carbohydrate and fat) was calculated for the last 30 minutes of each period (32). Glucose storage was the difference between total glucose disposal rate and glucose oxidation. Glucose utilization rates were expressed per Kg of FFM.

**Blood Analyses:** Blood samples were immediately centrifuged, aliquoted and frozen at -70°C. Plasma glucose was analyzed using a glucose oxygen electrode (Synchron CX7 Delta Systems; Beckman, Brea, CA). Plasma insulin was measured by chemiluminescent immunoassays on the Immulite 2000 analyzer (Diagnostic Product Corp., Los Angeles, CA). The intra and inter assay CV for insulin (at 50 µU/mL) was 1.75% and 3.6%, respectively. Plasma free fatty acid concentrations (FFA-s) were measured on a Beckman Synchron CX5 analyzer using a WAKO NEFA C kit (Denver, CO). All samples were analyzed in the Clinical Chemistry Laboratory at the Pennington Biomedical Research Center.

**Statistical Analyses:** Data was expressed and shown as mean ± SEM unless otherwise noted. For each variable, data was presented for completed, valid measurements both at baseline and after one year. Data was missing for men (out of 26) for liver, spleen and muscle attenuations (one man each) and for AT cell size (two men) and for women (out of
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Variables with significant deviation from normal distribution were log-transformed before analyses (insulin, FFA-s and their changes). ANOVA with repeated measures was used to assess significant changes over the one year of the intervention; interactions by gender were tested for significance. General linear models were built according to a priori hypotheses. Specifically we tested whether: a) changes in any of the regional fat measures were predictors of the one-year changes in metabolic variables independent of the change in overall weight or FM; b) the change in clamp FFA-s was a predictor of the changes in GDR or FG, independent of changes in weight, fat mass or any of the regional fat measures; and c) the change in GDR was a predictor of the change in FG, independent of changes in weight, fat mass or any of the regional fat measures. A P<0.05 was considered significant. Statistica v 6.0 (Statsoft Inc., Tulsa, OK) was used for analyses.

RESULTS

One-year changes in weight: Weight decreased significantly (P<0.00001) in both men and women (Table 1) but there was a wide range of weight change (-26.5 Kg to +3.5 Kg in men and -27.3 Kg to +0.9 Kg in women). Men lost a higher percentage of their initial weight than women (-12.1±1.2% vs. -8.1±1.1%, P<0.05). Higher one-year weight loss was predicted by higher weight at baseline in both sexes (beta -0.41, P<0.01) but was not related to baseline AT mass or distribution, nor was it related to fat cell size or metabolic variables.

One-year changes in AT mass and distribution, organ fat infiltration and mean abdominal subcutaneous fat cell size: FM and FFM decreased significantly by 27.7±2.6% and 5.5±0.8% and by 14.0±2.1% and 3.8±0.7%, in men and women, respectively (Table 1). The change in FM ranged from -20.4 to +0.2 kg and from -19.5 to +0.8 Kg, while the change in FFM ranged from -9.9 to +3.3 Kg and from -7.8 to +2.8 Kg, in men and women respectively, resulting in a significant decrease in body fat percentage (Table 1). There were a number of significant changes in various regional AT depots (Table 1). Upper body fat, VAT and deep abdominal SAT decreased significantly (Table 1), and significantly more in men than in women, independent of baseline values (P<0.01). There were also significant decreases in superficial abdominal SAT, gluteo-femoral fat, and in the lower extremity of the thigh measured cross-sectionally for both the sub-fascial and the superficial AT depots, but without sex effect (Table 1).

Due to the differential greater loss from the upper compared to the lower body, the fat distribution as measured by DXA changed significantly (Figure 1A), in both sexes. In men, VAT decreased more than the superficial abdominal SAT; this resulted in a significant change within abdominal AT distribution (Figure 1B). There was a similar trend evident in women, although to a lesser extent than in men (Figure 1B). A similar pattern was noted in the lower extremity: in men, the sub-fascial thigh AT decreased more than the superficial thigh SAT, resulting in a significant change in the thigh AT distribution (Figure 1C); a similar trend was observed in women (Figure 1C).

The ratio of the liver/spleen attenuation (L/S ratio) increased indicating a significant decrease in hepatic fat (P<0.00001) (Table 1), in both men (-18±5%) and women (-18±4%). The thigh muscle attenuation, a surrogate for intramuscular fat, did not change significantly (P=0.36) (Table 1). The thigh muscle area decreased on average by 4.6%; this decrease was similar in magnitude to the average decrease in FFM (4.6%).
Mean size of abdominal subcutaneous fat cells decreased in both men and women (Table 1). Lipid content per unit of AT together with the mean fat cell size was used to estimate the number of fat cells per unit of AT (see Methods). The calculated number of fat cells per unit of abdominal SAT increased in both sexes (Table 1), despite an average decrease in the overall size of the depot by approximately 17%.

One-year changes in fasting glucose (FG), FFA-s and GDR: FG decreased significantly in both men and women (Table 2), and significantly more in men than in women (-16.2±2.8% vs. -6.8±3.5%, P<0.05). The clamped glucose levels were not different after than before the intervention (Table 2). Fasting insulin decreased significantly and equally in both sexes (Table 2) while insulin levels at steady state during the clamp were lower after intervention in women only (interaction term P=0.05). Fasting FFA-s were significantly decreased (Table 2) and were suppressed by insulin to a significantly greater extent (P<0.0001) after (by 98±0.5% and 97±0.5%) than before the weight loss (by 95±1% and 94±1%, in men and women, respectively). Therefore, FFA levels at steady state of the clamp (clamp FFA-s) were significantly decreased after weight loss (Table 2), similarly in men and women (-54.9±8.5% vs. -41.2±15.7%). GDR increased significantly (Table 2), similarly in men and women (+63.3±8.1% vs. +43.1±8.6%), with improvement of both glucose oxidation and storage (Table 2).

Determinants of one-year changes in metabolic variables (GDR, FG and clamp FFA-s): The one–year improvement in insulin sensitivity (ΔGDR or as a % of baseline value, %Δ GDR) did not relate to any baseline variables but was significantly related to the decrease in weight (%Δ weight, r= -0.65, P=0.000002, Figure 2A) and fat mass (%Δ FM, r= -0.71, P=0.00004). It was also significantly related to the decreases in all regional fat depots (r range -0.65 to -0.50, P <0.01 for all) and in mean abdominal fat cell size (r=-0.27, P<0.05) as well as to the increase in the relative proportion of the superficial abdominal SAT (r=0.34, P<0.01). However none of these relationships were independent of the changes in weight or fat mass. In multiple regression analyses, the best predictive model for Δ GDR included %Δ FM and %Δ clamp-FFA (overall R^2=0.48, P=0.00049) and for %Δ GDR included independent contributions from the %Δ weight, %Δ L/S ratio and %Δ clamp-FFA with (overall R^2 = 0.52, P=0.000027).

Similar analyses were performed for the one–year improvement in FG and decreases in clamp FFA-s. Neither one was related to the changes in weight or fat mass. Among regional fat measures, Δ FG was only significantly related to the Δ L/S ratio (r= -0.37, P=0.006) and %Δ FG to the %Δ VAT (r=0.31, P=0.03). While Δ FG was also related to the decrease in clamp FFA-s (P<0.05), in multiple regression analysis, the best predictive model for Δ FG included independent contributions from the Δ L/S ratio and Δ GDR (overall R^2=0.32, P=0.009) and for %Δ FG included independent contributions from the %Δ GDR (R^2 = 0.31, P=0.005, Figure 2B). The one-year decrease in clamp FFA was only related to Δ L/S ratio or %Δ L/S ratio (r= -0.33, P=0.014 or r= -0.39, P=0.01, respectively) (Figure 2C), independently of changes in weight or fat mass.

DISCUSSION
After one year of Look AHEAD intensive lifestyle intervention (ILI) (22), participants with type 2 diabetes had greatly improved levels of peripheral insulin sensitivity, FG and FFA-s in parallel to significant weight and fat loss, improvement in AT distribution and decrease in hepatic fat. The changes in the peripheral insulin sensitivity were best predicted by the overall change in weight and
fat mass; the only regional fat measure independently predicting metabolic improvements was the decrease in hepatic fat. Several studies have examined the effect of weight loss on AT distribution and organ fat infiltration in type 2 diabetes (11-14, 33). These studies varied in duration from a few weeks to up to 6 months and reported variable changes in AT distribution and organ fat depending on the measurements done and the nature of the intervention leading to the weight loss. Our study is unique in that we have studied the subjects after a one year intervention, have measured all aspects of AT distribution and organ fat infiltration, and enrolled sufficient subject numbers to be able to report results separately for both men and women. In general, significant loss of visceral and hepatic fat has been consistently reported while a decrease in muscle fat has not been consistently observed (11-14, 33). In our study, muscle fat infiltration did not change; this could have been due to the CT measurement technique, which is less sensitive than intramyocellular lipid (IMCL) measurement by NMR spectroscopy (MRS), as well as to the duration and nature of the intervention. Previous studies have suggested that exercise may prevent the loss of IMCL during weight loss induced by caloric restriction (13, 34). Thus, the exercise component of our intervention could have had a similar effect over the one-year period. We also found that men in our study, and to lesser degree women, had favorable changes in AT distribution, from the upper to the lower and from the deeper to the more superficial depots. Such changes have not been previously reported during weight loss by dieting in type 2 diabetes; the exercise component of the ILI could have played a role (35-36). Changes in AT distribution could accompany significant improvements in the components of the metabolic syndrome in individuals with type 2 diabetes (35-36); however, we did not find that the relationships specifically between improvements in the AT distribution and improvements in the peripheral insulin sensitivity were independent of the change in overall weight and AT mass. We therefore confirmed our original hypothesis that, with the exception of the decrease in hepatic fat, weight loss and overall AT mass reduction was a better predictor of the improvement in peripheral insulin sensitivity than improvements in AT distribution. This finding is also in agreement to previous reports, of shorter duration, with smaller sample sizes and more homogenous weight loss (12-13).

The decrease in insulin-suppressed FFA levels and the decrease in hepatic fat were also independent determinants of improved peripheral insulin sensitivity. The latter finding is new for type 2 diabetes to the best of our knowledge, although cross-sectional independent associations between hepatic fat and insulin sensitivity have been previously described (2, 37). The causative direction and the underlying patho-physiology of this association could not be determined from the present study. Changes in insulin, glucose and FFA-s levels could all be potential mediators. The association between the decrease in the insulin-suppressed FFA levels and the improvement in peripheral insulin sensitivity was previously described (12) and the role of FFA-s in the etiology of insulin resistance in type 2 diabetes has been stressed in both cross-sectional (4) and weight loss studies (12). Both glucose phosphorylation and glucose transport in skeletal muscle are known to be affected by circulating FFA levels (21, 38) and, in turn, improve with weight loss in type 2 diabetes (39). We also found that the change in peripheral insulin sensitivity was related to the relative improvement in superficial AT distribution and the decrease in this depot’s mean fat cell size. These relationships were not independent of the change in body weight but
are significant in that they point out to the importance of the subcutaneous fat characteristics in the etiology of insulin resistance in type 2 diabetes (18-19). With regard to FG, our results are similar to those previously published (12) in that the best predictor for the improvement in FG was the improvement in insulin sensitivity (GDR). The changes in VAT and hepatic fat were associated with the improvement in FG independent of changes in overall AT mass but only the change in hepatic fat was related to the change in FG, independent of the change in GDR. We also report for the first time that the changes in insulin-suppressed FFA-s were related to the change in hepatic fat. The importance of hepatic fat as a determinant of metabolic parameters in type 2 diabetes has been underscored by cross-sectional associations with hepatic insulin resistance (12) and by associations with insulin requirements during insulin therapy in type 2 diabetes, independent of measured insulin action and FFA levels (40). In our study, a decrease in hepatic fat was associated with improvements in all three key metabolic variables studied. The exact mechanism is not known; among other possibilities is improved insulin clearance after weight loss (8), which in addition to the improved beta cell function, could result in a more physiologic insulin pattern and lower both plasma glucose and FFA-s (7, 41). Thus, we conclude that changes in hepatic fat play a key role in the improvement of metabolic parameters with weight loss in type 2 diabetes.

The changes in the oral hypoglycemic agents that occurred over the one-year intervention is a potential limitation for our study. We performed separate analyses excluding the two subjects who were on insulin-sensitizing agents at the one-year testing and not at baseline and adding discontinuation of any oral agents at one-year compared to baseline (yes or no) as a factor. Results were essentially unchanged with a notable exception: the gender differences in the overall weight or fat loss (Table 1) were not significant anymore once the discontinuation of the oral agents was accounted for. The peripheral insulin sensitivity changes (Table 2) and the AT distribution changes presented in Figure 1 were not affected. Therefore we speculate that, since more men discontinued oral agents than women, this may have accounted for the gender differences in the overall weight and fat loss. The baseline menopausal status of our women and its change over time could have also potentially influenced our results. Four women changed menopausal status over the course of the study; none changed HRT. Although we did not find interactions by menopausal status in our analyses (results not shown) the number of women in the different categories is too small to exclude a possible influence of baseline menopausal status on adipose tissue distribution changes over the one-year of the study.

Finally, the Look-AHEAD trial participants had measurements of fitness at baseline and then yearly throughout the ILI, as previously described (24-25). In our cohort, fitness improved by 40±8% and by 31±7%, in men and women respectively (P< 0.0001). The difference in magnitude compared to the fitness improvement of the entire ILI arm (25% and 18%, in men and women, respectively) (25) could be due to the special selection criteria for our study. Just for the entire ILI group (25), in our study the fitness improvement was significantly correlated with the degree of weight loss; in addition, it was significantly correlated with changes in FM, % body fat and GDR but not with changes in FG or FFA. The fitness improvement, however, did not predict changes in GDR independent of the overall weight or fat loss, consistent with results from other studies (42).

In conclusion, patients with type 2 diabetes undergoing a one-year lifestyle intervention
of diet and exercise had significant improvements in adipose tissue distribution, insulin sensitivity, fasting glucose and circulating free fatty acids. Changes in overall weight, adipose mass and hepatic fat were the most important associates of metabolic improvements.

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FIGURE LEGENDS
Figure 1 A-C: Absolute amounts in Kg (A) or cm$^2$ (B-C) on the Y axis, and the relative distribution as % of total fat (A) or % of total area (B-C) shown in figures as mean ± SEM, of upper body (UB-Fat) and lower body (gluteo-femoral, GF-Fat) fat by DXA (A), visceral (VAT), deep (Deep SAT) and superficial (Superficial SAT) abdominal AT areas measured at L4-L5, by CT (B); and sub-fascial (Sub-fascial AT) and superficial subcutaneous (Superficial SAT) AT areas measured at mid-thigh (one leg) by CT (C), before and after one-year of intervention; P values are shown for differences in the relative distribution.

Figure 2 A-C: The relationships between: A) the change in weight and the change in glucose disposal rate (GDR, mg/Kg/FFM), both expressed as a % of the baseline values ($r=-0.50$ $P=0.0006$), B) the change in glucose disposal rate (GDR, mg/Kg-FFM/min) and the change in fasting glucose (FG), both expressed as a % of the baseline values ($r=-0.37$, $P=0.005$); and C) the change in the liver/spleen attenuation ratio measured by CT and the change in plasma FFA-s at steady state during the clamp (clamp FFA-s, logged values)($r=-0.33$ $P=0.014$); correlation coefficients and P values shown are from models where gender and site were added as factors; men: closed triangles, women: open circles.
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### TABLE 1. Weight, Adipose Tissue (AT) Mass and Distribution, Organ Fat and Abdominal Subcutaneous Fat Cell Size Before and After One-year Lifestyle Intervention

|                  | Men n=26                  | Women n=32                  |
|------------------|---------------------------|-----------------------------|
|                  | Baseline                  | One Year                    | Baseline                  | One Year |
| Weight (Kg)¹     | 101.2±1.9                 | 88.8±1.8                    | 91.4±1.7                  | 83.9±1.7 |
| BMI (Kg/m²)²     | 32.4±0.5                  | 28.4±0.5                    | 34.8±0.6                  | 32.0±0.6 |
| Fat Free Mass (Kg)¹ | 70.9±1.1               | 66.9±1.0                    | 54.2±1.0                  | 52.1±0.9 |
| Fat Mass (Kg)¹   | 30.3±1.2                  | 22.0±1.2                    | 37.1±1.1                  | 31.8±1.1 |
| %Fat Mass (of weight)¹ | 29.8±0.8            | 24.5±0.9                    | 40.4±0.7                  | 37.5±0.8 |
| UB-Fat (Kg)²     | 21.4±0.9                  | 15.1±0.9                    | 24.4±0.9                  | 20.7±0.8 |
| GF-Fat(Kg)²      | 8.2±0.3                   | 6.2±0.3                     | 12.1±0.6                  | 10.6±0.5 |
| VAT (cm²)¹       | 311.7±18.3                | 216.5±18.3                  | 259.5±16.8                | 213.3±16.7 |
| Deep Abdominal SAT (cm²)¹ | 170.9±11.6          | 120.4±10.2                  | 148.2±10.6                | 130.6±9.3 |
| Superficial Abdominal SAT (cm²)² | 120.9±11.5       | 92.0±10.6                   | 237.1±10.6                | 206.8±9.7 |
| Sub-fascial Thigh AT (cm²)(one leg)² | 18.1±1.5              | 12.9±1.1                    | 22.7±1.5                  | 18.1±1.1 |
| Superficial Thigh SAT (cm²)(one leg)² | 84.7±7.9             | 66.8±7.5                    | 156.5±7.5                 | 138.4±7.1 |
| Liver Attenuation (HU)² | 51.2±2.1              | 59.7±1.8                    | 46.5±1.9                  | 54.6±1.7 |
| Spleen Attenuation (HU)² | 50.4±0.8              | 51.3±0.8                    | 47.6±0.8                  | 48.6±0.7 |
| L/S Attenuation Ratio² | 1.01±0.04             | 1.17±0.04                   | 0.99±0.04                 | 1.13±0.04 |
| Muscle area (cm²)(both legs)¹ | 311.5±6.8             | 292.7±6.9                   | 223.4±6.3                 | 215.2±6.4 |
| Muscle Attenuation (HU)² | 46.8±0.9              | 47.4±0.8                    | 45.0±0.8                  | 45.1±0.7 |
| Fat Cell Size²   | 0.73±0.05                 | 0.50±0.04                   | 0.96±0.04                 | 0.76±0.03 |
| Fat Cell Number² | 3756±271                  | 4897±370                    | 2982±239                  | 3345±325 |

Values are unadjusted means ± SEM; Fat free mass, fat mass, UB-Fat, upper body fat mass and GF-Fat, gluteal femoral fat mass, by DEXA; VAT and SAT, visceral and subcutaneous AT, abdominal and thigh SAT sub-compartments and organ (liver, spleen, muscle) attenuation by CT scan; ¹Significant change in both men and women (P range <0.05 to 0.00001) with significant interaction by sex (P range < 0.05 to 0.001 for the interaction term); ²Significant change in both men and women (P range < 0.05 to 0.00001) with no significant interaction by sex; ³Missing data for men (out of 26) for organ attenuations (one man each) and for fat cell size (two men); ⁴Missing data for women (out of 32) for abdominal AT measurements (one woman), thigh AT and organ attenuations (three women each) and for fat cell size (one woman).

### TABLE 2. Metabolic Parameters during the Euglycemic Hyperinsulinemic Clamp Before and After One-year Lifestyle Intervention

|                  | Men n=26                  | Women n=32                  |
|------------------|---------------------------|-----------------------------|
|                  | Baseline                  | One Year                    | Baseline                  | One Year |
| Post-absorptive State |                          |                             |                            |
| Glucose (µM)¹  | 8.2±0.4                   | 6.7±0.3                     | 7.8±0.3                   | 7.3±0.3 |
| Insulin (pM)¹  | 71.1±7.2                  | 52.5±8.9                    | 91.5±6.5                  | 80.0±8.0 |
| Free Fatty Acids (mM)¹ | 0.56±0.02              | 0.45±0.03                   | 0.79±0.03                 | 0.63±0.02 |
| Steady State During Clamp |                      |                             |                            |
| Glucose (µM)²  | 5.7±0.1                   | 5.7±0.1                     | 5.8±0.1                   | 5.8±0.1 |
| Insulin (pM)²  | 834.3±49.1                | 820.1±39.2                  | 982.9±44.2                | 859.5±35.3 |
| Free Fatty Acids (mM)² | 0.03±0.01               | 0.01±0.00                   | 0.05±0.06                 | 0.02±0.003 |
| GDR (mg/KgFFM/min)¹ | 5.7±0.4                | 8.9±0.5                     | 6.2±0.4                   | 8.4±0.5 |
| Glucose Oxidation (mg/KgFFM/min)² | 2.7±0.1              | 3.4±0.2                     | 3.1±0.2                   | 3.8±0.2 |
| Glucose Storage (mg/KgFFM/min)² | 3.0±0.3               | 5.5±0.4                     | 2.9±0.4                   | 4.4±0.5 |

Values are unadjusted means ± SEM; ¹Significant change in both men and women (P range <0.05 to 0.00001) with no significant interaction by sex; ²Significant change in women only (P < 0.05); ³Men= 26; Women=30
Weight loss in type 2 diabetes

**FIGURE 1A**

|        | Male | Female |
|--------|------|--------|
| Before | 73±1%| 68±1%  |
| After  | 27±1%| 11±1%  |

**FIGURE 1B**

|        | Male | Female |
|--------|------|--------|
| Before | 52±1%| 39±1%  |
| After  | 20±1%| 14±1%  |
Weight loss in type 2 diabetes

**FIGURE 1C**

|       | Male | Female |
|-------|------|--------|
| Before| 18 ± 1% | 82 ± 1% | 14 ± 1% | 86 ± 1% |
| After | 8 ± 1%  | 84 ± 1% | 87 ± 1% | 87 ± 1% |

% Change in weight

% Change in GDR

**FIGURE 2A**

% Change in GDR vs. % Change in weight
Weight loss in type 2 diabetes

FIGURE 2B

% Change in GDR

% Change in Fasting Glucose

FIGURE 2C

Change in clamp FFA-s (log)

Change in L/S ratio