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Intrahepatic lipid, not visceral or muscle fat, is correlated with insulin resistance in older, female Rhesus macaques

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Abstract

Objective—Little is known of the effect of body composition on glucose metabolism in the aging female non-human primate. We studied these variables in older female Rhesus macaques.

Design and Methods—Female Rhesus macaques (*Macacamulatta*, n = 19, age range 23–30 yrs) underwent magnetic resonance imaging and ^{1}H spectroscopy to quantify total abdominal fat, visceral fat (VF), subcutaneous fat (SF) area, extramyocellular lipid (EMCL), intramyocellular lipid (IMCL) and intrahepatic lipid (IHL) content, and DEXA scan for whole body composition. A subgroup (n=12) underwent a fasting blood draw and intravenous glucose tolerance test.

Results—SF correlated with homeostatic model assessment of insulin resistance ($HOMA_{IR}$) and quantitative insulin sensitivity check index (QUICKI), but not after adjustment for fat mass. IHL demonstrated the strongest correlation with $HOMA_{IR}$, QUICKI and calculated insulin sensitivity

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BK conceived the study design, analyzed and interpreted data

CK conceived the study design, collected, analyzed, and interpreted data, generated figures, and participated in writing of the manuscript

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index (CS_I), and remained significant after adjustment for fat mass. VF, IMCL, and EMCL did not correlate with any of our measures of insulin sensitivity.

Conclusions—Despite a greater amount of VF compared to SF, VF was not associated with markers of insulin resistance (IR) in the older female monkey. Instead, IHL is a marker for IR in the fasting and post-prandial state in these animals.

Keywords

Body Composition; Insulin Resistance; Magnetic Resonance; Fat Distribution; Obesity

Introduction

In the United States, the prevalence rates of obesity and diabetes have risen in parallel over the previous decade (1) and both are recognized as global health threats. Numerous studies have sought to understand the mechanisms by which obesity and insulin resistance are interrelated. Those with central obesity are characteristically insulin resistant and at a high risk for developing diabetes and heart disease (2–9). Using imaging methods to quantify fat in specific abdominal depots in humans, visceral fat (VF) has been shown to correlate more strongly with insulin resistance than subcutaneous fat (SF) (10); and, in patients with type 2 diabetes, increased visceral fat is associated with poorer glycemic control, decreased peripheral insulin sensitivity, and increased gluconeogenesis (11). Using the more sensitive technique of proton magnetic resonance spectroscopy (^{1}H MRS) to measure fat deposited ectopically into specific organs, insulin resistance has been shown to correlate positively with increased intramyocellular (IMCL) lipid levels (5, 6, 12), an association supported mechanistically by prospective studies demonstrating that free fatty acid infusions lead to accumulation of IMCL by ^{1}H MRS and insulin resistance (13). ^{1}H MRS has also been employed to demonstrate associations between intrahepatic lipid (IHL) content and insulin resistance in humans (14, 15). The ectopic accumulation of triglyceride in muscle and liver as a cause of insulin resistance is supported by animal studies (16–18) and by prospective studies in humans demonstrating that depletion of IMCL and liver fat with drug therapy predicts improved insulin sensitivity (19, 20). As a whole, these findings suggest that increased visceral adipose tissue and ectopic accumulation of lipids, including muscle and liver, are strongly linked with impaired glucose metabolism.

While rodents are convenient for studying the interactions between regional or ectopic fat accumulation and insulin resistance (21), non-human primates have been proposed as a more relevant model (22, 23) due to having similar genetic and metabolic traits to humans (24). In baboons, a negative correlation between obesity and insulin sensitivity has been demonstrated (24). Furthermore, intrahepatic triglyceride content measured by liver biopsies in obese insulin resistant baboons is positively associated with both hepatic and peripheral insulin resistance (25). To date, the relationships between visceral fat and ectopic fat, in liver and muscle, as measured by magnetic resonance imaging (MRI) and ^{1}H MRS, and insulin resistance has not been described in the non-human primate. Based on published findings in humans, we set out to explore the relationships between ectopic fat and insulin resistance in a primate model of aging. We hypothesized that increasing adiposity, especially visceral fat,

intrahepatic lipid, and intramyocellular lipid content, would positively correlate with insulin resistance in older female monkeys.

Methods and Procedures

Nineteen female Rhesus macaques (*Macacamulatta*) maintained at the Oregon National Primate Research Center (ONPRC) with a mean (range) age of 25 (23–30) years were included in this study. These monkeys underwent neurocognitive testing as part of an unrelated study investigating the effects of ovarian steroids on cognitive function. The monkeys were pair caged whenever possible, and maintained under controlled lighting (lights on from 7:00 – 19:00 h) and temperature (24 C). The animals were fed a complete balanced diet (Purina monkey chow; Purina Mills Inc., St. Louis, MO) supplemented with fresh fruit and vegetables. Animal care was provided by the ONPRC Division of Animal Resources (DAR) in accordance with the National Research Council *Guide for the Care and Use of Laboratory Animals*, and the experiments were approved by the Oregon Health and Science University (OHSU) Institutional Animal Care and Use Committee. All 19 monkeys underwent body composition analysis, and 13 underwent additional glucose tolerance testing. One of these animals which had insulin values > 10 times the standard deviation of the group was considered an outlier and was excluded from data analysis. One animal had missing fasting insulin measurements and another animal had missing dual energy x-ray absorptiometry (DEXA) data and missing MRS data on IMCL and EMCL content. In total there were 18 monkeys included for analysis of body composition and 12 monkeys included for analysis of body composition and glucose metabolism.

We compared outcomes between those with an intact hormonal status (intact ovaries or ovariectomy with replacement estrogen or estrogen plus progesterone) versus those after ovariectomy (n = 10 vs. 8, respectively for body composition measures, and n = 7 vs. 5, respectively for glucose metabolism measures). We found no significant differences in these measurements between the groups (data not shown) and, therefore, to improve the power of our analyses, all animals were combined into one group.

Intravenous Glucose tolerance test

All animals underwent an intravenous glucose tolerance test (IVGTT) in a fasting state as follows: glucose was given intravenously at a dose of 0.6 grams/kg, blood was taken for measurements of insulin and glucose at baseline (just prior to glucose injection) and then 1, 3, 5, 10, 20, 40, and 60 minutes post injection. Glucose was measured using a One Touch Ultra glucometer (LifeScan/Johnson & Johnson, Milpitas, CA, USA) (26). The same meter was used for all animals and internally validated against a YSI 2300 STAT (YSI) (Yellow Springs Instruments Incorporated, Yellow Springs, OH) using a subset of samples (n=100) (data not shown). Insulin concentrations were measured with a chemiluminescent immunoassay using the automated immulite system (Siemens Healthcare Diagnostics, Deerfield, IL). This method has an analytical sensitivity of 2.00 μU/ml and an inter-assay COV of 6.4%.

Body Composition

All animals underwent DEXA scanning (QDR Discovery model; Hologic, Bedford, MA) for measurements of total mass, fat mass and lean body mass, followed by magnetic resonance imaging (MRI) and spectroscopy on a 3T MRI instrument (Siemens Magnetom Trio; Siemens, Erlangen, Germany) for total abdominal fat, SF, and VF area; and ^{1}H MRS for IHL, IMCL and extramyocellular lipid (EMCL) content. To accomplish this, monkeys were anesthetized with ketamine, intubated, placed on a ventilator, and maintained on isofluorane during the MRI/MRS session. The use of a ventilator allowed for breath holds during image acquisition in order to reduce artifact. Using an extremity radiofrequency coil for 3T MRI, axial, sagittal and coronal localizer images of the abdomen were obtained followed by non-gated, high resolution anatomical imaging. 14 axial slices were obtained with 3-mm slice lengths and a 0.45-mm gap between slices. For abdominal fat content, a single transverse slice was analyzed at the level of the umbilicus with repetition time (TR) = 100ms, echo time (TE) = 2.66ms, flip angle = 70°, field of view = 240 x 180 mm^2, matrix 320 x 240mm, and 0.75 x 0.75 x 3.45 mm^3 voxels. Breath holds were performed on the ventilator while obtaining these data. Abdominal fat content was measured as VF and SF (Figure 1A) and are expressed as cross-sectional area (mm^2). Liver MRS data were obtained using a single voxel technique using stimulated echo acquisition mode (STEAM) sequences with voxel size = 10 x 10 x 10 mm^3 with care to avoid inclusion of major vessels in the liver, TR = 5000ms, TE = 30ms, and water suppression off. The number of signal averages for liver ^{1}H measurements was 1, and this measurement was repeated three times and lipid and water signals were fitted. Normalized IHL results reflect the average of up to three measurements. MRS data was processed using NUTS software (ACORN NMR Inc., Livermore, CA). Peak areas of water and lipid were obtained using scanner software analysis of the data (Figure 1B). Lipid signals were treated as one composite peak and integrated from 1.3 to 1.5 ppm. Water was integrated as a single peak at 4.7 ppm. The result was expressed as the ratio of total lipid / water peak (%) as a measure of IHL. Sagittal and coronal localizer images of the leg were obtained followed by a T_1 weighted high resolution anatomical image. Using a wrist coil, soleus muscle MRS data were obtained using a single voxel technique using STEAM sequences with voxel size = 10 x 10 x 10 mm^3, TR = 5000ms, TE = 30ms, NSA = 32, and water suppression on. Signal intensities were assessed by integration of peaks centered at 3.0 ppm for creatine, 1.5 ppm EMCL and 1.3 ppm for IMCL. The result was expressed as a ratio of total lipid / creatine peak (%) as a measure of IMCL and EMCL (Figure 1B). Reproducibility and repeatability rules were followed as previously described (27). Briefly, all MR images and liver and muscle lipid MRS analyses for each animal were performed by a single analyst who repeated the measures until at least two successive measures agreed with less than 5–10% variability. MRS values less than 0.5% were set to 0.5 %.

Statistical Methods

Area under the curve (AUC) values were calculated for glucose and insulin measurements from the IVGTT using the trapezoidal method. Insulin sensitivity was estimated by three methods: 1) using the Homeostatic model of assessment for insulin resistance ($HOMA_{IR}$) (glucose mg/dL x insulin μU/ml) / 405), (28); 2) Quantitative Insulin Sensitivity Check Index (QUICKI) (1 / (log(fasting insulin μU/ml) + log(fasting glucose mg/dL))) (29), both

of which primarily reflects insulin sensitivity at the level of the liver in the fasting state and have been validated against the hyperinsulinemic, euglycemic glucose clamp in Rhesus monkeys (30); and 3) using the IVGTT data to determine the calculated sensitivity index (CS_I), which has been validated against the minimal model and clamp methodologies in humans (31). Group means were compared using the Mann-Whitney Rank Sum Test. Correlational analysis was conducted using linear regression or multiple linear regression on untransformed data if normally distributed, or natural log-transformed data if non-normally distributed. Tertiles of AUC for glucose and insulin from the IVGTT were compared using Kruskal-Wallis One Way Analysis of Variance on Ranks. Statistical analyses were conducted using SigmaStat 3.5 (Systat Software Inc., San Jose, CA). $P < 0.05$ was considered statistically significant.

Results

Body composition

The mean (range) of body weight was 7.95 kg (6.45–9.40) (Table 1). Percent fat varied two-fold within the group as a whole. The average VF area was nearly two times greater than SF area (mean 4240 mm^2 vs. 2200 mm^2, $P = <0.001$) (Table 1). The mean IHL was 1.81 % with a range of 0.50–4.76% compared to a mean IHL of 4.69% and a range of 0–47.5% previously reported in humans (32). Increasing fat mass correlated with IHL (r = 0.64, $P = 0.004$), SF (r = 0.65, $P = 0.003$), and VF (r = 0.56, $P = 0.017$), but not IMCL (r = −0.13, $P = 0.62$) or EMCL (r = −0.01, $P = 0.97$). Increasing IMCL correlated with EMCL (r = 0.63, $P = 0.006$), but was not associated with IHL (r = 0.30, $P = 0.24$), VF (r = 0.26, $P = 0.31$), or SF (r = −0.02, $P = 0.93$). Increasing IHL correlated with VF (r = 0.55, $P = 0.019$), but not SF (r = 0.39, $P = 0.11$), IMCL (r = 0.30, $P = 0.24$) or EMCL (r = 0.12, $P = 0.65$).

Glucose Metabolism

The mean (range) of fasting glucose was 57 mg/dL (46–83 mg/dL) and mean (range) of fasting insulin was 19.8 μU/ml (5.9–36.7 μU/ml). Fat mass correlated with fasting insulin (r = 0.82, $P = 0.002$) (Table 2), IVGTT AUC glucose (r = 0.60, $P = 0.038$), and IVGTT AUC insulin (r = 0.69, $P = 0.018$), but not with fasting glucose (r = 0.44, $P = 0.15$). SF correlated with fasting insulin (r = 0.79, $P = 0.004$) and IVGTT AUC glucose (r = 0.73, $P = 0.008$) but not with fasting glucose (r = 0.44, $P = 0.16$) or IVGTT AUC insulin (r = 0.44, $P = 0.18$). IHL correlated with fasting glucose (r = 0.59, $P = 0.04$), fasting insulin (r = 0.86, $P < 0.001$), and AUC insulin (r = 0.74, $P = 0.009$), but not AUC glucose (r = 0.47, $P = 0.12$) (Table 2, Figure 2). We analyzed the glucose and insulin profiles of animals divided into tertiles by the greatest (n = 4) and lowest amount of IHL (n = 4). There was no significant difference between tertiles for AUC glucose (p = 0.20) but AUC insulin was significantly greater in highest tertile of IHL compared to the lowest tertile (p = 0.02) (Figure 2). Interestingly, neither IMCL, EMCL, nor VF correlated with any measure of insulin sensitivity (Table 2).

Fat mass, SF, and IHL all correlated positively with $HOMA_{IR}$ (r = 0.77, $P = 0.006$; r = 0.72, $P = 0.01$; and r = 0.81, $P = 0.002$ respectively) and negatively with QUICKI (r = −0.75, $P = 0.008$; r = −0.70, $P = 0.02$; and r = −0.78, $P = 0.005$ respectively) (Table 2 and Figure 3). As mentioned above, SF and IHL varied in proportion to fat mass. After adjusting SF for fat

mass, there was a loss of significance to $HOMA_{IR}$ (r = 0.48, $P = 0.14$) and QUICKI (r = −0.46, $P = 0.15$). On the other hand, the correlation of IHL adjusted for fat mass remained significantly associated with $HOMA_{IR}$ and QUICKI (r = 0.73, $P = 0.01$ and r = −0.68, $P = 0.02$ respectively) (Table 2 and figure 3). There was a non-significant trend towards a correlation of SF to CS_I (r = −0.49, $P = 0.11$), however this trend was lost after correcting for fat mass (r = −0.15, $P = 0.64$). Both IHL and fat mass were negatively associated with CS_I (r = −0.78, $P = 0.003$ and r = −0.78, $P = 0.003$ respectively). As with $HOMA_{IR}$ and QUICKI, IHL remained significantly correlated after adjusting for fat mass (r = − 0.61, $P = 0.03$).

Age did not directly correlate with any of the measures of glucose metabolism. Even so, including age as an independent variable using multiple linear regression analyses resulted in minor changes in the significance levels for SF and FM, however associations with IHL remained unchanged (data not shown).

Discussion

In the older female monkey, despite having greater amounts of VF compared to SF, SF was associated with insulin resistance (as estimated by $HOMA_{IR}$) and insulin sensitivity (as estimated by QUICKI and CS_I) but VF was not. This is in contrast to findings in humans, which have shown nearly three times more SF than VF in post-menopausal women (33) and independent associations between both VF and SF and insulin resistance (10). Of the measures of ectopic fat in liver and muscle, only IHL content was significantly correlated with $HOMA_{IR}$, QUICKI and CS_I. Following adjustment for total fat mass, only IHL, and not SF, remained significantly associated with $HOMA_{IR}$, QUICKI, and CS_I, indicating that the relationship between IHL and insulin resistance was independent of the effects of accumulation of total fat mass. $HOMA_{IR}$ and QUICKI calculations involve fasting levels of glucose and insulin and are thought to represent hepatic insulin sensitivity in a basal steady-state. The CS_I calculation makes use of 1-hour glucose tolerance test data and is a good approximation of minimal model analysis and clamp insulin sensitivity (31), essentially quantifying disappearance of glucose per rate change in insulin level. Thus, in our animals IHL was negatively associated with insulin sensitivity and glucose tolerance both in the fasting state and in response to a glucose load, suggesting that IHL is associated with both hepatic and peripheral insulin resistance. On the other hand, SF was negatively associated with insulin sensitivity only in the fasting state and these effects were not independent of the effects of fat mass on insulin sensitivity.

Mechanisms of insulin resistance can be approached from several perspectives, including analyses of adipose tissue depots, organ-specific lipid content, and finally, on a cellular basis. From an adipose tissue depot perspective, increased visceral fat in humans has historically been considered to have the strongest link to insulin resistance (10, 11) and the absence of associations between VF and our surrogate measures of insulin sensitivity in primates in our study was an unexpected finding. However, recent data in humans challenging a primary role for VF accumulation in the expression of insulin resistance may offer insight into this unanticipated finding. For example, Fabbrini et al. (34) studied one group of obese subjects who were matched on visceral adipose tissue but differed in their

intrahepatic triglyceride content as well as another group that were matched on intrahepatic triglyceride content but differed in their amount of visceral adipose tissue, thereby allowing for independent associations of intrahepatic triglyceride content and visceral adipose tissue on metabolic function. Using stable isotope, euglycemic-hyperinsulinemic clamps and tissue biopsies, it was demonstrated that intrahepatic triglyceride content was a better marker for both peripheral insulin resistance and hepatic glucose output than visceral adipose tissue (34). Furthermore, Lim et al. (35) showed that acute restriction of dietary energy intake in type 2 diabetics improved hepatic insulin sensitivity, but not peripheral insulin resistance, in association with decreased IHL content. Whether accumulation of IHL directly causes hepatic insulin resistance or is a marker cellular metabolic dysfunction as suggested by some studies (36, 37), our data in non-human primates is consistent with that in humans indicating increasing insulin resistance is more closely related to increasing IHL than VF.

Lipid content of skeletal muscle is also associated with insulin resistance. IMCL and EMCL content as measured by ^{1}H-NMR spectroscopy was significantly greater in obese adolescent humans compared to lean controls with IMCL inversely correlated with insulin sensitivity (4–7). We did not find an association with IMCL to any of our measures of insulin resistance in the non-human primate as has been shown in previous human studies. We chose to image the soleus muscle to measure IMCL content as it is the muscle typically evaluated in human studies (4, 7). However, because the lower limb muscle groups usage is much different in monkeys than humans it is possible that an alternative leg muscle group would show a positive relationship between IMCL content and insulin resistance in the non-human primate. Another factor of our study design that may have increased variability in our muscle lipid signals and reduced our ability to detect significance with measures of glucose metabolism was our choice to normalize the data to the creatine signal as previously reported (5, 6, 12, 13, 38, 39). An alternative for future studies approach that may reduce this variability would be to reference the lipid signal to the water signal instead.

Several additional limitations to our study should be noted. Only a single MR image was captured for quantification of SF and VF as opposed to whole abdominal region imaging. Previous studies have demonstrated the superiority of multi-slice 3-dimensional volumetric MRI measurements of intra-abdominal fat content over single slice quantitation, especially for inter-subject comparisons (40). Due to the small number of animals included in our study, the use of single slice MRI determinations may have led to reduced accuracy and increased variability in the SF and VF measurements, potentially reducing the strength of associations of the glucose metabolism measurements with these fat depots. We also relied on indirect measures of insulin resistance instead of more direct insulin clamp methodology, which limits our conclusions regarding specific relationships between fat accumulation and organ (muscle, liver) insulin sensitivity. A relatively small number of animals were included in the analysis of body composition and insulin sensitivity reported here. In order to achieve enough power to detect significant trends, primates assigned to treatment groups that varied by hormonal status (intact vs. ovariectomy; with and without estrogen/progesterone replacement therapy) and ages were combined. Although we could not find significant differences in body composition or ages and glucose metabolism outcomes between these treatment group assignments, studies involving larger numbers of animals by our group will address the role of early perimenopausal hormone replacement on body composition and

diabetes risk in the non-human primate. Lastly, our protocol did not include measures of cell-specific insulin signaling using liver or muscle biopsies, blood levels of cytokines or inflammatory markers, or lipid levels, which would provide more mechanistic links between adiposity and insulin resistance.

In summary, using DEXA, MRI, and ^{1}H MRS to assess whole body, regional, and ectopic adiposity, and $HOMA_{IR}$, QUICKI and CS_I as surrogate measures of insulin sensitivity, IHL, not VF or muscle fat, is a marker for insulin resistance in the fasting state as well as post-prandial insulin resistance in elderly female monkeys. Our findings suggest that insulin resistance associated with increased adiposity in primates may be mediated through the mechanisms that involve hepatic handling of lipid flux that lead to accumulation of liver fat.

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What is already known about this subject

- Visceral adiposity is linked with increased accumulation of ectopic fat, impaired glucose tolerance, and risk for diabetes in humans.
- Ectopic lipid accumulation in the liver and muscle is linked with impaired glucose metabolism.

What this study adds

- In an aging female non-human primate model, hepatic lipid content demonstrated the strongest link to insulin resistance compared to other fat depots.
- Visceral fat and intramyocellular fat was not significantly associated with insulin resistance in this model.

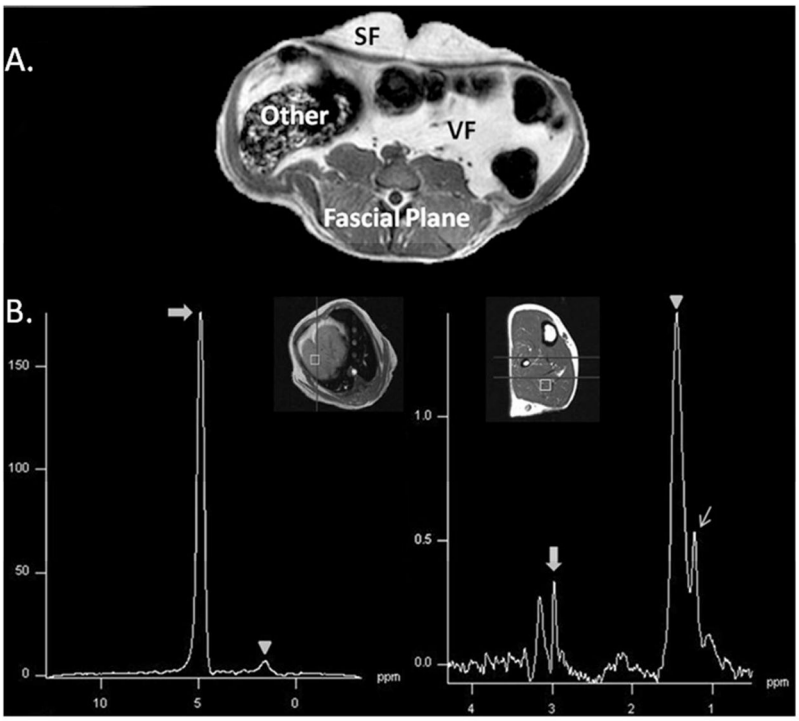

Figure 1.

A) Magnetic resonance imaging of abdomen of a female Rhesus macaque showing intra-abdominal fat and subcutaneous fat. The average amount of visceral fat in the group (n=18) was nearly twice that of subcutaneous fat ($p<0.001$) (Table 1). **B)** ^{1}H magnetic resonance spectroscopy. *Left:* Image of the liver for determination of intrahepatic lipid content. Thick arrow – Water peak at 4.7 parts per million (ppm), arrow head – intrahepatic lipid peak at 1.3–1.5 ppm. Intrahepatic lipid content is expressed as a percentage (lipid/water %). Smaller image depicting voxel capturing liver tissue in cross-section. *Right*: Image of the soleus muscle for determination of muscle lipid content. Thick arrow – creatine methyl peak at 3.0 parts per million (ppm), arrow head – extramyocellular (EMCL) peak at 1.5 ppm, narrow arrow – intramyocellular (IMCL) peak at 1.3 ppm. Muscle lipid content is expressed as a percentage (lipid/creatine %). Smaller image depicting voxel capturing the muscle tissue in cross-section.

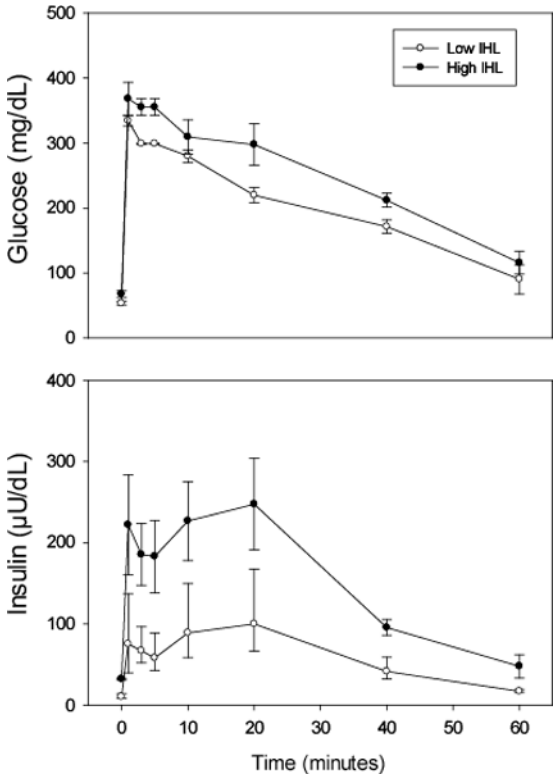

Figure 2.

Insulin and glucose profiles during intravenous glucose tolerance testing in female Rhesus macaques. Graphs show mean and standard error bars for those with the greatest amount of intrahepatic lipid (IHL) (top tertile, closed circle, n=4) and the least amount of intrahepatic lipid (lowest tertile, open circle, n = 4) by ^{1}H magnetic resonance spectroscopy (see also Table 1). *Top graph:* glucose (P = 0.20 for differences between tertile area under the curves). *Bottom graph:* insulin (P = 0.02 for difference between tertile area under the curves).

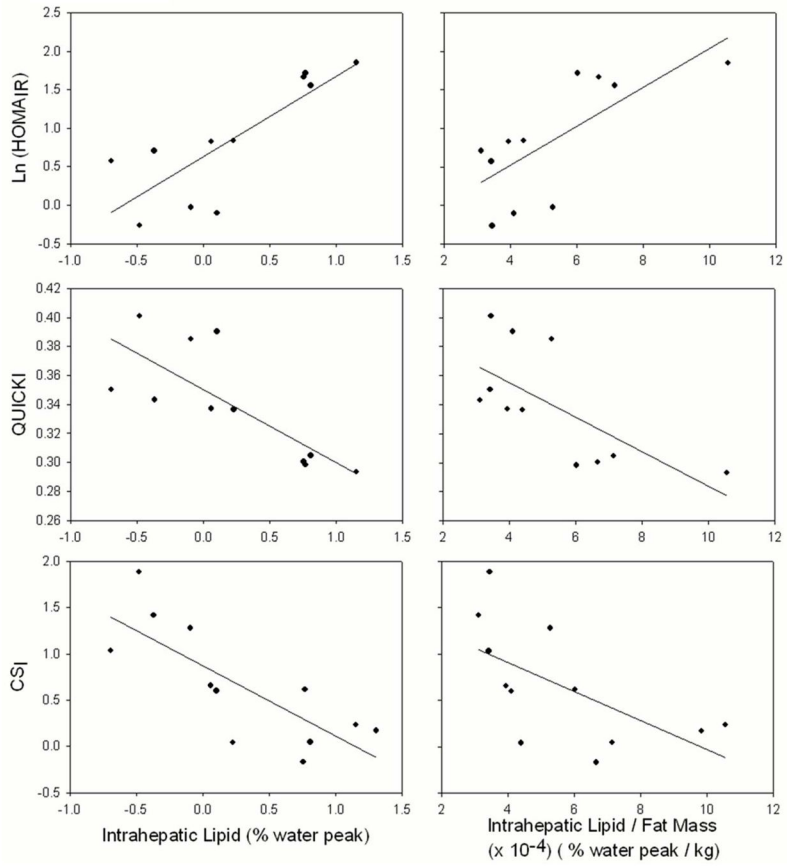

Figure 3.

Relationships between insulin sensitivity and intrahepatic lipid. Linear regression of: Ln IHL vs. $HOMA_{IR}$ (r = 0.81, *P* = 0.002) and IHL adjusted for fat mass vs. $HOMA_{IR}$ (r = 0.73, *P* = 0.01); IHL vs. QUICKI (r = −0.78, *P* = 0.005) and IHL adjusted for fat mass vs. QUICKI (−0.68, *P* = 0.02); IHL vs. CS_I (r = −0.78, *P* = 0.003) and IHL adjusted for fat mass vs. CS_I (r = −0.61, *P* = 0.03). Data that were non-normally distributed were natural log-transformed.

Table 1

Body composition (n=18) and insulin sensitivity (n=12) characteristics of primate subjects

All Subjects		**Bottom Tertile IHL (n = 4)**	**Top Tertile IHL (n = 4)**
Weight (kg)	7.95 (6.45–9.40)	7.46 (6.45–8.00)	8.62 (8.05–9.40)
Fat Mass (kg)	2.63 (1.46–3.76)	1.80 (1.46–2.22)	3.38 (3.01–3.76)
Lean Mass (kg)	5.20 (4.10–6.00)	5.47 (4.26–6.00)	4.94 (4.10–5.55)
Percentage fat mass (%)	33 (20–48)	25 (20–34)	41 (37–48)
Visceral fat area (mm^2)	4240 (2730–7090)	3500 (2730–4170)	6170 (4620–7090)
Subcutaneous fat area (mm^2)	2200 (1150–5190)	1440 (1150–1840)	3210 (2010–5190)
EMCL (lipid/creatine %)	9.76 (4.21–22.39)	13.47 (9.58–22.39)	13.87 (4.92–21.4)
IMCL (lipid/creatine %)	2.80 (1.49–5.34)	3.00 (2.07–5.34)	3.10 (2.01–4.82)
IHL (lipid/water %)	1.81 (0.50–4.76)	0.68 (0.50–0.91)	2.82 (2.16–3.70)
$HOMA_{IR}$	2.99 (0.77 – 6.35)	1.38 (0.77–2.03)	5.55 (4.73–6.35)
QUICKI	0.34 (0.29–0.40)	0.37 (0.34–0.40)	0.30 (0.29–0.31)
CS_I ($\times 10^{-4}$ min^{-1} $[\mu U/ml]^{-1}$)	2.34 (0.84–6.59)	4.28 (2.81–6.59)	1.34 (1.05–1.85)

EMCL, extramyocellular lipid; IMCL, intramyocellular lipid; IHL, intrahepatic lipid; $HOMA_{IR}$, homeostasis model assessment of insulin resistance; QUICKI, quantitative insulin sensitivity check index; CS_I, calculated insulin sensitivity index. Values are expressed as means (range).

Table 2

Linear regression analysis of measures of body composition and glucose metabolism. n = 12.

	Fasting insulin		AUC IVGTT Glucose		AUC IVGTT Insulin		$HOMA_{IR}$		QUICKI		CS_I	
Independent variables	**r**	***P***	**r**	***P***	**r**	***P***	**r**	***P***	**r**	***P***	**r**	***P***
IHL	0.86	**0.001**	0.47	0.12	0.74	**0.009**	0.81	**0.002**	−0.78	**0.005**	−0.78	**0.003**
SF	0.79	**0.004**	0.73	**0.008**	0.44	0.18	0.72	**0.01**	−0.70	**0.02**	−0.49	0.11
VF	0.38	0.25	0.27	0.40	0.20	0.56	0.38	0.25	−0.34	0.31	−0.33	0.30
Fat Mass	0.82	**0.002**	0.60	**0.04**	0.69	**0.02**	0.77	**0.006**	−0.75	**0.008**	−0.78	**0.003**
IHL / fat mass	0.73	**0.01**	0.25	0.44	0.61	**0.048**	0.73	**0.01**	−0.68	**0.02**	−0.61	**0.03**
SF / fat mass	0.54	0.09	0.45	0.15	0.19	0.57	0.48	0.14	−0.46	0.15	−0.15	0.64
VF / fat mass	−0.36	0.28	−0.27	0.39	−0.44	0.18	−0.32	0.34	0.35	0.30	0.44	0.15

Non-normally distributed data were log transformed prior to linear regression analysis. IHL, Intrahepatic lipid; SF, Subcutaneous fat area; VF, Visceral fat area; AUC IVGTT, Area under the curve intravenous glucose tolerance test; $HOMA_{IR}$, Homeostasis model assessment of insulin resistance; QUICKI, Quantitative insulin sensitivity check index; CS_I, Calculated insulin sensitivity index. Bold numbers indicate significant P-values (< 0.05).