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ORIGINAL ARTICLE

Arzu Kovanlikaya
Steven D. Mittelman
Andrette Ward
Mitchell E. Geffner
Frederick Dorey
Vicente Gilsanz

Obesity and fat quantification in lean tissues using three-point Dixon MR imaging

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A. Kovanlikaya · V. Gilsanz (✉)
Department of Radiology, MS#81,
Children's Hospital of Los Angeles,
4650 Sunset Blvd., Los Angeles,
CA 90027, USA
E-mail: vgilsanz@chla.usc.edu
Tel.: +1-323-6694571
Fax: +1-323-6667816

S. D. Mittelman · A. Ward · M. E. Geffner
Division of Endocrinology,
Diabetes and Metabolism,
Children's Hospital of Los Angeles,
Los Angeles, CA, USA

F. Dorey
Department of Pediatrics,
Children's Hospital of Los Angeles,
Los Angeles, CA, USA

Abstract ***Background***: It has been suggested that increased hepatic and intramuscular fat is associated with insulin resistance, and that increased pancreatic fat is related to impaired insulin secretion. ***Objective***: We postulated that in obese nondiabetic teenagers insulin levels would be directly related to increases in intramuscular and hepatic fat and inversely related to increases in pancreatic fat. ***Materials and methods***: MRI was used to assess the percentage of fat in the liver, muscle and pancreas in 15 healthy Mexican-American girls, 14–17 years old, with body mass indexes (BMIs) ranging from 17.7 kg/m^2 to 46 kg/m^2. ***Results***: Strong correlations were observed between BMI and fat content in the liver, muscle, and pancreas (r^2s between 0.50 and 0.89; $P < 0.003$). Serum insulin levels were closely associated with fat measures in the muscle and liver ($r^{2s} = 0.63$ and 0.29, and $P = 0.001$ and $P = 0.023$, respectively). In contrast to our hypothesis, fat content in the pancreas was also directly related to insulin secretion ($r^2 = 0.74$; $P = 0.001$). ***Summary***: We conclude that in nondiabetic teenagers, obesity is associated with an increased accumulation of fat in the pancreas without impairment of insulin secretion.

Keywords Obesity · Adolescence · Fat quantification · Pancreas · MR

Introduction

Worldwide, obesity has reached epidemic proportions. More than 30% of children in the United States are overweight or obese, and there is no evidence that the rise in obesity incidence will plateau or decline in the coming years [1, 2]. Insulin resistance commonly occurs in overweight and obese children and is associated with type 2 diabetes, which accounts for a large number of the new-onset cases of diabetes in children [3]. The increasing incidence of type 2 diabetes parallels the increasing occurrence and severity of pediatric obesity [4–6].

In overweight adolescents, excess fat in the muscle cells and hepatocytes interferes with insulin signaling, leading to insulin resistance [7]. As obesity develops, insulin secretion increases parallel to insulin resistance in order to maintain normal glucose homeostasis. Although most people are capable of compensating for insulin resistance by increasing insulin secretion, thus maintaining normal glucose levels, patients predisposed to diabetes fail to compensate adequately for the greater

insulin requirements [8]. It has been suggested that the eventual impairment of insulin secretion in subjects with type 2 diabetes is related to an overload of adipose tissue in the pancreas [9, 10]. Indeed, data from experimental animals indicate that the ectopic deposition of lipids in the pancreas is associated with destruction of islet cells and impaired insulin secretion [11, 12].

Whether variations in pancreatic fat accumulation also account for differences in insulin secretion in humans is unknown because of technical difficulties in measuring fat stores in intra-abdominal organs in vivo. Magnetic resonance (MR) spectroscopy has been used to assess lipid content in skeletal muscle and, less frequently, in the liver, but inaccuracies related to motion have limited its use in the pancreas. The availability of novel magnetic resonance imaging (MRI) techniques that can provide noninvasive assessments of pancreatic composition might allow delineation of the phenotypic characteristics of subjects at risk for type 2 diabetes.

In this study, we used an MR technique that was described by Dixon [13] and provides accurate measures of fat content in any tissue to assess which lean tissues stockpile the most fat in lean and obese subjects. We postulated that in nondiabetic teenagers, increasing body mass would be related to increased fat accumulation in the muscle, liver, and pancreas. We also postulated that even though insulin levels in teenagers would be correlated with the percentage of fat in the liver and muscle (because of compensation for insulin resistance), beyond a threshold, pancreatic fat content would be inversely related to insulin levels (because of impaired insulin secretion).

Materials and methods

Experimental subjects

Fifteen nondiabetic, healthy Mexican-American girls, 14–17 years of age, were recruited from a lifestyle intervention program for overweight youth (Kids N Fitness) in the Division of Endocrinology, Diabetes and Metabolism at Children's Hospital of Los Angeles (CHLA), as well as schools in the area. All obese subjects enrolled in the study had fasting glucose levels and hemoglobin A1c (HbA1c) as part of their participation in the Kids N Fitness program to ensure no presence of type 2 diabetes. Subjects were excluded if they had been diagnosed with any major illness, including pancreatitis, had a condition or had taken medications known to influence body composition, insulin action, or insulin secretion, or if they had a family history of tpe 2 diabetes among first-degree relatives. The investigational protocol was approved by the institutional review board for clinical investigations at CHLA, and informed consent was obtained from all subjects and their parents.

Physical examination

All participants underwent a physical examination by a pediatric endocrinologist to determine their general health and stage of sexual development. Tanner stage of sexual maturity was assessed based on breast development [14]. Only girls in Tanner stage 5 were included in the study. Measurements of total height were obtained to the nearest 0.1 cm using the Harpenden stadiometer (Holtain, Crymmych, Wales), and measurements of weight were obtained to the nearest 0.1 kg using the Scale-Tronix (Scale-Tronix,, Wheaton, Ill., USA). Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters.

Biochemical determinations

Blood was drawn and serum levels of fasting glucose, serum insulin, HbA1c and lipid profiles, including triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and cholesterol were obtained using validated methods. Fasting glucose levels were measured with an YSI 2300 Stat glucose/lactate analyzer (YSI, Inc., Yellow Springs, Ohio, USA). Serum insulin was determined by RIA [15]. HbA1c was measured by high-pressure liquid chromatography [16] using the fully automated Glycosylated Hemoglobin Analyzer System (Bio-Rad Laboratories, Inc., Richmond, Calif., USA). Total cholesterol, HDL- and LDL-cholesterol and triglycerides were measured as previously described [17].

Three-point Dixon MR imaging

Subjects underwent imaging examinations of the liver, pancreas,and soleus muscle in the supine position with the use of a 1.5 T GE MR unit (GE Medical Systems, Milwaukee, Wis., USA). The soleus muscle was selected because it is prevalently composed of slow-twitch oxidative fibers (fiber type I), which have the greatest insulin sensitivity [18]. After positioning the abdomen in a phased array coil, axial images were acquired from the level of the liver and the pancreas (Fig. 1) using the three-point Dixon sequence (not widely commercially available). Thereafter, the subjects were repositioned, and three-point Dixon images from the right calf (Fig. 2) at the level of the largest circumference were acquired using the knee coil. The entire procedure, including positioning and scanning, was completed within 30 min.

After completion of the automated reconstruction of the three-point Dixon images, the images were transferred to a GE Advantage Workstation for analysis. The signal intensity in the images was calculated with operator-defined regions of interest (ROIs) at the same

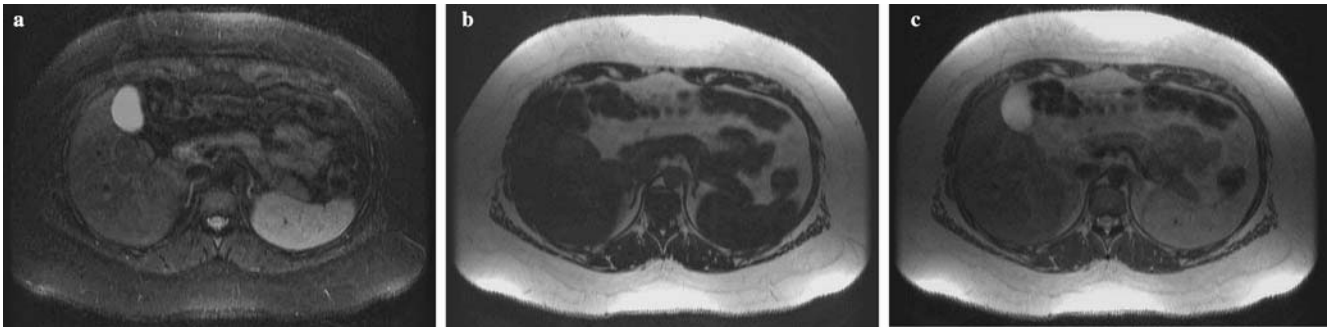


Fig. 1 Dixon technique of liver and pancreas. Water (**a**), fat (**b**), and combined water and fat images (**c**) of the liver and pancreas

location in both fat and water images. A total of three ROIs per organ (liver, pancreas, soleus muscle) were acquired and averaged, with care given to placement of the ROIs to avoid confounding anatomy (e.g., large blood vessels). Fat/water ratios were calculated on a pixel-by-pixel basis by dividing the signal intensity calculated from the fat image by the signal intensity calculated from the water image.

The use of decomposing fat and water signals to discriminate between fat and water protons based on their resonant frequency difference was first introduced by Dixon [13]. This method uses two acquisitions with a delay between the radiofrequency (RF) and gradient echoes, such that the phase shift between water and fat is either 0 or radians (in-phase and out-of-phase, respectively). Separate water and fat images can be obtained by adding and subtracting the in-phase and out-of-phase images. This method was subsequently enhanced to accommodate magnetic field (B_0) and RF inhomogeneity through the use of a third acquisition, leading to the three-point Dixon method [19, 20]. The resulting sequence produced three registered volumes with the refocusing pulse time shifted to produce phase differences of 0, π, and $-\pi$ radians. The pulse sequence parameters were: TR/TE = 4,000/54 ms, echo train length = 10, slice thickness 3 mm, matrix = 512×192, NEX = 1, and a total scan time 8:48 min. In vitro reproducibility of the quantitative fat fraction measurements in phantom models using this technique has been calculated to be 1.27% [21]; in vivo reproducibility in the liver, muscle, and pancreas was between 2.3% and 4.6% in normal volunteers.

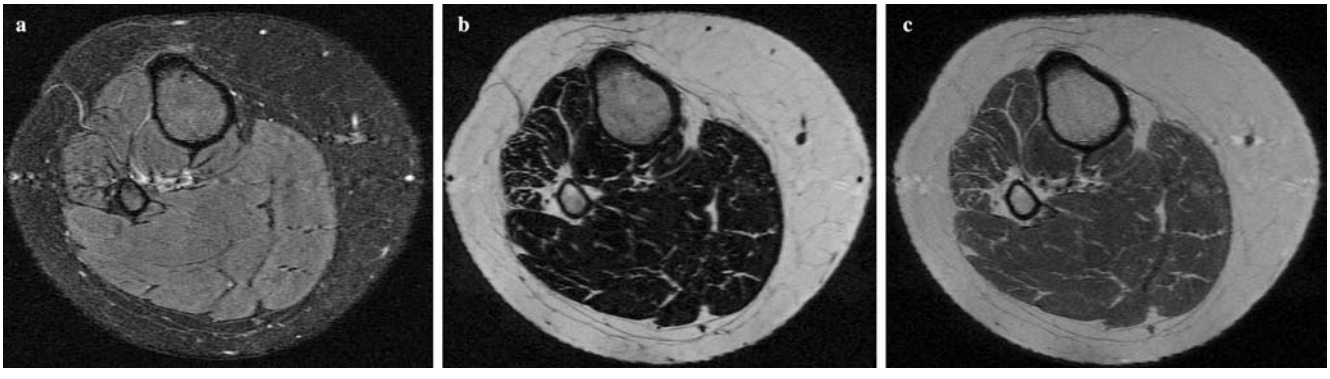
Statistical analysis

Statistical analysis was carried out using STATA for windows (version 8.0, STATA, College Station, Tex., USA). Data are expressed as means ± SD. Statistical analyses included the Pearson correlation and linear regression. Comparison of the slopes of the fat content in liver, muscle, and pancreas was made using the difference in the fat content as the dependent variable and either BMI or insulin as the independent variable. Analyses were conducted for all subjects together and for lean and obese teenagers independently. For the purpose of this study, lean subjects were defined as those who had a BMI of <25, and obese subjects as those with a BMI of ≥ 25.

Results

Age, anthropometric and metabolic data, and the percentage volumes of fat in the liver, muscle, and pancreas

Fig. 2 Dixon technique of muscle. Water (**a**), fat (**b**), and combined water and fat images (**c**) of the calf muscles



in all subjects and in lean and obese subgroups are summarized in Table 1. There was a wide range of values for BMI in study subjects, from 17.7 to 46.0. Moderate correlations were found between weight and BMI and serum levels of triglycerides, HDL, and HgA1C (Table 2). Strong correlations were observed between BMI and the liver ($R^2 = 0.50$), muscle ($r^2 = 0.80$) and pancreas ($r^2 = 0.89$) fat contents; the strongest associations were seen with muscle and pancreatic fats (Fig. 3). BMI was also strongly correlated with serum insulin, implying a state of compensated insulin resistance in the obese subjects (Fig. 4). As expected, the levels of serum insulin were closely associated with measures of adiposity in the muscle and the liver. However, in contrast to our hypothesis, serum insulin levels in the teenagers studied were also positively associated with fat content in the pancreas (Fig. 5). Because of the high correlation between adiposity in the pancreas and in the muscle ($R^2 = 0.82$), it was not possible to determine which variable was more influential in relation to insulin levels; multivariate analyses indicated that when either muscle or pancreatic fat was taken into account, fat content in the other tissues did not enter into the equation.

Lean and obese nondiabetic teenagers had similar values for fasting glucose, HgA1C and cholesterol. In contrast, measures for fasting serum insulin were significantly higher, and those for HDL were significantly lower in obese subjects than in lean subjects. On average, obese teenagers stored 50–150% more fat in the liver, muscle, and pancreas than lean subjects. Significant correlations were present between BMI and measures of fat in the liver, muscle, and pancreas of the obese and lean subgroups; the strongest were seen between BMI and muscle fat content; r^2 $s = 0.59$ and 0.69 for lean and obese subgroups, respectively. In obese subjects, strong correlations were seen between values for fasting serum insulin and measures of adiposity in the muscle and the pancreas; r^2 $s = 0.50$ and 0.52, respectively.

Table 2 Correlations between biochemical determinations and weight and BMI in 15 teenagers

Variable	Weight		BMI	
	r value	*P* value	*r* value	*P* value
Fasting glucose (mg/dl)	0.313	0.256	0.239	0.388
Hemoglobin A1C (mg/dl)	0.574	0.025	0.537	0.039
Serum insulin (μIU/ml)	0.823	0.001	0.796	0.001
Triglycerides (mg/dl)	0.540	0.038	0.497	0.060
Cholesterol (mg/dl)	0.018	0.948	0.041	0.885
HDL (mg/dl)	−0.513	0.050	−0.496	0.060
LDL (mg/dl)	0.155	0.581	0.184	0.511

Discussion

The findings of this study corroborate data indicating that obese subjects have greater intramuscular and hepatic fat and increased insulin resistance, resulting in increased insulin levels. Our results also demonstrated that when compared with normal-weight subjects, obese teenagers had greater fat accumulation in the pancreas. However, in contrast to our hypothesis, the greater proportion of pancreatic fat in the obese nondiabetic subjects studied was associated with increased, not decreased, insulin levels.

We postulated that the ability of the pancreas to maintain a high insulin secretory rate declines with an increasing accumulation of pancreatic fat. This supposition was based on evidence that the clinical onset of type 2 diabetes is preceded by years of insulin resistance and a shorter period of decreased insulin secretion. Al-

Table 1 Mean values for age, anthropometric measures, fasting biochemical determinations, and MR fat percentage in ectopic fat depots in all subjects and in lean and obese groups (*BMI* body mass index)

Variable	All ($n = 15$)	Lean (BMIn = 6)	Obese (BMI≥25) ($n = 9$)	*P* value
Age (years)	16.6 ± 1.2	17.1 ± 0.7	16.2 ± 1.4	
Anthropometric				
Weight (kg)	84.9 ± 31.5	53.6 ± 7.7	105.8 ± 21.7	–
BMI (kg/m^2)	30.7 ± 10.2	20.5 ± 2.3	37.5 ± 6.9	–
Biochemical				
Glucose (mg/dl)	81.2 ± 7.2	78.7 ± 8.3	82.9 ± 6.4	0.285
Hemoglobin A1C (mg/dl)	5.3 ± 0.3	5.1 ± 0.2	5.3 ± 0.3	0.116
Serum insulin (μIU/ml)	20.9 ± 14.2	8.7 ± 5.2	29.0 ± 12.2	0.002
Triglycerides (mg/dl)	104.3 ± 33.6	84.7 ± 31	117.3 ± 29.9	0.062
Cholesterol (mg/dl)	170.9 ± 30.0	173.8 ± 34.3	168.9 ± 28.8	0.767
HDL (mg/dl)	50.1 ± 13.6	60.2 ± 14.6	43.4 ± 8.1	0.013
LDL (mg/dl)	99.8 ± 25.7	96.5 ± 27.5	102 ± 25.9	0.700
MR fat (%)				
Liver	23.7 ± 8.8	17.3 ± 4.1	28 ± 8.7	0.016
Muscle	19.6 ± 5.9	14.9 ± 2.7	22.8 ± 5.3	0.007
Pancreas	30.1 ± 14.6	15.6 ± 2.6	39.7 ± 10.4	0.002

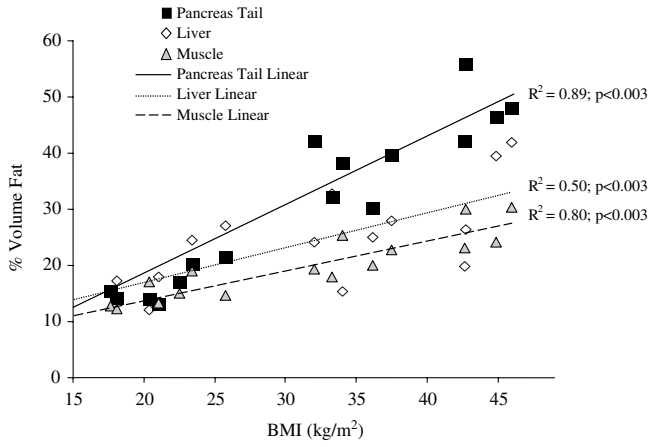


Fig. 3 The correlation between BMI and % volume fat in the liver, pancreas, and muscle in lean and obese subjects

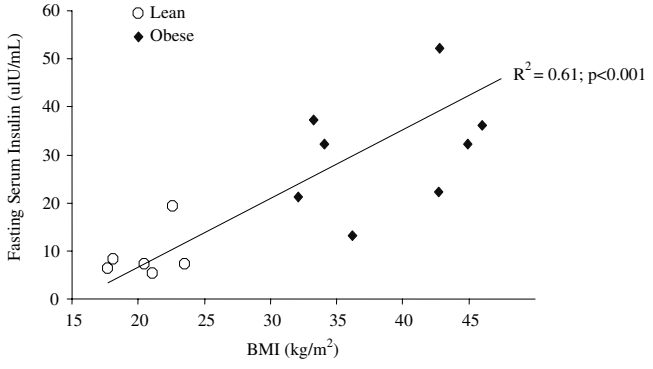


Fig. 4 The correlation between BMI and fasting serum insulin in lean and obese subjects

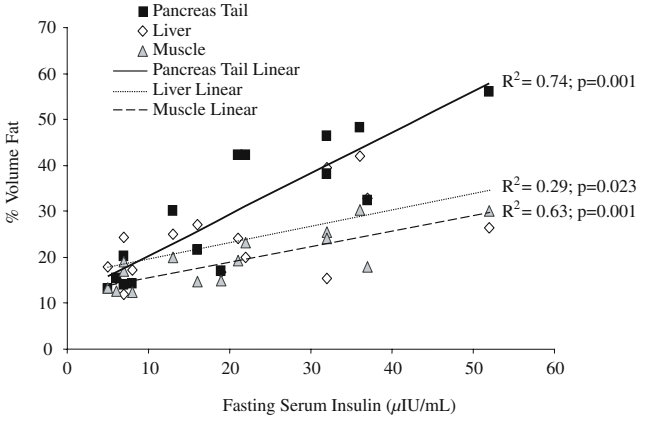


Fig. 5 The correlation between fasting serum insulin and percentage of volume fat in the liver, pancreas and muscle in lean and obese subjects

though the mechanism for the failure of insulin production has not been well-delineated, it might be related to lipotoxicity of β-cells [22]. Data from in vitro studies suggest that ectopic deposition of lipids in the pancreas is associated with destruction of islet cells and impaired insulin secretion [23]. In addition, studies in obese rats indicate that increases in triglyceride content in β-cells result in β-cell damage [24]. Our hypothesis was also supported by the knowledge that progressive fat replacement in the pancreas of patients with cystic fibrosis (CF) coincides with increasing disruption of islet morphology and β-cell apoptosis; in cases of CF, diabetes is observed in 9% of children, 26% of adolescents, 35% of adults ages 20–29%, and 43% of adults age 30 and older [25, 26]. Lastly, there is increasing evidence from studies in experimental animals that the use of thiazolidinediones (TZDs) leads to decreased islet triglyceride stores [27] and increased islet cell insulin [28, 29]. Several recent trials of diabetes prevention in humans have shown that TZDs preserve β-cell function, and it has been proposed that the mechanism of this effect is a decrease in lipotoxicity in the pancreatic β-cell [22].

There are several possible explanations for our unexpected results. One might be that the degree of pancreatic fat accumulation had not reached a hypothetical threshold, above which insulin secretion deteriorates. Another is the possible high variability in pancreatic fat accumulation among obese teenagers. However, the cohort studied included markedly obese subjects, and there was a strong correlation between pancreatic fat and weight, minimizing the likelihood of the above possibilities. It is more probable that there is variability in genetic susceptibility to lipotoxicity, or the deleterious effect of adipose tissue deposition in the pancreas might require a long time before manifesting in impaired β-cell function. Indeed, it has been estimated that pancreatic β-cell damage is present for more than a decade before diabetes is diagnosed [30]. Thus, there might be a lengthy period in which lipotoxicity-induced β-cell damage is associated with normal β-cell function.

Until recently, studies supporting the concept that lipotoxicity in humans contributes to a decline in β-cell function through increased apoptosis have been limited to extrapolations from observations of experimental animal models. In this study, the three-point Dixon MR technique was used to obtain quantitative measures of hepatic, muscular, and pancreatic fat. Because the number of islets of Langerhans in the pancreas is about two times greater in the tail of the pancreas than in the head, we specifically measured the percentage of fat in the tail [31]. Previously, studies using multiple phantoms with various fat volumes (0–50%) conducted at our institution indicated that measures of fat obtained with the three-point Dixon technique correlated strongly with true fat ratios ($r^2 = 0.98$; $P < 0.001$) [21].

It should be noted that MR measures of pancreatic fat using the three-point Dixon method are influenced by both the fat content in the islet cells, which is believed to be related to lipotoxicity, and extracellular fat accumulation. Although it is not possible to quantify adipose tissue independently in these two compartments with this technique, studies in experimental animals suggest that, with obesity, the intraislet lipid content increases disproportionately to the extracellular fat [24]. In humans, autopsy studies show that the amount of adipose tissue correlates positively with age and body weight [32] and that, with aging, more fat accumulates in the pancreatic β-cells than in the exocrine pancreas [33, 34]. More recently, studies with I^{125}-labeled LDLs have shown the presence of high-affinity LDL receptors on β-cells, but not on neighboring α-cells [33]. Because with progressive obesity the proportion of fat that accumulates in the intracellular compartment increases, MR measures of total fat using the three-point Dixon technique likely reflect intracellular fat content.

In summary, this study represents the first in vivo measurement of the ratio of pancreatic fat and water in lean and obese subjects. Further studies are needed to establish the degree to which fat accumulation in the pancreas is associated with impaired β-cell function and decreased insulin secretion. The future application of novel MRI techniques to assess noninvasively the composition of the pancreas might eventually be useful in delineating the phenotypic characteristics of subjects at risk for type 2 diabetes.

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