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Sex and Depot Differences in Palmitoleic Acid Content of Human Blood and Fat

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Abstract

Background.—Palmitoleic acid has been classified as an insulin-sensitizing lipokine, but evidence for this from human studies has been inconsistent. We hypothesized that this is related to either the types of samples or conditions under which samples are collected.

Methods.—We measured plasma palmitoleic acid and total free fatty acids (FFA) using ultraperformance liquid chromatography in blood samples collected from 34 adults under a variety of conditions. We collected duplicate samples of adipose (n=10), FFA (n = 9) and VLDL-triglyceride (n=7) to measure the palmitoleic acid as a percent of total fatty acids. We tested whether percent palmitoleic acid was correlated with insulin resistance as measured by homeostatic model of insulin resistance (HOMA-IR). Adipose stearoyl-coenzyme A desaturase 1 (SCD-1) protein was measured by capillary Western.

Results.—FFA-palmitoleic acid % increased as a function of total FFA and was greater ($P<0.005$) in females than males. Adipose palmitoleic acid % was greater in females than males ($P<0.001$), as was adipose SCD-1. Palmitoleic acid was greater in femoral than abdominal fat in both females and males ($P<0.001$), and correlated positively with HOMA-IR only in females. The test-retest reliability values for % palmitoleic acid were $7 \pm 10\%$ for adipose, $24 \pm 26\%$ for VLDL and $53 \pm 31\%$ for FFA.

Conclusions.—Because FFA-palmitoleic acid % varies as a function of total FFA, investigators should re-evaluate how palmitoleic acid data is presented. The positive relationship between adipose palmitoleic acid and HOMA-IR in females suggests it is not a potent, insulin-sensitizing lipokine in humans.

Keywords

ultra-performance liquid chromatography (UPLC); free fatty acids; femoral adipose tissue; abdominal adipose tissue

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Author contributions.

P.R. - analyzed samples and data, interpreted results of experiments, prepared figures, drafted manuscript, edited and revised manuscript, approved final version of manuscript. N.B. - performed experiments, analyzed samples and data, interpreted results, edited and revised manuscript, approved final version of manuscript. M.D.J. - Conception and design of research, interpreted results of experiments, edited and revised manuscript, approved final version of manuscript.

Conflict of interest.

The authors have no conflict of interest to disclose.

INTRODUCTION

The experimental elevation of plasma free fatty acids (FFA) in normal weight, healthy individuals can induce many of the metabolic abnormalities of obesity (Belfort, 2005, Boden, 1994, Lewis, 1995). However, recent data suggests that not all fatty acids are equal with regards to metabolic responses. For example, palmitoleic acid, the omega-7 cis-monounsaturated derivative of palmitic acid is suggested to act as an insulin sensitizing lipokine based upon animal experiments (Cao, 2008) and correlative studies in humans (Pinnick, 2012). Because there is little dietary palmitoleic acid, some speculate that much of the blood and adipose tissue palmitoleic acid is generated by the desaturation of palmitic acid via the endogenous enzyme stearoyl-coenzyme A desaturase 1 (SCD-1) (Gong, 2011, Stefan, 2010).

The beneficial effects of palmitoleic acid were convincingly demonstrated in experimental animals (Cao, 2008), however human studies suggest a more complicated picture. A positive correlation between adiposity and relative palmitoleic acid concentrations in humans has been described (Gong, 2011, Iggman, 2010, Paillard, 2008). The varying associations between palmitoleic acid and metabolic risk factors (Bergman, 2013, Fabbrini, 2011, Pinnick, 2012) may be related to the confounding effects of sex (Pinnick, 2012, Stefan, 2010), obesity, or differences in fatty acid clearance, each of which can impact total FFA concentration, as well as different sources (liver or adipose tissue, exogenous or endogenous) of fatty acids. The goal of our studies was to understand whether palmitoleic acid is associated with metabolic health, and whether the sample source (adipose, VLDL-triglyceride fatty acids, plasma FFA) and sex need to be considered when testing for these associations.

MATERIALS AND METHODS

Participants.

We collected blood from two groups of volunteers (Figure 1). The first group was prospectively recruited to measure plasma palmitoleic acid content. The group consisted of 6 volunteers (3 males), who we identified as being in good health on no medications (IRB:12-003576). Each volunteer reported to the Endocrine Research Unit, Mayo Clinic, Rochester, MN in the morning weekly for 4 weeks where a venous blood sample was drawn (total of 5 samples). The second group included a convenience sample of 28 volunteers (15 premenopausal females) who had participated in related studies of FFA metabolism (IRB:2089-03, 2090-03, 2091-03 and 2092-03). These volunteers were likewise taking no medications, were weight stable for 3 months before the study, and were recruited to represent a wide range of BMI (20–36 kg/m²). The samples were selected to assure a balance between males and females, obese and non-obese, and samples from studies collected in the fasting and fed conditions. A complete blood count, electrolyte panel, liver and kidney function tests and routine urinalysis were documented as normal. The study was approved by the Mayo Clinic Institutional Review Board. Informed written consent was obtained from all volunteers.

Study Design.

The second group of volunteers had body composition measured using dual energy x-ray absorptiometry. In order to obtain an especially broad range of plasma FFA concentrations, samples from these groups were selected for analysis because they were collected under fasted (Koutsari, 2011) or fed (Koutsari, 2012) conditions, and because they had undergone adipose biopsies as part of the studies. Analyses were performed using 3 blood samples collected over a 30 minute interval from 6 lean males and 5 lean females under fasted (Koutsari, 2011) or fed (Koutsari, 2012) conditions, as well as 7 obese males and 10 obese females under fasted or fed (Koutsari, 2012) conditions. The fed condition consisted of volunteers consuming small portions of a combination of fat-free frozen yogurt, skim milk, Beneprotein (Nestlé Nutrition, Florham Park, NJ), Polycose (Abbott Nutrition, Columbus, OH), and frozen unsweetened strawberries at 20 minute intervals to achieve steady state suppression of FFA. One blood sample was used for analysis of VLDL-triglyceride fatty acids. Abdominal and femoral subcutaneous fat biopsies were collected under local anaesthesia using sterile technique within 24 h of the blood samples. Ten of these volunteers (5 males) participated in two studies, allowing us to collect duplicate abdominal and femoral subcutaneous adipose biopsies, duplicate measures of plasma FFA (under different conditions) and duplicate measures of VLDL-triglyceride fatty acid content ($n = 7$); the studies were at least 2 months apart. Blood samples were collected under fasting conditions from 13 of the males and 14 of the females to measure plasma glucose and insulin concentrations for calculation of fasting homeostasis model assessment of insulin resistance (HOMA-IR).

Measurement of fatty acids with UPLC.

Because not all laboratories have access to mass spectrometry, we developed an ultraperformance liquid chromatography (UPLC) UPLC approach that involves UV detection to measure palmitoleic acid by modifying our previously published HPLC approach (Miles, 1987). We measured fatty acid concentrations using an Acquity UPLC System (Waters Corporation, Milford, MA) coupled with a heated (55° C) Acquity UPLC BEH C18 column (1.7 μ m; 2.1 $\times$ 150 mm; Waters Corporation, Milford, MA) and a ACQUITY UPLC Tuneable UV (TUV) detector set at 254 nm. The mobile phase was 72 % acetonitrile:28% water at a flow rate of 0.6 ml/min. The internal standard [$^2\text{H}_{31}$]palmitic acid (2 μ g/sample) was used to allow measurement of arachidonic, eladic, linoleic, linolenic, myristic, palmiteladic, palmitic, palmitoleic, oleic, and stearic acids. The mixed FFA concentration standard solution was newly created for the purpose of this study using pure (as measured by LC/MS), gravimetrically measured fatty acids (Sigma-Aldrich, St. Louis, MO) suspended in fatty acid free bovine serum albumin (BSA) (Sigma-Aldrich).

We also measured plasma FFA concentrations on 30 of the same plasma samples using liquid chromatography/mass spectrometry (Persson, 2010, Song, 2019) for comparison with UPLC with UV detection.

Plasma samples.

To 0.5 ml of each plasma sample and each of the standard curve samples we added 20 μ l of the internal standard ([$^2\text{H}_{31}$]palmitate) as previously described (Jensen, 1988); the fatty

acids were extracted using a Dole solution (isopropyl alcohol 40 parts: heptane 10 parts: 1N H₂SO₄ 1 part). The fatty acids were converted to their phenacyl derivatives using α -bromoacetophenone, triethylamine as previously described (Miles, 1987). The samples were dried and re-suspended in 30 μ L acetonitrile for injection onto the UPLC. The personnel who analysed the samples did not have access to clinical data from the volunteers.

Adipose tissue biopsies and analysis.

Subcutaneous adipose tissues biopsies were collected from the abdominal (just lateral to the umbilicus) and the femoral region (on the anterior-lateral aspect of the mid-thigh) as previously described (Koutsari, 2008, Shadid, 2007). The samples were thoroughly rinsed with saline through Nitex Nylon Fiber 250/50 and frozen at -80° until being processed. To extract the adipose tissue lipids we first shook ~ 10 mg of each sample in 15 ml of CHCl₃:methanol (2:1) and then placed them in a dark cold room at 4 degrees Celsius for two days. We added 3.75 ml of 0.88% KCl to each sample and after 20 min the samples were centrifuged for 10 min at 914 g. The lower layer was removed and dried. The lipid classes were fractionated using Superclean cartridges (LC-NH2 500 mg/3 mL; Supelco – Sigma Aldrich; St. Louis, MO) per manufacturing instructions to collect the triglyceride (TG) fraction, which were hydrolysed with 2 ml (80:20) methanol:heptane (with 5 μ l 10N NaOH added per ml) for 30 min at 60°C. Extraction of samples and standards, derivatization and measurement by UPLC were performed as described above. The personnel who analysed the samples did not have access to clinical data from the volunteers.

Measurement of VLDL-triglyceride fatty acids.

2 ml of plasma samples were placed in the bottom of an ultracentrifuge tube, covered with 3.6 mL of 1.006 g/mL density solution and centrifuged at 145,606 g for 19.5 h at 4°C in a Beckman ultracentrifuge (Optima™, LE-80K, Beckman Instruments, Spinco Division, Palo Alto, CA). Afterwards the top 2 cm of the tube, containing the VLDL particles were collected. The VLDL were extracted with the Dole solution (see above), the lipids fractionated and the TG's hydrolysed, extracted, derivatized and measured by UPLC as described above.

Plasma glucose concentrations were measured using the glucose oxidase method (YSI, Yellow Springs, OH). A chemiluminescence assay with reagents (Access Assay; Beckman Coulter Inc., Chaska, MN) was used to measure insulin concentrations.

Measurement of SCD-1 protein.

This assay was performed largely as previously described (Heinemann, 1998). In brief, adipose tissue was stored at -70 degrees C until processed by homogenization and then centrifugation to remove nuclei and lipid. Total protein was measured using BCA analysis. Capillary western blotting was carried out using a Protein Simple Wes® system. Immunoblotting was performed with a 1:100 dilution SCD-1 rabbit primary antibody (Abnova, Walnut, CA). The peak intensity was calculated and compared to the intensity of a calibrator sample. The amount of SCD in each patient sample was reported as a relative amount compared to the control sample. SCD-1 showed a peak at ~ 40 kDa. Minor shifting is commonly seen in capillary western blotting. Because we measured the weight of the tissue

and lipid and protein content of each sample, we are able to report this relative amount of SCD-1 per mg tissue.

Calculations.

We used the percent palmitoleic acid in FFA for a single sample for the first group of volunteers and the mean of the 3 samples for the second group. Adipose tissue and VLDL-triglyceride percent palmitoleic acid is a single analysis during each study. HOMA-IR was calculated as fasting serum insulin (mU/mL) $\times$ fasting plasma glucose (mmol/L)/22.5. There was no time interval between the samples collected for palmitoleic acid and those for the HOMA-IR and there were no clinical interventions

Statistics.

All values are provided as mean $\pm$ SD unless otherwise stated. The percent palmitoleic acid values in the female and male groups were normally distributed (Shapiro-Wilk test). HOMA-IR and total FFA were not normally distributed, thus non-parametric statistical approaches were used. Comparisons between males and females were performed with a non-paired T-test whereas comparisons between adipose depots from the same individual were done using a paired T-test. The reproducibility (test-retest reliability) assessment for the percent of palmitoleic acid in adipose, VLDL-TG and FFA is presented as the absolute difference between the two values and their mean. Our primary hypothesis was that the percent palmitoleic acid would be negatively associated with insulin resistance, as measured by HOMA-IR, using linear regression analysis. Because the percent palmitoleic acid in FFA was different between females and males and varied as a function of total FFA concentrations, we adjusted for this effect by normalizing each participant's percent FFA-palmitoleic acid to their sex-specific norms relative to total FFA concentrations. This was done by describing the linear relationship between total FFA and % palmitoleic acid for males and females separately to determine whether an individual's palmitoleic acid content was greater or less than predicted (their individual residual variance). Each individual's residual variance was calculated by subtracting the predicted % palmitoleic acid from the observed % palmitoleic acid. It was this residual FFA-palmitoleic acid value, not the raw concentration or % palmitoleic acid, that was used to test for relationships between plasma FFA palmitoleic acid and HOMA-IR using linear regression. For comparisons between VLDL and adipose % palmitoleic acid we used sex-specific Pearson's linear regression analysis.

The power calculations for this analysis were based upon the following estimates: we wished to limit the probability of a type I error to 0.10 and the probability of a type II error to 0.05. We found the technical SD of the % palmitoleic acid averaged 9% and the biological plus technical variability of the adipose % palmitoleic acid averaged 5%. We hypothesized a negative correlation between % palmitoleic acid and HOMA-IR with a slope of -0.50 . An n of 25 would allow us to detect a correlation of -0.556 with a standard deviation of the regression of 0.075.

RESULTS

Volunteer characteristics.

The 6 volunteers (3 males) undergoing weekly blood samples to assess inter-individual variability were 29 ± 6 years old (mean $\pm$ SD) with BMI of 26.0 ± 2.9 kg/m². These volunteers underwent only these measurements. The salient characteristics of the volunteers participating in the second set of studies are presented in Table 1. The non-obese and obese volunteers were matched for age and, as expected, obese volunteers had significantly greater fasting triglyceride concentrations and were more insulin resistant as measured by HOMA-IR. The proportions of males and females were comparable in the non-obese and obese groups.

Reproducibility of the measurement.

The technical variability in palmitoleic acid concentration was assessed by examining the results from a quality control plasma sample run on 12 separate days; the concentration was 8 ± 1 μ mol/L (mean $\pm$ SD), which equalled $2.2 \pm 0.2\%$ of total FFA (CV = 9%). The percent of FFA that were palmitoleic acid as measured by UPLC/UV detection and by LC/MS was highly correlated ($n = 30$ samples, $r = 0.96$, $P < 0.0001$).

For the 10 volunteers with duplicate adipose biopsies, palmitoleic acid was $4.2 \pm 0.9\%$ (mean $\pm$ SD, range 2.7 – 5.7%) of fatty acids in abdominal triglyceride and $5.8 \pm 1.1\%$ (range 4.2 – 8.8%) of femoral triglyceride. The test-retest reliability between the first and second biopsies was $7 \pm 10\%$ of the mean. For the 7 volunteers (4 males) with replicate VLDL-triglyceride fatty acid analysis, palmitoleic acid was $5.9 \pm 2.1\%$ of fatty acids and the test-retest reliability was $24 \pm 26\%$ of the mean. Six volunteers had blood drawn in the fasting state each week for 4 weeks (total of 5 samples); palmitoleic acid as a % of total FFA is plotted vs. total FFA in the right panel of Figure 2. Palmitoleic acid constituted $3.3 \pm 0.5\%$ of total FFA for these volunteers and the CV of the 5 single measures on 5 separate occasions averaged $19 \pm 11\%$. We noted that, even in this small set of data, the within individual the percent palmitoleic acid was greater when plasma total FFA concentrations were greater.

Palmitoleic acid was $2.4 \pm 0.9\%$ of plasma FFA for the 9 from volunteers who provided both fasted and fed samples. The absolute test-retest difference between the fasted and fed samples was $1.1 \pm 0.6\%$, which was a test-retest difference of $53 \pm 31\%$ of the mean. We were unable to chromatographically separate palmitoleic acid from arachadonic acid from the fed and fasted blood samples collected from one volunteer despite repeated attempts, and thus this volunteer's data was not able to be included in the final analysis.

Plasma FFA.

The percent of fatty acids that were palmitoleic acid in plasma, adipose tissue (abdominal and femoral fat) and VLDL-TG are presented in Table 2. For the larger group of volunteers in group 2, plasma palmitoleic acid ranged from 1.0 – 4.4% of total FFA concentrations and were greater ($P < 0.005$) in females than males (Table 2). The percentage of palmitoleic acid varied as a function of total FFA concentrations, increasing as total FFA concentrations

increased (Figure 2, left panel). The same pattern was observed in samples obtained from the study of group 1 volunteers, which included the typical range of fasting plasma FFA (Figure 2 right panel). Potentially because of this relationship, the % plasma palmitoleic acid was not correlated with HOMA-IR when the data from both non-obese and obese females and males were analysed separately or pooled together. We therefore used sex-specific relationships between plasma total FFA concentrations and the percentage of palmitoleic acid to calculate each individual's residual plasma palmitoleic FFA (corrected total FFA concentration); there remained no relationship between corrected % palmitoleic acid-FFA and insulin resistance as measured by HOMA-IR.

Plasma VLDL-triglycerides.

The percent of VLDL-triglyceride fatty acids that were palmitoleic was not different between males and females (Table 2, $P = 0.43$). There was no correlation between VLDL-triglyceride palmitoleic acid % and HOMA-IR in females ($r = 0.001$, $P > 0.7$) or males ($r = -0.39$, $P = 0.19$). The VLDL-triglyceride palmitoleic acid % was greater ($P < 0.0001$) than in plasma FFA % palmitoleic acid and the two variables were not significantly correlated ($r = 0.31$, $P = 0.13$).

Adipose tissue triglyceride fatty acids.

The percentage palmitoleic acid in adipose tissue lipids is also provided in Table 2. Palmitoleic acid was greater in femoral than abdominal fat in males and females (both $P < 0.001$) and palmitoleic acid was greater in females than males in both depots ($P < 0.001$ for both depots). Palmitoleic acid was greater in obese than non-obese volunteers ($P = 0.006$) in abdominal, but not femoral ($P = 0.43$) fat. The adipose tissue triglyceride palmitoleic acid content was positively correlated with HOMA-IR for both abdominal ($r = 0.74$, $P = 0.002$) and femoral ($r = 0.65$, $P = 0.01$) in females (Figure 3). There was not a significant relationship between adipose tissue palmitoleic acid content and HOMA-IR in males ($r = 0.04$, $P > 0.7$).

For those with plasma samples collected under fasting conditions, the % plasma palmitoleic acid was correlated with both abdominal ($r = 0.56$, $P = 0.002$) and femoral ($r = 0.60$, $P < 0.001$) adipose tissue triglyceride palmitoleic acid content (Figure 4).

SCD-1.

We measured SCD-1 protein in 6 abdominal and 6 femoral adipose tissue samples from males ($BMI = 32.2 \pm 3.0$) and females ($BMI = 30.5 \pm 1.0$). SCD-1 protein/mg tissue was greater in femoral than abdominal adipose tissue in females ($P = 0.02$). Both femoral ($P = 0.001$) and abdominal ($P = 0.005$) adipose SCD-1 protein was greater in females than males (Figure 5).

DISCUSSION

Our goal was to assess the variability in measures of adipose tissue, VLDL-triglyceride and plasma FFA palmitoleic acid content as well as to the relationship between palmitoleic acid and insulin sensitivity in humans. The assay variability of palmitoleic acid as the % of total

fatty acids using UPLC with UV detection was close to the adipose tissue variability from duplicate biopsies, whereas the variability in VLDL-TG fatty acids and plasma FFA was 2 – 4 times greater than the assay variability. One reason for the greater variability in the percentage of plasma palmitoleic acid FFA is likely to be differences in clearance of various FFA at high concentrations (Nelson, 2013). This variability will make it difficult to detect associations between FFA palmitoleic acid and insulin sensitivity and might even lead investigators to misinterpret data if samples are collected under conditions of low vs. high FFA. Likewise, given the sex differences in plasma FFA palmitoleic acid found by us and others (Pinnick, 2012, Stefan, 2010), an additional adjustment for sex may be necessary. We found no association between plasma FFA or VLDL-triglyceride palmitoleic acid content and HOMA-IR, a crude measure of insulin resistance. However, there was a relatively robust, positive association between adipose palmitoleic acid content and HOMA-IR in females, consistent with the report of Fabbrini et al (Fabbrini, 2011).

We used UPLC to reduce the analysis time and improve the separation of fatty acids compared to the HPLC technique we have used for many years (Miles, 1987). The amount of fatty acids in plasma and adipose are more than sufficient to quantitate using phenacyl derivatives with UV detection, avoiding the need for more expensive mass spectrometry detection approaches (Cao, 2008, Fabbrini, 2011, Gong, 2011, Paillard, 2008). This approach should allow investigators without access to mass spectrometry to investigate how variations in plasma FFA, VLDL and adipose content of “minor” fatty acids relate to health and disease.

New attention focused on palmitoleic acid following the report that mice deficient in the fatty acid binding protein aP2 have increased plasma and adipose palmitoleic acid (Cao, 2008) together with improved peripheral insulin sensitivity (Hudgins, 2008). It was suggested that the increased plasma and adipose palmitoleic acid suppressed liver SCD-1 and increased skeletal muscle glucose uptake (Cao, 2008). Interestingly, greater SCD-1 activity is seen with obesity in animal models (Enser, 1975, Enser, 1979, Park, 1997, Smith, 1999), whereas SCD-1 deficient mice are protected against diet induced obesity (Miyazaki, 2001, Ntambi, 2002). It has been suggested that palmitoleic acid acts in a counter-regulatory feedback loop to reduce de novo fatty acid synthesis in the liver (Cao, 2008). Finally, studies using animal models suggest a beneficial effect of palmitoleic acid on adipocytes, macrophages, skeletal muscle and pancreatic beta-cells (Dimopoulos, 2006, Maedler, 2003, Sauma, 2006).

However, the results of clinical investigations are not as straightforward. Some authors report that greater plasma palmitoleic acid is associated with improved insulin sensitivity (Bergman, 2013, Pinnick, 2012, Stefan, 2010), whereas others (Fabbrini, 2011, Gong, 2011, Iggman, 2010) do not. Adults in the United States get very little palmitoleic acid in the diet; it is estimated that of the typical 100 g of fat in the diet only 1.2% are in the form of palmitoleic acid (Vannice, 2014). This contrasts with the 3.5 – 6.6 % range of palmitoleic acid in adipose tissue and VLDL-TG (Table 2), indicating that desaturation of palmitic acid likely accounts for the vast majority of palmitoleic acid in humans. For our studies of FFA metabolisms we place our volunteers on diets controlled for macronutrient composition for 3–5 days prior to the experiments, which would obviate short-term effects of food intake on

these results. Although there may have been longer-term effects of diet on the fatty acid profiles of our volunteers, for the studies we've conducted that included survey assessments of food intake, we do not see major differences between females and males, strongly suggesting that our observations don't reflect dietary responses. Although there are nutritional sources, including some foods (macadamia nuts) and nutritional supplements (sea buckthorn oil - *Hippophae rhamnoides*) that provide a high percentage of palmitoleic acid, desaturation of palmitic acid by SCD1 is considered to be the main source in humans. Our findings regarding adipose palmitoleic acid content are consistent with the greater adipose SCD1 mRNA expression in females than men and in femoral than abdominal adipose observed previously (Pinnick, 2012) and confirmed by our Western blot analysis. We note that insulin is one of the factors that stimulates SCD1 expression (Mauvoisin, 2011), which may explain the positive association between hyperinsulinemia and adipose palmitoleic acid content observed by Fabbrini et al (Fabbrini, 2011) and ourselves.

There are some limitations to our study. The HOMA-IR is not a good a measure of insulin resistance as an insulin clamp, although the results of the two measures are positively correlated. That said, the finding that adipose palmitoleic acid was positively associated with HOMA-IR, and our hypothesis was for a negative correlation. This strongly suggests we didn't miss a negative association between palmitoleic acid and insulin resistance measures.

In summary, our findings that FFA and adipose palmitoleic acid concentrations are greater in females than males are consistent with previous reports (Pinnick, 2012, Stefan, 2010), as is our finding of greater femoral than abdominal adipose content (Pinnick, 2012). The observation that percent plasma FFA palmitoleic acid varies as a function of total FFA concentration indicates that investigators will need to take this into consideration. Our data do not support a role for palmitoleic acid as a potent insulin sensitizer in humans. Rather, our data is more consistent with the known role of insulin as a stimulator of SCD-1 (Mauvoisin, 2011), at least in females.

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Figure 1:
Flowchart of how participants and samples were selected for the studies that included measurements of palmitoleic acid content of plasma and adipose lipids.

Figure 2.

Plasma FFA concentrations vs. % palmitoleic acid. Left panel - plasma total free fatty acid (FFA) concentrations are plotted on the x-axis and the percentage of plasma FFA as palmitoleic acid is plotted on the y-axis, which serves as the y-axis values for the right panel also; the values are samples from females and males who were fasting or fed. The symbols for obese and non-obese men and women for the left panel are provided in the figure legend. The relationships between plasma total FFA concentrations and % palmitoleic acid were positive for both women ($r = 0.70$, $P = 0.0006$) and men ($r = 0.048$, $P = 0.049$). Right panel - plasma FFA as palmitoleic acid as a percent of plasma total free fatty acid (FFA) concentrations in fasting blood samples collected at weekly intervals for 4 weeks (5 samples each) from 3 females and 3 males. The 5 data points from each volunteer is represented by a unique symbol; open symbols are females and closed symbols are males. The regression formula, r value and P value refer to the combined dataset.

Figure 3.

Adipose palmitoleic acid vs. HOMA-IR. Adipose tissue palmitoleic acid content is plotted vs. HOMA-IR for females (left panels) and males (right panels). The percentage adipose tissue palmitic acid was positively correlated with insulin resistance in females ($n = 14$) for both abdominal ($r = 0.74$, $P = 0.002$) and femoral ($r = 0.65$, $P = 0.01$) fat; no significant associations were found for males.

Figure 4.

Femoral and abdominal adipose tissue palmitoleic acid vs. plasma palmitoleic acid %. Adipose tissue palmitoleic acid content is plotted vs. plasma palmitoleic acid % for abdominal (left panels) and femoral (right panels) adipose tissue. Plasma FFA % palmitoleic acid was correlated with both abdominal ($r = 0.56$, $P = 0.002$) and femoral ($r = 0.60$, $P < 0.001$) adipose tissue triglyceride palmitoleic acid content.

Figure 5.

Capillary Western measurements of adipose tissue SCD-1. Top - Representative WES blot of SCD-1. All samples were normalized to total protein. Bottom - Relative SCD-1 protein content per mg abdominal or femoral tissue weight. # - $P = 0.001$ vs. males femoral; * - $P = 0.018$ vs. abdominal females. & - $P = 0.0057$ vs. abdominal males.

Table 1.

Characteristics of volunteers participating in the second set of studies

	Non-obese (n = 11)	Obese (n = 17)
Age (years)	32 ± 10	37 ± 7
BMI (kg/m ²)	22.6 ± 1.7	32.3 ± 3.0
Body fat (%)	23 ± 8	39 ± 7
Fasting glucose (mmol/L)	4.8 ± 0.4	5.1 ± 0.5
HOMA-IR	0.6 ± 0.3	2.1 ± 1.3 *
Fasting triglycerides (mmol/L)	0.79 ± 0.28	2.05 ± 1.91 †

All values are mean ± SD. BMI and body fat are not compared between groups because they were selected to be different and therefore not subject to statistical testing.

* P < 0.001 vs. non-obese;

† P < 0.05 vs. non-obese.

Table 2.

% Palmitoleic Acid in Plasma and Adipose Tissue

	% Palmitoleic Acid			
		Adipose Tissue		
	Plasma FFA	Femoral	Abdominal	VLDL- Triglyceride
Females	3.1 ± 0.9 [#]	6.0 ± 1.4 ^{##†}	4.3 ± 1.2 ^{#†}	5.7 ± 2.5 [†]
Males	2.3 ± 0.7	4.5 ± 1.0 ^{*†}	3.5 ± 0.8 [†]	5.2 ± 5.2 [†]
Non obese	2.6 ± 1.1	5.2 ± 0.8 [†]	3.5 ± 0.7 ^{†&}	6.6 ± 2.3 ^{&†}
Obese	2.9 ± 1.2	5.6 ± 1.7 [†]	4.4 ± 1.1 [†]	4.7 ± 1.3 [†]

All values are mean ± SD. FFA – free fatty acids; VLDL – very low density lipoprotein.

* P < 0.001 vs. Abdominal;

[#] P < 0.005 vs. males;

[&] P < 0.05 vs obese.

[†] P < 0.001 vs plasma