Effect of Calorie Restriction With or Without Exercise on Insulin Sensitivity, β-Cell Function, Fat Cell Size, and Ectopic Lipid in Overweight Subjects

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OBJECTIVE — The purpose of this article was to determine the relationships among total body fat, visceral adipose tissue (VAT), fat cell size (FCS), ectopic fat deposition in liver (intrahepatic lipid [IHL]) and muscle (intramyocellular lipid [IMCL]), and insulin sensitivity index (S_i) in healthy overweight, glucose-tolerant subjects and the effects of calorie restriction by diet alone or in conjunction with exercise on these variables.

RESEARCH DESIGN AND METHODS — Forty-eight overweight volunteers were randomly assigned to four groups: control (100% of energy requirements), 25% calorie restriction (CR), 12.5% calorie restriction +12.5% energy expenditure through structured exercise (CREX), or 15% weight loss by a low-calorie diet followed by weight maintenance for 6 months (LCD). Weight, percent body fat, VAT, IMCL, IHL, FCS, and S_i were assessed at baseline and month 6.

RESULTS — At baseline, FCS was related to VAT and IHL ($P < 0.05$) but not to IMCL. FCS was also the strongest determinant of S_i ($P < 0.01$). Weight loss at month 6 was 1 ± 1% (control, mean ± SE), 10 ± 1% (CR), 10 ± 1% (CREX), and 14 ± 1% (LCD). VAT, FCS, percent body fat, and IHL were reduced in the three intervention groups ($P < 0.01$), but IMCL was unchanged. S_i was increased at month 6 ($P = 0.05$) in the CREX (37 ± 18%) and LCD (70 ± 34%) groups ($P < 0.05$) and tended to increase in the CR group (40 ± 20%, $P = 0.08$). Together the improvements in S_i were related to loss in weight, fat mass, and VAT, but not IHL, IMCL, or FCS.

CONCLUSIONS — Large adipocytes lead to lipid deposition in visceral and hepatic tissues, promoting insulin resistance. Calorie restriction by diet alone or with exercise reverses this trend.

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Insulin resistance is an early metabolic abnormality that precedes the development of hyperglycemia, hyperlipidemia, and overt type 2 diabetes. Both insulin resistance and β-cell dysfunction are associated with obesity (1–3). Although total fat mass and subcutaneous abdominal adipose tissue (SAT) are associated with insulin resistance, visceral adipose tissue (VAT) is generally considered to be the most significant determinant (4). The causal link between visceral fat accumulation and insulin resistance, however, remains unclear. A commonly accepted view is that fatty acids released from visceral fat into the portal vein have direct effects on hepatic metabolism. Another hypothesis, however, is that visceral fat may simply covary with other causal factors that affect insulin sensitivity, namely, fat cell size (FCS) and ectopic fat in muscle and liver. Previous studies have shown that increased FCS, a marker of impaired adipogenesis, is related to insulin resistance and predicts the development of type 2 diabetes (5). Whether increased FCS affects insulin sensitivity by increased spillover of triglyceride into visceral fat or into muscle, liver, or other nonadipose tissues is unclear.

Calorie restriction reduces fat mass, delays the development of age-associated diseases such as type 2 diabetes, and increases lifespan in rodents. In obese humans, it is well established that calorie restriction, weight loss, and exercise improve insulin sensitivity (6–11), although the additional benefits of increased exercise on insulin sensitivity are debated. Moreover, the extent that these interventions alter ectopic fat accumulation in muscle and liver has not been explored. Muscle lipid content is reduced with severe weight reduction (~15–24%) in obese and morbidly obese individuals undergoing an 800-kcal diet (12) or gastric bypass surgery (13). Moderate weight loss, by diet alone or in combination with exercise, however, does not alter muscle lipid depots, despite significant improvements in insulin sensitivity (6,14,15). Hepatic fat, on the other hand, was significantly lowered by moderate weight reduction in obese women and in type 2 diabetes (16,17). To our knowledge no study has yet determined the effect of caloric restriction with or without exercise on ectopic fat in nonobese individuals.

The goal of this study was, therefore, to determine in healthy nonobese, glucose tolerant subjects *1*) the relationships among total body fat, visceral fat, FCS,

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Abbreviations: AIR_g, acute insulin response to glucose; CALERIE, Comprehensive Assessment of the Long-term Effects of Reducing Intake of Energy; DSAT, deep subcutaneous abdominal adipose tissue; FCS, fat cell mass; IHL, intrahepatic lipid; IMCL, intramyocellular lipid; SAT, subcutaneous abdominal adipose tissue; VAT, visceral adipose tissue.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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intramyocellular lipid (IMCL), intrahepatic lipid (IHL), and insulin sensitivity index (S_i) and 2) the effects of a calorie-restricted diet alone or in conjunction with exercise on ectopic fat, visceral fat, FCS, insulin sensitivity, and β-cell function.

RESEARCH DESIGN AND METHODS

— Details of this study are reported elsewhere (18). Briefly, healthy male (25–50 years) and female (25–45 years) overweight participants ($25 \leq$ BMI ≤ 30 kg/m^2) were recruited for the Comprehensive Assessment of the Long-term Effects of Reducing Intake of Energy (CALERIE) trial. Participants were excluded if they smoked; exercised more than twice per week; were pregnant, lactating, or postmenopausal; had a personal history of obesity (BMI never >32 kg/m^2), cardiovascular disease, or diabetes; or regularly used medications (except birth control). The study was approved by the Pennington Biomedical Research Center Institutional Review Board and the CALERIE Data Safety Monitoring Board, and all subjects provided written informed consent.

Baseline

To carefully determine individual energy requirements, total daily energy expenditure was measured by two 14-day measures of doubly labeled water, once while participants followed their usual diet at home and once while they were being provided with a weight maintenance diet by the metabolic kitchen. Patients were then admitted to the ward for 5 days of metabolic testing.

Intervention

After baseline testing, 48 participants were randomly assigned to one of four groups for 6 months: control (healthy diet for weight maintenance), 25% calorie restriction of baseline energy requirements (CR), 12.5% calorie reduction + 12.5% increase in total energy expenditure by structured exercise (CREX), and low-calorie diet until a 15% reduction in body weight, followed by maintenance of the new lower body weight (LCD). Participants were provided with all food for the first 12 weeks and for weeks 22–24. Diets were based on American Heart Association recommendations (≤30% fat). For weeks 13–22, participants self-selected their diet based on their calorie targets. LCD participants were placed on 890 kcal/day (HealthOne, Health and Nutrition Technology, Carmel, CA). Once target weight loss (−15%) was achieved, LCD participants were refed to an energy level that maintained this new body weight. CREX participants increased their energy expenditure by 12.5% above resting by undergoing structured exercise (i.e., walking, running, or stationary cycling) 5 days per week. Participants were required to conduct three sessions per week under supervision. For unsupervised sessions, participants wore a portable heart rate monitor (Polar S-610, Polar Beat, Port Washington, NY) with heart rate and exercise duration recorded. For support, all participants attended weekly group meetings that were led by clinical psychology professionals.

Metabolic tests

All metabolic tests were performed during inpatient stays at baseline and month 6 following a 12-h overnight fast and at least 48 h after the last bout of exercise. Body fat was measured by dual-energy X-ray absorptiometry (QDA 4500A; Hologics, Bedford, MA) and multislice computed tomography scanning of the abdominal region (GE High Speed Plus; General Electric, Fairfield, CT) was performed to quantify abdominal fat compartments (19). Muscle and liver lipid stores were determined by proton magnetic resonance spectroscopy using point-resolved spectroscopy (20). Subcutaneous abdominal needle biopsies were performed, and FCS was determined by the Multisizer-3 counter (Beckman Coulter, Fullerton, CA) as previously described (21). Insulin sensitivity was determined by the insulin-modified frequently sampled intravenous glucose tolerance test (22,23). Baseline blood samples were drawn before 300 mg/kg body weight of glucose (50% dextrose; Hospira, Lake Forest, IL) was injected and 32 blood samples were collected over 180 min. At 20 min, a bolus injection of insulin (0.03 units/kg Humulin; Eli Lilly, Indianapolis, IN) was given. The S_i and acute insulin response to glucose (AIR_g) were calculated by the minimal model (23). Because of illness or problems with intravenous lines, four tests could not be analyzed at month 6. Glucose was analyzed using a Synchron CX7 (Beckman-Coulter, Brea, CA) and insulin was analyzed via immunoassay on the DPC 2000 (Diagnostic Product Corporation, Los Angeles, CA).

Statistical analysis

Data are expressed as means ± SE and the level of significance for all statistical tests was set at $P < 0.05$. SAS version 9.1 was used for analysis, and all analyses were performed by biostatisticians in the Pennington Biomedical Research Center Biostatistics Core. Pearson or Spearman rank order correlations were used where appropriate, and general linear regression was used to identify any interactions of the changes with sex. To assess the effect of the intervention among the four groups, the change from baseline to month 6 was computed, and an ANCOVA was performed with baseline values included in the model as covariates and adjusted with respect to Tukey-Kramer. Two subjects withdrew during the study; one was a control subject who withdrew for personal reasons and the other subject, who was following the LCD diet, was lost to follow-up. Data are therefore presented on 46 subjects.

RESULTS

— Characteristics of the subjects at baseline are reported in Table 1. Subjects were generally in good health with fasting glucose, insulin, and blood pressure within recommended ranges; 30 Caucasians, 15 African Americans, and 1 Asian were examined.

Baseline

Relationships between distribution of fat and insulin sensitivity. At baseline, IHL and VAT were positively correlated ($r = 0.57$, $P < 0.0001$), and both fat depots were related to FCS (Fig. 1). IMCL in the soleus was not correlated with FCS, VAT, or IHL. S_i was significantly related to IHL ($r = -0.31$, $P = 0.04$) and FCS ($r = -0.36$, $P = 0.01$), but not VAT, DSAT, or IMCL. A forward stepwise regression analysis, showed that S_i was best explained by FCS ($P < 0.01$), whereas fat mass, IHL, VAT, and IMCL were not additional independent determinants. All of the above correlations were also statistically significant at month 6 (data not shown).

Response to intervention

The impact of the intervention can be seen in Table 1 by comparing results at month 6 versus baseline.

Body composition and fat distribution. Body weight was significantly reduced in the CR (−8 ± 1 kg, 10 ± 1%), CREX (−8 ± 1 kg, 10 ± 1%), and LCD (−11 ± 1 kg, 14 ± 1%) groups compared with the

Table 1—*Physical characteristics of the subject groups at baseline and following 6 months of calorie restriction*

	Control (n = 11)		CR (n = 12)		CREX (n = 12)		LCD (n = 11)		
n	Baseline	Month 6	Baseline	Month 6	Baseline	Month 6	Baseline	Month 6	P*
Body composition									
Weight (kg)	81.8 ± 2.8	81.9 ± 2.8	81.0 ± 3.3	72.6 ± 3.1†	82.0 ± 3.0	73.9 ± 2.8†	81.0 ± 3.3	70.0 ± 3.0†	<0.0001
Body (kg)	31.3 ± 1.8	30.9 ± 2.1	31.0 ± 2.4	26.6 ± 2.4†	32.6 ± 2.2	27.6 ± 2.4†	33.0 ± 2.4	26.5 ± 2.7†	<0.0001
Fat mass (kg)	25.5 ± 1.2	25.0 ± 1.7	24.9 ± 1.8	19.1 ± 1.7†	26.4 ± 1.7	20.1 ± 1.7†	26.5 ± 1.9	18.4 ± 1.9†	<0.0001
Fat-free mass (kg)	56.8 ± 3.1	56.5 ± 3.1	56.3 ± 3.5	53.7 ± 3.3†	55.6 ± 3.5	53.6 ± 3.2†	54.6 ± 3.4	51.5 ± 3.3†	<0.0001
Fat distribution									
VAT (kg)	2.9 ± 0.4	2.8 ± 0.4	3.2 ± 0.5	2.3 ± 0.4†	2.8 ± 0.4	2.0 ± 0.4†	2.9 ± 0.5	1.8 ± 0.3†	<0.0001
SAT (kg)	7.7 ± 0.6	7.8 ± 0.7	7.8 ± 0.7	5.8 ± 0.6†	8.2 ± 0.8	6.0 ± 0.8†	8.2 ± 0.8	5.6 ± 0.7†	<0.0001
DSAT (kg)	3.6 ± 0.3	3.6 ± 0.3	3.8 ± 0.3	2.7 ± 0.3†	3.8 ± 0.4	2.7 ± 0.4†	3.9 ± 0.4	2.5 ± 0.3†	<0.0001
Fat cell size (μl)	0.73 ± 0.05	0.72 ± 0.04	0.65 ± 0.05	0.51 ± 0.02†	0.70 ± 0.04	0.52 ± 0.06†	0.67 ± 0.06	0.51 ± 0.05†	<0.001
IHL (% of oil phantom)	1.60 ± 0.39	2.36 ± 0.81†	1.66 ± 0.56	0.69 ± 0.13†	1.17 ± 0.21	0.65 ± 0.10†	1.16 ± 0.29	0.55 ± 0.09†	<0.003
IMCL (%of oil phantom)	4.05 ± 0.50	4.20 ± 0.92	3.79 ± 0.33	3.54 ± 0.43	2.88 ± 0.26	2.84 ± 0.29	4.00 ± 0.86	3.33 ± 0.70	NS
Insulin sensitivity									
Fasting glucose (mg/dl)	90.2 ± 1.2	91.8 ± 2.1	89.3 ± 1.8	88.0 ± 2.3	92.0 ± 1.8	91.8 ± 2.0	89.1 ± 0.8	89.7 ± 1.0	NS
Fasting insulin (μU/ml)	12.4 ± 1.0	13.0 ± 1.8	9.4 ± 1.5	6.6 ± 0.9†	9.8 ± 1.0	7.0 ± 0.7†	11.0 ± 0.9	9.4 ± 1.3†	<0.05
S_i (10^{-4} mU · l^{-1} · min^{-1})	2.8 ± 0.4	2.5 ± 0.4	3.3 ± 0.5	4.2 ± 1.0	3.4 ± 0.4	5.3 ± 0.8†	3.1 ± 0.6	4.7 ± 0.9†	0.09
AIRg (mU · l^{-1} · min^{-1})	750 ± 135	685 ± 98	815 ± 136	558 ± 71†	729 ± 175	440 ± 115†	892 ± 231	587 ± 161†	0.09

Data are means ± SE. *Differences between treatment groups for the change scores using an ANCOVA with the absolute change as the dependent variable and the baseline score as a covariate. †Significant change from baseline.

control group, and each intervention group had significant losses of fat mass (CR 24 ± 3%, CREX 25 ± 3%, and LCD 32 ± 3%) and fat-free mass (CR 5 ± 1%, CREX 3 ± 1%, and LCD 6 ± 1%). Similar reductions in VAT (CR 28 ± 4%, CREX 27 ± 3%, and LCD 36 ± 3%; $P < 0.005$), SAT (CR 26 ± 4%, CREX 28 ± 3%, and LCD 34 ± 3%; $P < 0.005$), and DSAT (CR 29 ± 5%, CREX 30 ± 3%, and LCD 37 ± 3%; $P < 0.005$) were observed in each intervention group. The intervention also induced a significant ($P < 0.001$) reduction in FCS (CR 19 ± 4%, CREX 26 ± 5%, and LCD 26 ± 4%). The changes in body composition and abdominal fat were not dependent on whether the caloric deficit was achieved by exercise and diet (CREX) or diet alone (CR and LCD). IHL was significantly ($P < 0.01$) reduced by the intervention but was not additionally influenced by exercise (CR 37 ± 10%, CREX 29 ± 15%, and LCD 40 ± 10%). The intervention did not change the percentage of IMCL in the soleus for either the CR (−8 ± 7%) or CREX groups (3 ± 11%); however, it tended to decrease in the LCD group (−12 ± 6, $P = 0.07$).

Insulin sensitivity. After the 6-month intervention, there was a significant improvement in S_i in the CREX (37 ± 18%, $P < 0.01$) and LCD groups (70 ± 34%, $P < 0.04$), which also tended to increase in the CR group (40 ± 20%, $P = 0.08$). The improvement in S_i was not different among the three intervention groups. Similarly, AIR_g was significantly decreased from baseline in each of the treatment groups (CR 29 ± 7%, CREX 30 ± 8%, and LCD 28 ± 9%; $P < 0.01$).

Relationships among the changes in body composition, fat distribution, and insulin sensitivity

The decrease in FCS was correlated with the decrease in weight ($r = 0.61$, $P < 0.001$), percent fat ($r = 0.61$, $P = 0.0001$), VAT ($r = 0.38$, $P < 0.01$), SAT ($r = 0.54$, $P = 0.0001$), and fasting serum triglyceride levels ($r = 0.44$, $P < 0.005$). Similarly the decrease in IHL was related to the reduction in weight ($r = 0.46$, $P = 0.001$), percent fat ($r = 0.37$, $P < 0.001$), VAT ($r = 0.49$, $P < 0.001$), and fasting serum triglyceride levels ($r = 0.60$, $P < 0.005$). The improvement in S_i was related to the reduction in percent fat ($r = -0.46$, $P = 0.002$) (Fig. 2*A*), VAT ($r = -0.51$, $P < 0.01$) (Fig. 2*B*), SAT ($r = -0.32$, $P < 0.05$), and DSAT ($r = -0.47$, $P < 0.01$), but it was not related to the change in FCS ($P = 0.11$) (Fig. 2*C*), IHL ($P = 0.59$) (Fig. 2*D*), or IMCL ($P = 0.52$). A forward stepwise regression analysis showed that 25% of the improvement in S_i was attributed to the reduction in VAT ($P = 0.002$). The changes in IMCL, IHL, FCS, and the other abdominal fat depots were not additional independent determinants. These correlation analyses were repeated with the control group removed. The significance of the relationship between the changes in FCS and VAT and the changes in IHL and percent fat was lost, but no other relationships were affected.

CONCLUSIONS — In this study we examined the relationships between S_i and various indexes of body fat in overweight, glucose-tolerant subjects before and after calorie restriction. At baseline, we found that *1*) fat deposition in liver was related to the accumulation of fat in the abdominal visceral area and to enlarged subcutaneous abdominal adipocytes and *2*) increased FCS but not ectopic fat deposition in muscle and liver was independently associated with reduced insulin sensitivity. In response to 6 months of calorie restriction, we found that *1*) weight, visceral fat, and FCS are reduced with improvements in S_i and reduced AIR_g and *2*) fat deposition in liver but not muscle was reduced by the intervention, but the changes were not associated with improvements in S_i.

Whether ectopic lipid deposition in skeletal muscle and/or liver is related to total body fat is debated. Several studies have suggested that ectopic fat accumulation is independent of whole-body adiposity (16,24–30). However, other studies have noted that lipid accumulation in both muscle (27,31–33) and liver (34–37) increases as a function of obesity,

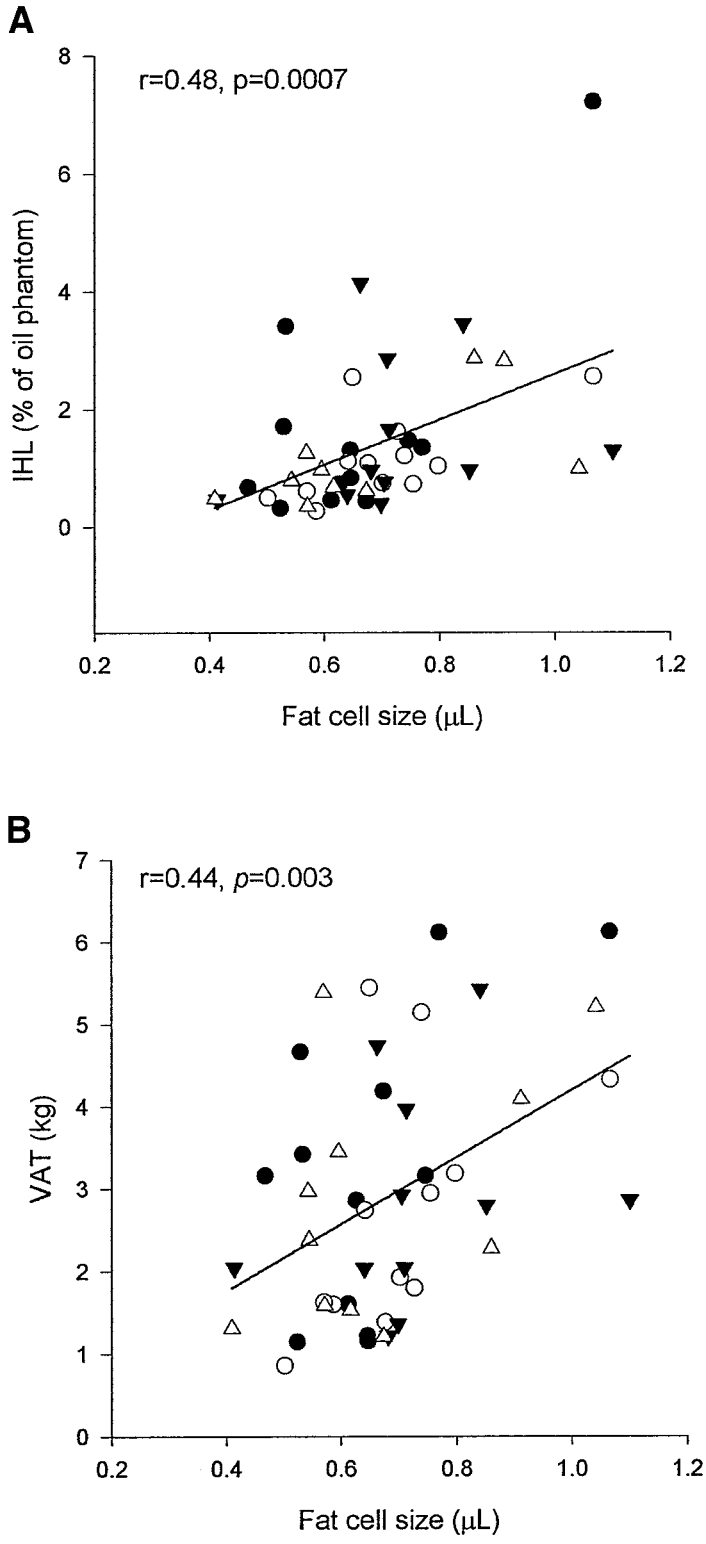

Figure 1—*In healthy overweight men and women at baseline, there was a strong positive correlation between abdominal subcutaneous FCS and VAT* (A) *and abdominal subcutaneous FCS and IHL* (B). *Groups were pooled for analysis.* ●, *CR;* ○, *CREX;* △, *LCD;* ▼, *control.*

providing that subjects with a wide range of adiposity are studied. In this study, we observed that lipid deposition in liver (but not muscle) was related to both total and abdominal adiposity. Specifically, our findings indicate that ectopic fat in the liver may be related to visceral fat stores. This relationship between liver lipid and visceral adiposity has been noted in some (34,38) but not all (29,30) studies. Most interestingly, we observed that liver lipid infiltration tended to be greater in overweight individuals who had enlarged adipocytes and increased visceral abdominal adiposity. Furthermore, visceral fat was related to FCS. These findings support the hypothesis that inadequate subcutaneous adipose stores result in lipid overflow into visceral fat and other nonadipose tissues (39). In this regard, visceral fat could be considered as a marker of ectopic fat.

At baseline and at month 6, large fat cells were also the strongest determinant of insulin resistance in these nondiabetic subjects. This finding prompts speculation that impaired adipogenesis may be the primary defect in insulin resistance, and the hypothesis is supported by findings that humans with partial or complete loss of adipose tissue are extremely insulin resistant (40), that surgical replacement of adipose stores in the fatless mouse restores insulin sensitivity (41), and that expression of *Wnt* signaling genes and adipogenic transcription factors are reduced in nondiabetic subjects with a family history of type 2 diabetes (42). Large fat cells have also been shown to have a different pattern of adipocytokine secretion than smaller fat cells (43), which may contribute to the strong association between large FCS and insulin sensitivity.

In contrast to previous studies (24,26–28,31,44,45), we observed that IMCL was not related to insulin sensitivity. Furthermore, IMCL was not related to adipocyte size. Our results are consistent with the hypothesis that IMCL stores alone are not sufficient to account for impaired insulin action (46–48). Liver lipid, on the other hand, was inversely related to insulin sensitivity. Liver lipid content has previously been reported to correlate with measures of whole-body insulin sensitivity in individuals with and without diabetes (30,34,35,38,49), but this relationship is difficult to explain mechanistically because most ingested (or infused) glucose is taken up by muscle. Theoretically, IHL is expected to correlate with reduced hepatic insulin sensitivity (impaired insulin suppression of glucose rate of appearance) and not necessarily with whole-body insulin action. However, the accumulation of hepatic triglyceride has been hypothesized

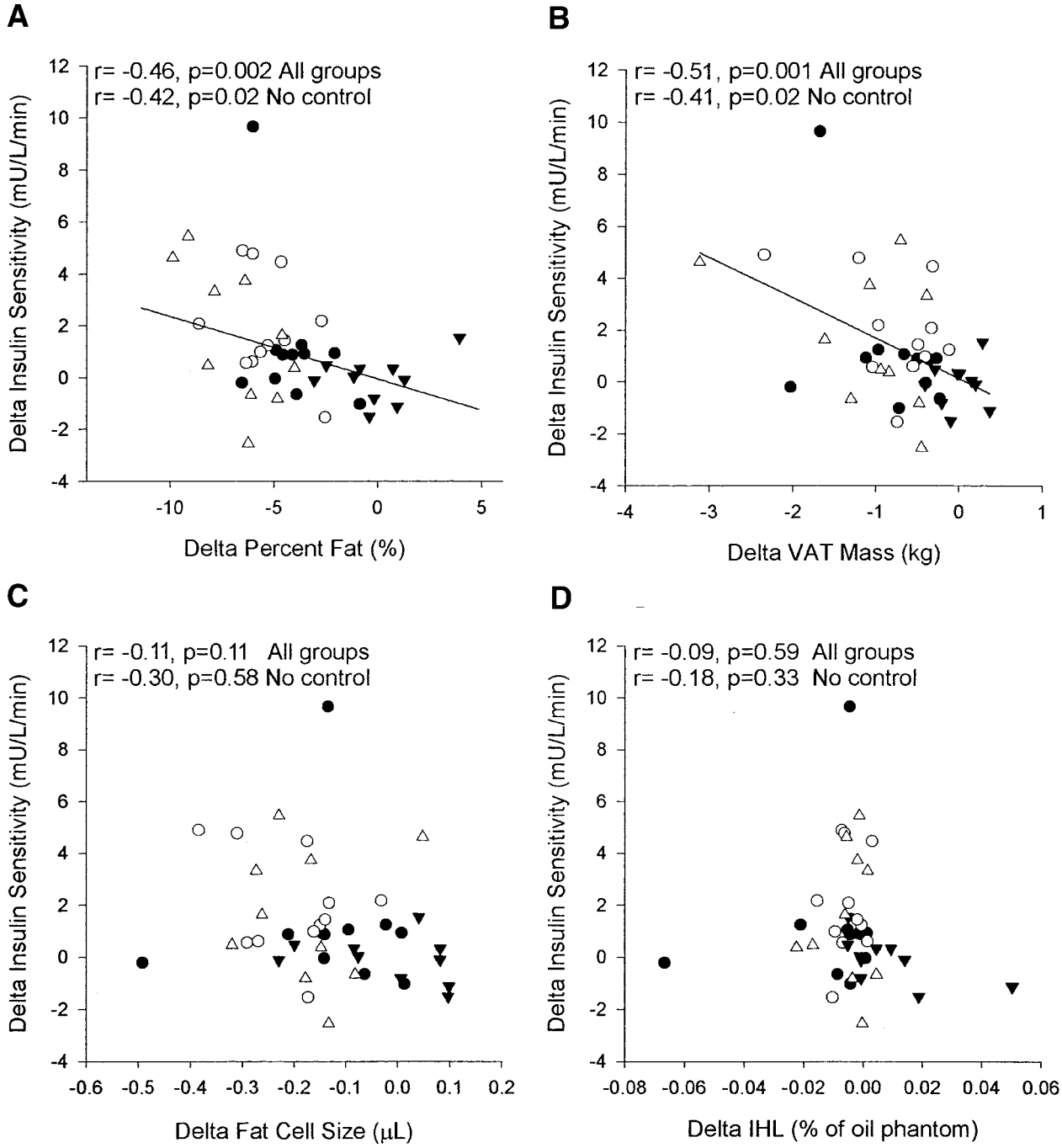

Figure 2—*The improvement in insulin sensitivity with 6 months of calorie restriction was significantly associated with the loss of fat mass* (A) *and abdominal VAT depots* (B) *but not to the change in subcutaneous abdominal FCS* (C) *and IHL* (D). ●, *CR*; ○, *CREX*; △, *LCD*; ▼, *control. Analyses are reported with and without the control group included.*

to reduce insulin clearance and lead to peripheral insulin resistance via a down-regulation of insulin receptors (34,50). Clearly, prospective human studies that define whether lipid accumulation in liver precedes insulin resistance would be of interest.

Contrary to some previous studies (51,52), we observed that diet alone or with exercise produced identical reductions in weight, fat mass, and abdominal fat mass. These conflicting results may be due to inaccurate calculations of the energy costs of the prescribed activity in those studies, which would lead to differences in energy deficits among groups. We also observed that FCS was reduced in response to an energy deficit, but we could not detect an additional effect of exercise. Our study was underpowered to detect differences in FCS among groups and our results contrast with the reports of You et al. (53), who found that whereas total body fat reduction was equivalent among groups, abdominal adipocyte size was reduced only with the combination of diet and exercise. The current study is

also the first to simultaneously measure ectopic fat stores in both muscle and liver in response to a calorie restriction intervention. We found that the calorie restriction alone or with exercise did not affect IMCL in the soleus. These results are consistent with previous studies (6,14,15) and (together with the findings that IMCL was not independently related to S_i) suggest that IMCL accumulation alone is not likely to be a causal factor leading to acquired insulin-signaling defects in muscle. Many other factors, including lipid droplet size, location of lipid droplets relative to mitochondria, and muscle oxidative capacity, are all potential determinants of insulin resistance (15,48,54). An alternate hypothesis is that the capacity for lipid metabolism is an important mediator in the association between IMCL and insulin resistance. We observed, however, that IHL was sensitive to calorie restriction being reduced by an average 29–40% in the intervention groups. Caution must be exercised when interpreting these results because the study may have been underpowered to detect small differences in IHL among groups. The reduction in liver lipid levels is consistent with results of Tiikkainen et al. (16), who reported a 39–49% reduction in IHL with a simultaneous reduction in body mass of 8% in obese nondiabetic women. In addition, we also observed parallel reductions in IHL and abdominal visceral fat.

In summary, calorie restriction by diet alone or in conjunction with exercise leads to similar improvements in insulin sensitivity and reductions in β-cell sensitivity in overweight, glucose-tolerant subjects. The study also provides support for the hypothesis that the underlying pathologic cause of insulin resistance is related to abnormal partitioning of fat among adipose, hepatic, muscle, and pancreatic tissues, probably as a result of an inability to make new fat cells. However, the finding that IMCL was not responsive to weight loss (despite improvements in insulin sensitivity) suggests that intracellular fat accumulation is not a causal factor in insulin resistance in muscle. Overall, this study provides new evidence to suggest that impaired adipogenesis and increased liver lipid infiltration occur early in the pathogenesis of insulin resistance.

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References

1. Forsey RJ, Thompson JM, Ernerudh J, Hurst TL, Strindhall J, Johansson B, Nilsson BO, Wikby A: Plasma cytokine profiles in elderly humans. *Mech Ageing Dev* 124:487–493, 2003
2. Matsumoto K, Sera Y, Abe Y, Ueki Y, Tominaga T, Miyake S: Inflammation and insulin resistance are independently related to all-cause of death and cardiovascular events in Japanese patients with type 2 diabetes mellitus. *Atherosclerosis* 169: 317–321, 2003
3. Utzschneider KM, Carr DB, Hull RL, Kodama K, Shofer JB, Retzlaff BM, Knopp RH, Kahn SE: Impact of intra-abdominal fat and age on insulin sensitivity and β-cell function. *Diabetes* 53:2867–2872, 2004
4. Kelley DE, Thaete FL, Troost F, Huwe T, Goodpaster BH: Subdivisions of subcutaneous abdominal adipose tissue and insulin resistance. *Am J Physiol* 278:E941–E948, 2000
5. Weyer C, Foley JE, Bogardus C, Tataranni PA, Pratley RE: Enlarged subcutaneous abdominal adipocyte size, but not obesity itself, predicts type II diabetes independent of insulin resistance. *Diabetologia* 43: 1498–1506, 2000
6. Goodpaster BH, Katsiaras A, Kelley DE: Enhanced fat oxidation through physical activity is associated with improvements in insulin sensitivity in obesity. *Diabetes* 52:2191–2197, 2003
7. Goodpaster BH, Kelley DE, Wing RR, Meier A, Thaete FL: Effects of weight loss on regional fat distribution and insulin sensitivity in obesity. *Diabetes* 48:839–847, 1999
8. Niskanen L, Uusitupa M, Sarlund H, Siitonen O, Paljarvi L, Laakso M: The effects of weight loss on insulin sensitivity, skeletal muscle composition and capillary density in obese non-diabetic subjects. *Int J Obes Relat Metab Disord* 20:154–160, 1996
9. Dengel DR, Pratley RE, Hagberg JM, Rogus EM, Goldberg AP: Distinct effects of aerobic exercise training and weight loss on glucose homeostasis in obese sedentary men. *J Appl Physiol* 81:318–325, 1996
10. Ross R, Dagnone D, Jones PJ, Smith H, Paddags A, Hudson R, Janssen I: Reduction in obesity and related comorbid conditions after diet-induced weight loss or exercise-induced weight loss in men: a randomized, controlled trial. *Ann Intern Med* 133:92–103, 2000
11. Franssila-Kallunki A, Rissanen A, Ekstrand A, Ollus A, Groop L: Weight loss by very-low-calorie diets: effects on substrate oxidation, energy expenditure, and insulin sensitivity in obese subjects. *Am J Clin Nutr* 56:247S–248S, 1992
12. Goodpaster BH, Theriault R, Watkins SC, Kelley DE: Intramuscular lipid content is increased in obesity and decreased by weight loss. *Metabolism* 49:467–472, 2000
13. Greco AV, Mingrone G, Giancaterini A, Manco M, Morroni M, Cinti S, Granzotto M, Vettor R, Camastra S, Ferrannini E: Insulin resistance in morbid obesity: reversal with intramyocellular fat depletion. *Diabetes* 51:144–151, 2002
14. He J, Goodpaster BH, Kelley DE: Effects of weight loss and physical activity on muscle lipid content and droplet size. *Obes Res* 12:761–769, 2004
15. Malenfant P, Tremblay A, Doucet E, Imbeault P, Simoneau JA, Joanisse DR: Elevated intramyocellular lipid concentration in obese subjects is not reduced after diet and exercise training. *Am J Physiol* 280: E632–E639, 2001
16. Tiikkainen M, Bergholm R, Vehkavaara S, Rissanen A, Hakkinen AM, Tamminen M, Teramo K, Yki-Jarvinen H: Effects of identical weight loss on body composition and features of insulin resistance in obese women with high and low liver fat content. *Diabetes* 52:701–707, 2003
17. Petersen KF, Dufour S, Befroy D, Lehrke M, Hendler RE, Shulman GI: Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. *Diabetes* 54:603–608, 2005
18. Heilbronn LK, de Jonge L, Frisard MI, DeLany JP, Larson Meyer DE, Rood J, Nguyen T, Martin CK, Volaufova J, Most MM, Greenway FL, Smith SR, Williamson DA, Deutsch WA, Ravussin E, the Pennington CALERIE Team: Effect of 6-month calorie restriction on biomarkers of longevity, metabolic adaptation, and oxidative stress in overweight sub-

jects: a randomized controlled trial. *JAMA* 295:1539–1548, 2006
19. Smith SR, Lovejoy JC, Greenway F, Ryan D, deJonge L, de la Bretonne J, Volafova J, Bray GA: Contributions of total body fat, abdominal subcutaneous adipose tissue compartments, and visceral adipose tissue to the metabolic complications of obesity. *Metabolism* 50:425–435, 2001
20. Larson-Meyer DE, Smith SR, Heilbronn LK, Kelley DE, Ravussin E, Newcomer BR: Muscle-associated triglycerides measured by computed tomography and magnetic resonance spectroscopy. *Obes Res* 14:73–87, 2006
21. Heilbronn LK, Rood J, Janderova L, Albu JB, Kelley DE, Ravussin E, Smith SR: Relationship between serum resistin concentrations and insulin resistance in nonobese, obese, and obese diabetic subjects. *J Clin Endocrinol Metab* 89:1844–1848, 2004
22. Lovejoy JC, Smith SR, Bray GA, Veldhuis JD, Rood JC, Tulley R: Effects of experimentally induced mild hyperthyroidism on growth hormone and insulin secretion and sex steroid levels in healthy young men. *Metabolism*:1424–1428, 1997
23. Boston RC, Stefanovski D, Moate PJ, Sumner AE, Watanabe RM, Bergman RN: MINMOD Millennium: a computer program to calculate glucose effectiveness and insulin sensitivity from the frequently sampled intravenous glucose tolerance test. *Diabetes Technol Ther* 5:1003–1015, 2003
24. Krssak M, Falk Petersen K, Dresner A, DiPietro L, Vogel SM, Rothman DL, Roden M, Shulman GI: Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a ^{1}H NMR spectroscopy study. *Diabetologia*:113–116, 1999
25. Pan DA, Lillioja S, Kriketos AD, Milner MR, Baur LA, Bogardus C, Jenkins AB, Storlien LH: Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes* 46:983–988, 1997
26. Perseghin G, Scifo P, De Cobelli F, Pagliato E, Battezzati A, Arcelloni C, Vanzulli A, Testolin G, Pozza G, Del Maschio A, Luzi L: Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: a ^{1}H–^{13}C nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents. *Diabetes* 48:1600–1606, 1999
27. Sinha R, Dufour S, Petersen KF, LeBon V, Enoksson S, Ma Y-Z, Savoye M, Rothman DL, Shulman GI, Caprio S: Assessment of skeletal muscle triglyceride content by ^{1}H nuclear magnetic resonance spectroscopy in lean and obese adolescents: relationships to insulin sensitivity, total body fat, and central adiposity. *Diabetes* 51:1022–1027, 2002
28. Virkamaki A, Korsheninnikova E, Seppala-Lindroos A, Vehkavaara S, Goto T, Halavaara J, Hakkinen AM, Yki-Jarvinen H: Intramyocellular lipid is associated with resistance to in vivo insulin actions on glucose uptake, antilipolysis, and early insulin signaling pathways in human skeletal muscle. *Diabetes* 50:2337–2343, 2001
29. Seppala-Lindroos A, Vehkavaara S, Hakkinen AM, Goto T, Westerbacka J, Sovijarvi A, Halavaara J, Yki-Jarvinen H: Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *J Clin Endocrinol Metab* 87:3023–3028, 2002
30. Tiikkainen M, Tamminen M, Hakkinen AM, Bergholm R, Vehkavaara S, Halavaara J, Teramo K, Rissanen A, Yki-Jarvinen H: Liver-fat accumulation and insulin resistance in obese women with previous gestational diabetes. *Obes Res* 10:859–867, 2002
31. Forouhi NG, Jenkinson G, Thomas EL, Mullick S, Mierisova S, Bhonsle U, McKeigue PM, Bell JD: Relation of triglyceride stores in skeletal muscle cells to central obesity and insulin sensitivity in European and South Asian men. *Diabetologia* 42:932–935, 1999
32. Kautzky-Willer A, Krssak M, Winzer C, Pacini G, Tura A, Farhan S, Wagner O, Brabant G, Horn R, Stingl H, Schneider B, Waldhausl W, Roden M: Increased intramyocellular lipid concentration identifies impaired glucose metabolism in women with previous gestational diabetes. *Diabetes* 52:244–251, 2003
33. Thamer C, Machann J, Bachmann O, Haap M, Dahl D, Wietek B, Tschritter O, Niess A, Brechtel K, Fritsche A, Claussen C, Jacob S, Schick F, Haring HU, Stumvoll M: Intramyocellular lipids: anthropometric determinants and relationships with maximal aerobic capacity and insulin sensitivity. *J Clin Endocrinol Metab* 88:1785–1791, 2003
34. Banerji MA, Buckley MC, Chaiken RL, Gordon D, Lebovitz HE, Kral JG: Liver fat, serum triglycerides and visceral adipose tissue in insulin-sensitive and insulin-resistant black men with NIDDM. *Int J Obes Relat Metab Disord* 19:846–850, 1995
35. Ryysy L, Hakkinen AM, Goto T, Vehkavaara S, Westerbacka J, Halavaara J, Yki-Jarvinen H: Hepatic fat content and insulin action on free fatty acids and glucose metabolism rather than insulin absorption are associated with insulin requirements during insulin therapy in type 2 diabetic patients. *Diabetes* 49:749–758, 2000
36. Drenick EJ, Simmons F, Murphy JF: Effect on hepatic morphology of treatment of obesity by fasting, reducing diets and small-bowel bypass. *N Engl J Med* 282:829–834, 1970
37. Luyckx FH, Lefebvre PJ, Scheen AJ: Non-alcoholic steatohepatitis: association with obesity and insulin resistance, and influence of weight loss. *Diabetes Metab* 26:98–106, 2000
38. Goto T, Onuma T, Takebe K, Kral JG: The influence of fatty liver on insulin clearance and insulin resistance in non-diabetic Japanese subjects. *Int J Obes Relat Metab Disord* 19:841–845, 1995
39. Danforth E Jr: Failure of adipocyte differentiation causes type II diabetes mellitus? *Nat Genet* 26:13, 2000
40. Petersen KF, Oral EA, Dufour S, Befroy D, Ariyan C, Yu C, Cline GW, DePaoli AM, Taylor SI, Gorden P, Shulman GI: Leptin reverses insulin resistance and hepatic steatosis in patients with severe lipodystrophy. *J Clin Invest* 109:1345–1350, 2002
41. Colombo C, Cutson JJ, Yamauchi T, Vinson C, Kadowaki T, Gavrilova O, Reitman ML: Transplantation of adipose tissue lacking leptin is unable to reverse the metabolic abnormalities associated with lipoatrophy. *Diabetes* 51:2727–2733, 2002
42. Yang X, Jansson P-A, Nagaev I, Jack MM, Carvalho E, Sunnerhagen KS, Cam MC, Cushman SW, Smith U: Evidence of impaired adipogenesis in insulin resistance. *Biochem Biophys Res Commun* 317:1045–1051, 2004
43. Havel PJ: Update on adipocyte hormones: regulation of energy balance and carbohydrate/lipid metabolism. *Diabetes* 53 (Suppl. 1):S143–S151, 2004
44. Jacob S, Machann J, Rett K, Brechtel K, Volk A, Renn W, Maerker E, Matthaei S, Schick F, Claussen CD, Haring HU: Association of increased intramyocellular lipid content with insulin resistance in lean nondiabetic offspring of type 2 diabetic subjects. *Diabetes* 48:1113–1119, 1999
45. Stettler R, Ith M, Acheson KJ, Decombaz J, Boesch C, Tappy L, Binnert C: Interaction between dietary lipids and physical inactivity on insulin sensitivity and on intramyocellular lipids in healthy men. *Diabetes Care* 28:1404–1409, 2005
46. Perseghin G, Scifo P, Danna M, Battezzati A, Benedini S, Meneghini E, Del Maschio A, Luzi L: Normal insulin sensitivity and IMCL content in overweight humans are associated with higher fasting lipid oxidation. *Am J Physiol* 283:E556–E564, 2002
47. van Loon LJ, Koopman R, Manders R, van der Weegen W, van Kranenburg GP, Keizer HA: Intramyocellular lipid content in type 2 diabetes patients compared with overweight sedentary men and highly trained endurance athletes. *Am J Physiol Endocrinol Metab* 287:E558–E565, 2004
48. Goodpaster BH, He J, Watkins S, Kelley DE: Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. *J Clin Endocrinol Metab* 86:5755–5761, 2001

49. Hwang JH, Stein DT, Balent B, Barillas L, Tonelli J, Rosenbaum M, Hawkins M: Simultaneous quantitative assessment of intrahepatic triglycerides (IHTG) and intramyocellular lipids (IMCL) using ^{1}H MRS in non-diabetic subjects: relationship to insulin sensitivity. In *Proceeedings of the International Society for Magnetic Resonance in Medicine, Honolulu, Hawaii*. International Society for Magnetic Resonance in Medicine, 2002
50. Bjorntorp P: Liver triglycerides and metabolism. *Int J Obes Relat Metab Disord* 19: 839–840, 1995
51. Giannopoulou I, Ploutz-Snyder LL, Carhart R, Weinstock RS, Fernhall B, Goulopoulou S, Kanaley JA: Exercise is required for visceral fat loss in postmenopausal women with type 2 diabetes. *J Clin Endocrinol Metab* 90:1511–1518, 2005
52. Okura T, Nakata Y, Lee DJ, Ohkawara K, Tanaka K: Effects of aerobic exercise and obesity phenotype on abdominal fat reduction in response to weight loss. *Int J Obes (Lond)* 29:1259–1266, 2005
53. You T, Yang R, Lyles MF, Gong D, Nicklas BJ: Abdominal adipose tissue cytokine gene expression: relationship to obesity and metabolic risk factors. *Am J Physiol* 288:E741–E747, 2005
54. Simoneau JA, Colberg SR, Thaete FL, Kelley DE: Skeletal muscle glycolytic and oxidative enzyme capacities are determinants of insulin sensitivity and muscle composition in obese women. *FASEB J* 9:273–278, 1995