Acquired obesity is associated with increased liver fat, intra-abdominal fat, and insulin resistance in young adult monozygotic twins

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Study of monozygotic (MZ) twin pairs discordant for obesity offers a unique opportunity to determine whether acquired obesity increases liver fat. Because MZ twins are perfectly matched for genes as well as for age, sex, and ethnicity and MZ twins also often share the same environment, study of young adults within a narrow age range would seem particularly informative. In the present study, we determined the effects of acquired (subcutaneous) obesity, as defined by body mass index (BMI), percent body fat, and abdominal subcutaneous fat on intra-abdominal and liver fat as well as on insulin resistance in young adulthood by studying 19 MZ twin pairs discordant or concordant for obesity. These twin pairs were identified among 658 MZ pairs from a longitudinal population-based twin cohort.

MATERIALS AND METHODS

Subjects. The study participants were recruited from the FinnTwin16 cohort (13, 31), a population-based, longitudinal study of five consecutive birth cohorts (1975–1979) of Finnish twins and their siblings and parents, identified through the national population registry of Finland. The baseline data collection of all twin pairs, made within 60 days of their 16th birthdays, was initiated in 1991, resulting in 5,661 responses (2,733 full pairs). The response rates were 88 and 93% for boys and girls, respectively. All respondent twins were surveyed again at 17, 18, and 22–27 yr of age. On the basis of responses to items on current weight and height in the latest follow-up questionnaire, twin pairs were recruited to the present study. Zygosity had been earlier assigned on the basis of validated questionnaire items (34). After all monozygotic (MZ) twin pairs (n = 658) were screened, we found 14 pairs with a reported BMI difference of ≥ 4 kg/m², such that one cotwin was nonobese (BMI ~ 25 kg/m²), whereas the other was obese (BMI ~ 30 kg/m²). All 14 pairs were invited, and 10 (5 male and 5 female pairs) participated in the clinical studies. In addition to these discordant pairs, we studied nine concordant MZ pairs with a reported BMI difference of < 2 kg/m² (2 male and 2 female overweight pairs; 3 male and 2 female normal-weight pairs). The growth in height and weight gain from birth to early adulthood in this sample has been described recently (27). All participants were considered healthy (except for obesity) and without medication (except contraceptives) and were not pregnant, based on clinical examination by a physician (K. H. Pietilainen) and a structured psychiatric interview for major psychiatric disorders, including depression, eating disorders, and substance abuse. Their weight had been stable for ≥ 3 mo before the study. Females were scheduled to attend during the follicular phase of their menstrual cycle. Monozygosity was confirmed by genotyping of multiple, informative genetic markers.

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D3S1358 (10 alleles), VWA (12 alleles), FGA (20 alleles), AMEL (2 alleles), THO1 (7 alleles), TPOX (8 alleles), CSF1PO (10 alleles), D5S818 (11 alleles), D13S317 (9 alleles), and D7S820 (11 alleles) at the Paternity Testing Laboratory at the National Public Health Institute, Helsinki, Finland. The purpose, nature, and potential risks of the study were explained to the subjects before their written informed consent was obtained. The protocol was designed and performed according to the principles of the Helsinki Declaration and was approved by the Ethics Committee of the Helsinki University Central Hospital.

Study protocol. The subjects were enrolled from all over Finland. The subjects arrived at the clinical research center the day before the studies. All subjects were instructed by a nutritionist to consume an isocaloric diet and to avoid strenuous exercise for 2 days before admission. To ascertain the type of diet the subjects were consuming, dietary intake was recorded using 3-day food diaries, which were kept within a week after the experiments from Thursday to Saturday, including two working days and one weekend day. The diaries were analyzed by using the program DIET32, which is based on a national database for food composition (Fineli; http://www.ktl.fi/fineli/). Habitual weekly alcohol consumption was assessed from a structured questionnaire.

In each subject, insulin sensitivity was measured using the euglycemic clamp technique, liver fat by proton spectroscopy, intra-abdominal and subcutaneous fat by magnetic resonance imaging (MRI), as detailed below, and the percent whole body fat by using dual-energy X-ray absorptiometry (20) (software version 2.15; Lunar Prodigy, Madison, WI). Waist circumference was measured midway between the spina iliaca superior and the lower rib margin and hip circumference at the level of the greater trochanters (18). Because in both obese (BMI 30–40 kg/m²) and lean (BMI <25 kg/m²) subjects subcutaneous fat accounts for 84–85% of fat mass (42), BMI, percent whole body fat, and subcutaneous fat mass were used as measures of obesity.

Insulin sensitivity. Whole body insulin sensitivity of glucose metabolism (M-value) was determined by using the euglycemic hyperinsulinemic clamp technique (8). The study was performed after a 12-h overnight fast starting at 8:00 AM. Two 18-gauge catheters (Venflon; Viggo-Spectramed, Helsingborg, Sweden) were inserted, one in the antecubital vein in the nondominant arm for infusion of insulin and glucose and the other in a retrograde position in a heated dorsal hand vein on the same arm for withdrawal of arterialized venous blood (21). Insulin (Actrapid Human; Novo Nordisk, Copenhagen, Denmark) was infused in a primed continuous manner for 120 min. The rate of the continuous infusion was 40 mU·m⁻²·min⁻¹. Normoglycemia was maintained by adjusting the rate of 20% glucose infusion based on measurements of plasma glucose, which were performed every 5 min from arterialized venous blood. The M-value (expressed as mg·kg⁻¹·min⁻¹ or mg·kg⁻¹·free mass⁻¹·min⁻¹) was calculated from the glucose infusion rate after correction for changes in the glucose pool size (8). Because hepatic glucose production is already maximally suppressed in nondiabetic subjects at an insulin concentration achieved during infusion of insulin at a rate of 0.5 mU·kg⁻¹·min⁻¹ (50), the M-value mostly reflects glucose uptake. Even if hepatic glucose production were incompletely suppressed, the M-value provides an exact measure of whole body insulin sensitivity, although it does not allow partitioning of insulin action to stimulation of glucose utilization and inhibition of hepatic glucose production. We used fasting insulin as a surrogate of hepatic insulin sensitivity as it has been shown to correlate with insulin suppression of hepatic glucose production (35). Blood samples were taken at 30-min intervals for measurement of serum free insulin and free fatty acid (FFA) concentrations.

Plasma glucose concentrations were measured in duplicate with the glucose oxidase method using a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA) (12). Serum free insulin concentrations were determined with radioimmunoassay (Phadeseph Insulin RIA; Pharmacia and Upjohn Diagnostics, Uppsala, Sweden) after precipitation with polyethylene glycol (9). FFA concentrations were analyzed by a fluorometric method (22).

Liver fat content (proton spectroscopy). Localized single-voxel (2 × 2 × 2 cm³) proton spectra were recorded using a 1.5-T whole body system (Siemens Magnetom Vision, Erlangen, Germany), which consisted of the combination of the body coil and a loop surface coil with a diameter of 19 cm for radiofrequency transmitting and signal receiving. T1-weighted high-resolution magnetic resonance images were used for localization of the voxel of interest within the right lobe of the liver. The proximity of vascular structures and subcutaneous fat tissue was avoided in localization of the voxel. Subjects were lying on their stomachs on the surface coil, which was embedded in a mattress to minimize abdominal movement due to breathing. The single-voxel spectra were recorded by using the stimulated-echo acquisition mode sequence with an echo time of 20 ms, a repetition time of 3,000 ms, a mixing time of 30 ms, and 1,024 data points over 1,000-kHz spectral width with 32 averages. Water-suppressed spectra with 128 averages were also recorded to detect weak lipid signals. The short echo time and the long repetition time were chosen to ensure a fully relaxed water signal, which was used as an internal standard. Chemical shifts were measured relative to water at 4.80 ppm. The methylene signal, which represents intracellular triglyceride, was measured at 1.4 ppm. Signal intensities were quantified by using the analysis program VAPRO-MRUI (http://www.mrui.uab.es/mrui/). Spectroscopic intracellular triglyceride content was expressed as methylene/(water + methylene) signal area × 100. This measurement of hepatic fat by proton spectroscopy has been validated against chemically determined lipid content of liver biopsies in humans (43) and against estimates of fatty infiltration by computed tomography (17, 32). All spectra were analyzed by a physicist (A.-M. Håkkinen), who was unaware of any of the clinical data. The reproducibility of repeated measurement of liver fat in nondiabetic subjects studied on two occasions in our laboratory is 11% (39).

Intra-abdominal and subcutaneous fat (MRI). MRIs were recorded using the body coil as the transmitter and receiver. A series of T1-weighted transaxial scans for the determination of intra-abdominal and subcutaneous fat were acquired from a region extending from 8 cm above to 8 cm below the 4th and 5th lumbar interspace (16 slices, field of view 375 × 500 mm², slice thickness 10 mm, breath-hold repetition time 138.9 ms, echo time 4.1 ms). Intra-abdominal and subcutaneous fat areas were measured using an image analysis program (Alice 3.0; Parexel, Waltham, MA). A histogram of pixel intensity in the intra-abdominal region was displayed, and the intensity corresponding to the lowest intensity between the lean and fat peaks was used as a cut point. Intra-abdominal adipose tissue was defined as the area of pixels in the intra-abdominal region above this cut point. For calculation of subcutaneous adipose tissue area, a region of interest was first manually drawn at the demarcation of subcutaneous adipose tissue and intra-abdominal adipose tissue, as previously described (39). The reproducibility of repeated measurements of subcutaneous and intra-abdominal fat is 3 and 5% (39).

Statistical analyses. Natural logarithmic transformation was performed on data that were not normally distributed. The paired t-test was used to compare means between the leaner and the heavier cotwins. Pearson correlation coefficients were calculated to quantify the association between intrapair differences in the different measures of obesity, as well as between intrapair differences in obesity and intrapair differences in insulin resistance. When twins are analyzed as individuals rather than as pairs, the observations and their error terms between members of a pair may be correlated. Therefore, we adjusted for twin clustering and used survey estimation procedures [SVYMEAN, SVYTEST (Wald test for equality of means), and SVYREG] to derive the proper standard errors, variances, confidence intervals, and P values. All calculations were performed using the Stata statistical software (release 8.0; Stata, College Station, TX). Data are shown as means ± SE unless indicated otherwise. A P value of < 0.05 was considered statistically significant.
RESULTS

Body composition, fat distribution, and liver fat. Physical characteristics of the study groups are shown in Table 1. In all twins, intrapair differences in body weight ranged from 0.1 to 24.7 kg. In the discordant pairs, there was an average 16.4-kg difference in body weight between the cotwins, whereas that in the concordant pairs averaged 2.3 kg. The intrapair differences in BMI ranged from 3.8 to 10.1 kg/m² in the discordant pairs (range of individual BMIs 22.8–33.9 kg/m²) and from 0.0 to 2.3 kg/m² in the concordant pairs (20.0–30.4 kg/m²).

In the discordant pairs, the heavier cotwins had 64% more abdominal subcutaneous fat, 93% more intra-abdominal fat, and 284% more liver fat than the leaner cotwins. Fat distribution was comparable between the cotwins of the concordant pairs (Table 1 and Fig. 1). Examples of MRI scans and liver spectra of discordant and concordant pairs are shown in Fig. 2.

As expected (as the majority of body fat is located in subcutaneous tissues), intrapair differences in measurements of obesity were highly correlated: \( r = 0.83, P = 0.0001 \), between BMI and percent body fat, and \( r = 0.97, P = 0.0001 \), between BMI and subcutaneous abdominal fat. To examine whether the differences in intra-abdominal and liver fat were due to acquired obesity, we calculated correlations between intrapair differences in these parameters and other indexes of obesity. Intrapair differences in all of the different measurements of subcutaneous obesity (BMI, %body fat, subcutaneous abdominal fat) were positively correlated with those in intra-abdominal and liver fat (Fig. 3). From the correlation coefficients, it can be calculated that intrapair differences in BMI, percent body fat, and abdominal subcutaneous fat account for 67, 42,
and 56% of the variation in intra-abdominal fat, but only 33, 12, and 29%, respectively, of the intrapair differences in liver fat. Intrapair differences in intra-abdominal fat accounted for 27% of the variation of the intra-pair differences in liver fat.

Dietary intake and measures of obesity. In the discordant pairs, the leaner and the heavier cotwins, respectively, did not differ with respect to energy intake, the percent energy (E%) from protein (14 ± 1 vs. 15 ± 1 E%), carbohydrate (49 ± 1 vs. 49 ± 2 E%), total (33 ± 2 vs. 30 ± 2 E%), or saturated fat (13 ± 1 vs. 13 ± 1 E%) in the 3-day diaries. However, the leaner cotwins consumed more monounsaturated (9.8 ± 0.5 vs. 8.6 ± 0.5 E%) and polyunsaturated (4.6 ± 0.4 vs. 3.5 ± 0.3 E%) fatty acids, respectively, than the heavier cotwins (P = 0.04 for both). The habitual weekly intake of alcohol was similar in the leaner (3.4 ± 0.9 doses) and heavier (3.8 ± 1.3 doses) cotwins (P = 0.75). In the concordant cotwins, the energy and macronutrient intake and weekly intake of alcohol were similar. In all pairs, there were no significant correlations between intrapair differences in dietary intake and intrapair differences in the measurements of obesity (data not shown).

Components of dietary intake (alcohol intake or %energy from fat, saturated fat, carbohydrate, or protein) were not correlated with measurements of subcutaneous obesity or intra-abdominal fat when all subjects were analyzed as one group of individuals. However, percent energy from fat was significantly correlated with percent liver fat (r = 0.37, P = 0.02). Similarly, percent energy from saturated fat was significantly correlated with percent liver fat (r = 0.38, P = 0.005).

Acquired obesity and insulin resistance. In the discordant pairs, the heavier cotwins had significantly lower M-values and higher levels of fasting insulin than the leaner cotwins, whereas there were no differences between the concordant cotwins (Fig. 1).

The intrapair differences in BMI (r = −0.67, P = 0.002), percent body fat (r = −0.80, P = 0.0001), and abdominal
subcutaneous fat ($r = -0.72, P = 0.001$) correlated negatively with intrapair differences in the M-value, implying that acquired obesity measured by these indexes (which are highly interrelated and all mainly reflect subcutaneous fat mass) accounted for 45, 64, and 52% of the variation in insulin sensitivity. Intrapair differences in the M-value correlated with those in intra-abdominal fat ($r = -0.55, P = 0.015$) but not liver fat ($r = -0.20, P = 0.40$).

The intrapair differences in BMI ($r = 0.70, P = 0.001$), percent body fat ($r = 0.49, P = 0.04$), subcutaneous fat ($r = 0.60, P = 0.008$), intra-abdominal fat ($r = 0.75, P = 0.0001$), and liver fat ($r = 0.49, P = 0.048$) were significantly correlated with those in fasting insulin.

**DISCUSSION**

In the present study, we were able to identify only 14 pairs out of 658 MZ pairs in whom BMI differed by more than 4 kg/m². This finding itself is consistent with previous data demonstrating that obesity is a strong genetic trait (3). Similar data are not available for liver fat. However, by study of the genetically matched cotwins of MZ pairs who were discordant for BMI, we could document that acquired (subcutaneous) obesity is associated with significant increases in intra-abdominal and liver fat and decreases in insulin sensitivity.

Rönnemaa et al. (30) found that, similar to the present data, intrapair differences in intra-abdominal fat were positively correlated with those in fasting insulin in middle-aged identical twins discordant for obesity. Our results document that this relationship is already present in early adulthood. Contrary to the previous study, our findings demonstrate that pairwise differences in overall and subcutaneous obesity are also closely correlated with insulin resistance. Moreover, this is the first study to estimate the contribution of acquired obesity on liver fat.

Although obesity (i.e., an increase in body weight mainly due to an increase in subcutaneous fat mass) was, independent of genetic background, associated with a decrease in insulin sensitivity and an increase in liver fat and other metabolic consequences, this does not necessarily imply a cause and effect relationship. This is because obesity is accompanied by many metabolic alterations that have been shown to cause insulin resistance. One of these in the present study was the increase in intra-abdominal fat. According to the “portal hypothesis,” this leads to an increased flux of FFA into the portal vein and to hepatic insulin resistance (10). Direct proof of this hypothesis has been lacking until recently, when direct measurements of hepatic FFA delivery from visceral adipose tissue lipolysis were shown to be correlated with the amount of visceral fat (26). However, in this study, there were no differences between total FFA flux between obese and lean women or men (26). Not all fat in the liver, however, needs to be derived from the visceral depot. Recent studies have shown that the fraction of FFA that is available for direct storage postprandially following intravascular lipolysis in tissues other than adipose tissue and skeletal muscle is much larger than was previously thought (23). Another mechanism that could cause insulin resistance in both subjects with too much and those with too little subcutaneous fat is adiponectin deficiency, which characterizes both conditions (1, 41). Insulin resistance could also be the consequence of increased local cortisol production in adipose tissue. Overexpression of 11β-hydroxysteroid dehydrogenase-1, the enzyme producing cortisol in adipose tissue, leads to insulin resistance in animals (19) and characterizes both obese (48, 49) and lipoatrophic (40) human adipose tissue. These data thus also suggest that both too much and too little subcutaneous fat may lead to insulin resistance by means of the same mediators.

Several previous studies, especially the pivotal overfeeding experiments in twins (4), have suggested that intra-abdominal fat is controlled more by genetic factors than by subcutaneous fat. We found acquired subcutaneous obesity to explain roughly twice as much of the variation in intra-abdominal (42–67%) than liver fat (12–33%) in the present study. The coefficient of variation of repeated measurements of liver fat was 11% and that of intra-abdominal fat 5% (39). Even when for this difference is accounted for, liver fat still seems to be less influenced by acquired obesity than intra-abdominal fat. This is not unexpected, given that liver fat can be increased even in the absence of subcutaneous fat in both humans (28, 39) and mice (2, 5, 15, 29, 36).

Even if liver fat seems less influenced by acquired obesity than intra-abdominal fat, this does not necessarily imply that liver fat is regulated more by obesity-associated genetic factors than intra-abdominal fat. Acquired factors other than obesity could explain variation in liver fat. In the present study, we found a correlation between the percent energy from total and saturated fat in the diet and liver fat. These correlations may even be deflated, because the 3-day food diary method is liable to underreporting, especially in overweight individuals (11). Nevertheless, the correlations were virtually identical to those we previously found in a group of obese women (44). Consistent with these human data, dogs placed for 6 wk on an isocaloric diet with a modest increase in fat content develop marked hepatic insulin resistance and an increase in serum fasting insulin concentrations with very little change in body weight and no change in peripheral insulin sensitivity (16). Similar data were recently reported in rats (33). Regarding intake of saturated fat and liver fat content, intervention studies in humans have suggested that insulin sensitivity is impaired with a diet rich in saturated fatty acids compared with a diet rich in monounsaturated (46) or polyunsaturated (38) fatty acids.

In conclusion, study of MZ twin pairs discordant for obesity showed that obesity, defined as an increase in body weight that is mostly due to subcutaneous fat mass is associated with increases in intra-abdominal and liver fat and insulin resistance. On the basis of previous studies, it seems possible that the increase in intra-abdominal fat could contribute to the increase in liver fat and that this would lead to hepatic insulin resistance.

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